

An Assessment of Solute Kinetics and The
Application of Mathematical Modelling in the
Haemodialysis Process

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A Thesis for the Degree of Doctor of Medicine

University of Edinburgh

Presented 2007

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List of Abbreviations

AAMI	American Association of Medical Instrumentation
AGE	Advanced Glycosylation End-product
A β 2m	Beta-2-microglobulin amyloid
β 2m	Beta-2-microglobulin
C	Solute concentration
C ₀	Initial solute concentration
Ca ²⁺	Calcium
C _{b_i}	Concentration in blood at dialyser inlet
C _{b_o}	Concentration in blood at dialyser outlet
C _e	Extracellular concentration
C _{eq}	Solute concentration after re-equilibrium has occurred
C _F	Concentration of solute in dialysate
C _i	Intracellular concentration
CKD	Chronic Kidney Disease
DCOR	Dialysis Outcomes Revisited Study
DRA	Dialysis Related Amyloid
eGFR	Estimated glomerular filtration rate
eKt/V	Estimated Kt/V
EqKt/V	Equilibrated Kt/V
FDA	Food and Drug Administration
F _p	Plasma fraction of whole blood
F _r	Red cell fraction of whole blood
G	Generation rate of solute
GH	Growth hormone
HCO ₃ ⁻	Bicarbonate
Hct	Haematocrit
HD	Haemodialysis
HDF	Haemodiafiltration
HFHD	High-flux haemodialysis
IFHD	Intermediate-flux haemodialysis
K _d	Dialyser clearance
K ⁺	Potassium
kD	kiloDaltons
K _{diff}	Diffusive dialyser clearance
K-DOQI	Kidney Disease Outcomes Quality Initiative
K _{ie}	Intracellular to extracellular mass transfer coefficient
K _{nr}	Non-renal clearance of solute
K _r	Renal clearance of solute
KRU	Residual renal clearance of urea
K _{uf}	Convective clearance of solute
LFHD	Low-flux haemodialysis
M	Mass of solute
M _e	Extracellular mass of solute
M _i	Intracellular mass of solute

NCDS	National Co-operative Dialysis Study
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
PO_4^{2-}	Phosphate
PTH	Parathyroid hormone
Q_b	Blood flow
Q_d	Dialysate flow
Q_f	Total fluid flux across membrane
Q_{HDF}	Volume of reinfusion fluid in HDF
Q_u	Ultrafiltration rate
S	Sieving coefficient
SAP	Serum Amyloid P
spKt/V	Single pool Kt/V
StdKt/V	Standardised Kt/V
t	Time
TAC	Time averaged clearance
T_d	Total dialysis time
T_r	Transmittance coefficient
UF	Ultrafiltration volume
URR	Urea reduction ratio
USRDS	United States Renal Disease Survey
V	Volume
V_0	Initial volume

Acknowledgements

Several people have assisted me greatly with different aspects of this thesis. I would particularly like to thank my supervisor Dr Ken Farrington who has given his support and guidance throughout my career and particularly during this period of research.

The series of tutorials on biological modelling provided by Dr Paul Chamney permitted me to conduct the studies involving mathematical modelling.

The assistance of the nursing and technical staff at the Lister, St Albans, Luton, Royal Free and Barnet Hospital dialysis units is much appreciated. Richard Peto and Priya Pandya helped with some of the blood sampling during dialysis, and Richard Humber modified many pieces of equipment to make the studies possible.

The staff of the National Amyloidosis Centre assisted in the performance and interpretation of the SAP scans.

The study of diurnal variation was made possible by the provision of accommodation at the Roche Pharmaceutical Clinical Pharmacology Unit for which I would like to thank Dr Christopher Kilpatrick. My colleagues Paula Maclaren, Jack Galliford and Roshni Gokhal assisted in the blood sampling during the 24-hour period.

Dr Mandy Donaldson at the Hammersmith Hospital analysed the hormonal assays.

Finally, I would like to thank all the patients who participated in the studies.

Declaration

The concepts underlying the work presented in this thesis were conceived during discussions between Dr Ken Farrington, Dr Paul Chamney and myself over a number of years. The studies represent work I have personally undertaken and the thesis has been composed by me.

Where other individuals have contributed to the execution of studies this is clearly stated in the text. All sources of information are acknowledged specifically and permission sought for the replication of figures.

This thesis has not been submitted or accepted as a previous degree to either Edinburgh University or elsewhere, although individual components have been presented at scientific meetings or have been submitted as papers to peer-reviewed journals.

Elaine M Spalding

Summary

Aim

The aim of this thesis is to enhance knowledge about solute clearance during haemodialysis and to provide insight into factors that may influence dialysis efficiency. By improving the understanding of the kinetics of solute removal the limitations of current dialysis therapy will be better understood, and suggestions can be made for future improvements in the delivery of dialysis.

Methods

The history of dialysis technique and adequacy measurement is detailed. The origin and potential problems with urea kinetic modelling, including the effect of high haematocrit on adequacy, are explored.

Mathematical modelling is utilised to provide potential explanations for the clearance characteristics of phosphate and beta2-microglobulin during chronic dialysis. The phosphate model is explored further with studies in acute renal failure and the effect of dialysis on intra-erythrocytic phosphate concentrations is assessed. Diurnal variation in phosphate concentration is explored.

The effect of different dialysis modalities on beta2-microglobulin levels and symptoms of dialysis related amyloid is studied. Dialysis related amyloid deposition is investigated by a scintigraphic imaging technique.

Results

Haematocrit. High haematocrit is not found to have a significant effect on the clearance of solutes across a wide range of molecular size.

Phosphate. A four-pool model that can be applied in both acute and chronic renal failure is proposed to explain the observed kinetic behaviour of phosphate. Studies of intracellular phosphate concentrations fail to demonstrate release of phosphate from erythrocytic stores during dialysis. Diurnal variation in phosphate concentration is demonstrated in subjects with normal renal function and also in advanced chronic kidney disease.

Beta2-microglobulin. A multi-pool model explains the kinetic behaviour of beta2-microglobulin during dialysis. Beta-2-microglobulin deposition is assumed to be a staged process with some deposits easily accessible during dialysis and some more resistant to depuration. Patients receiving high-flux dialysis or haemodiafiltration are shown to have lower circulating beta2-microglobulin levels and less symptomatic dialysis related amyloid, but evidence of amyloid deposition is still found when assessed by a scintigraphic imaging technique. Age and duration of dialysis are shown to be the best predictors of symptomatic amyloid deposition.

Conclusions

The results of the studies in this thesis indicate that, for solutes such as phosphate and beta2-microglobulin which have complex intra-dialytic kinetics, current dialysis techniques are insufficient to achieve adequate solute removal. It will be necessary to deliver longer or perhaps more frequent dialysis therapy in order to achieve this goal. Mathematical modelling facilitates understanding of the pathophysiology of the dialysis process and provides a platform for the development and monitoring of improved dialysis strategies.

Section 1

History of Haemodialysis Technique and Measurement of Dialysis Adequacy

Chapter 1

History of Haemodialysis

History of Dialysis

1.1 The Development of Theory

The concept of using dialysis for long-term replacement of renal function is still a relatively new idea. The Greeks were clearly aware of the pathology of renal failure when they described “uraemia” which literally means urine in the blood. Their fascination with steam baths to “purify” the body of toxins was probably not entirely without basis.

In 1855 a German physiologist Adolf Fick first described the process of diffusion^{1,2}, but it was Thomas Graham, a professor at Glasgow’s Anderson University, who first used the term “dialysis”. He described the process of diffusion across an ox bladder that served as a semi-permeable membrane³. The pioneering work of these scientists formed the basis of the later application of these theories to human dialysis.

The first artificial kidney was utilised in 1913 when Abel and his colleagues removed blood from a rabbit, poisoned with salicylic acid, and passed this through a dialyser made of colloidin tubes derived from cotton, encased within a glass container filled with saline. The blood was removed via an arterial cannula and returned to a vein and the animals survived at least in the short term. Although they considered such a device for human use, development was not continued due to the outbreak of war and problems with obtaining a supply of leeches to act as anticoagulant⁴.

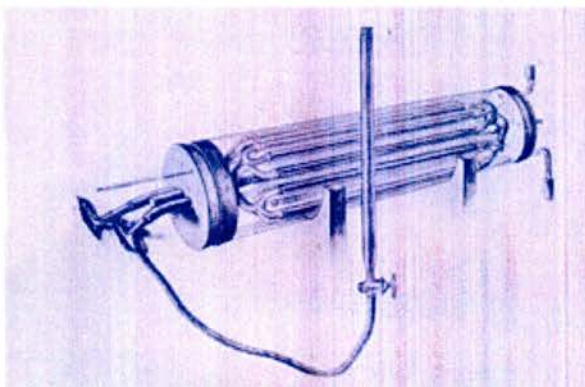


Figure 1.1. The dialysis apparatus used by Abel in 1913.

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After the war, interest in dialysis was rekindled and, in 1924, George Haas performed the first human dialysis treatment. He dialysed his patient for 15 minutes with no adverse effects during the treatment. He dialysed a total of seven patients over a four-year period all of whom ultimately died of renal failure. He achieved anticoagulation in the early days using hirudin obtained from the saliva of leeches. This caused many problems when administered to humans and latterly Haas used heparin. This anticoagulant was isolated from dog livers by an American named Maclean in 1916⁶. The ability to produce heparin in large quantities in purified form was achieved by 1937⁷.

1.2 The Development of Acute Dialysis 1940s-1960

In 1943, two Dutch nephrologists, Kolff and Berk, built the first practical haemodialysis machine. Kolff had originally become interested in developing a kidney machine as a young man, and based his ideas on the work of Abel. He was restricted by the German occupation during the war and was forced to use any available materials including the drum of a washing machine. The discovery of cellophane (used commercially as sausage skin) gave Kolff an ideal dialysis membrane. The machine he finally devised consisted of 30-40 meters of cellophane tubing on a rotating drum contained in a 100 litre stationary tank. When the drum rotated, blood was drawn through the cellophane tubes and dialysis occurred as the tubes were exposed to the dialysis bath.

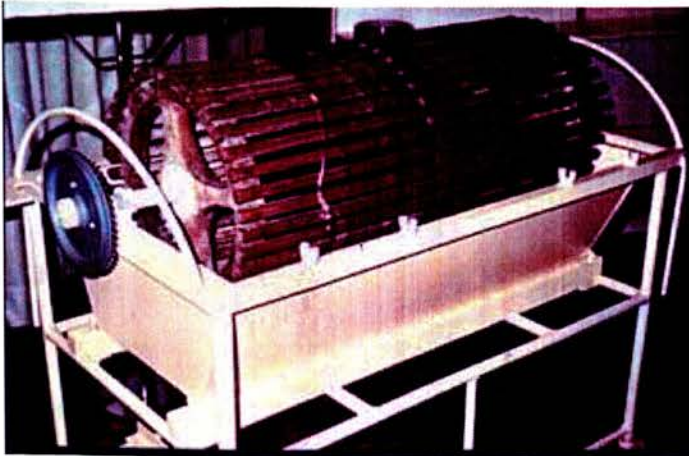


Figure 1.2. The Kolff rotating drum dialyser.

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Although the first sixteen of Kolff's patients died, he is credited with performing the first successful human dialysis in 1945. The patient, a woman with septicaemia and acute renal failure, survived and died several years later of unrelated causes.

In 1946, Alwall produced a modified version of the Kolff rotating drum that, for the first time, permitted controlled ultrafiltration to occur. It consisted of 10 metres of cellophane stretched over a metal frame suspended in a stationary tank. The cellophane was more able to withstand pressure because of its encasement between metal sheets and the containment of the entire apparatus within a closed container permitted differential pressure in the blood and dialysate compartments to be maintained.

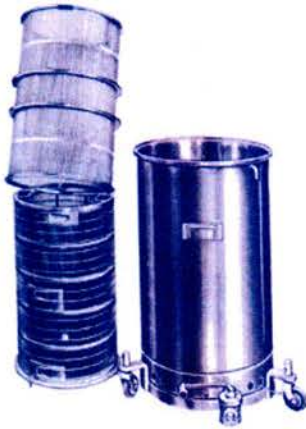


Figure 1.3.
The Alwall dialyser

a) [Reproduced with permission from⁵]

b) Edren: www.edren.org
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Haemodialysis was used for the first time in the UK at the Hammersmith Hospital after the Second World War. Kolff donated one of the five rotating drum dialysers he had made to permit nephrologists in the UK to become familiar with his renal replacement therapy. A report of the successful treatment of 12 cases of acute renal failure was published by Bywater and Joekes in 1946⁸. Despite their initial success, dialysis was still viewed with suspicion. The traditional therapies for renal failure of protein and fluid restriction remained dominant⁹ and dialysis was not pursued as a potential therapy in the UK for another 10 years.

Kolff's dialysis machine was also taken to the Peter Brent Brigham Hospital in Boston at the invitation of George Thorn. There, Carl Walters and John Merrill modified it and it became the Kolff-Brigham machine. This was the first widely used machine and was taken overseas during the Korean War (1950-53) to successfully treat 31 cases of acute renal failure in the field. This helped to establish dialysis as a

worthwhile treatment and, with retrospect, was the first indication that more frequent treatment was beneficial.

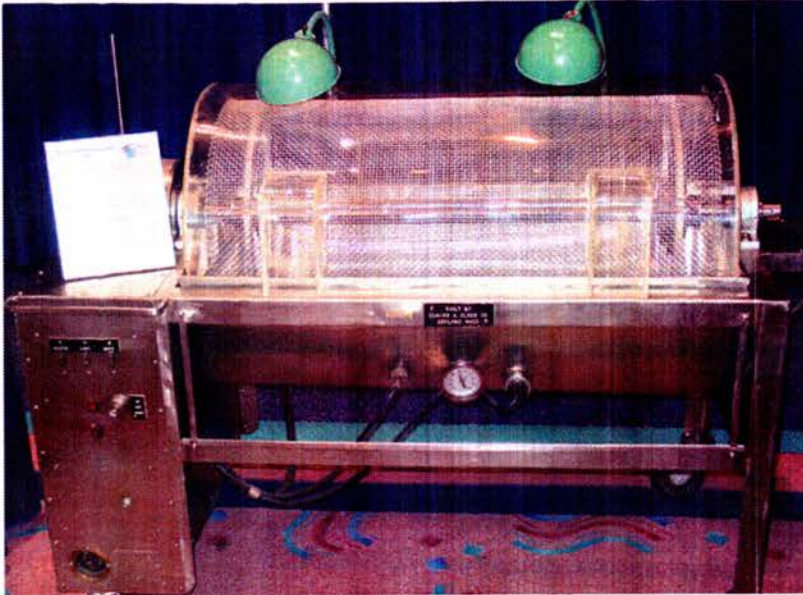


Figure 1.4.
The Kolff-Brigham dialysis machine
Photo by Jim Curtis
www.homedialysis.org
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In 1956, after spending some time with John Merrill in Boston, Frank Parsons returned to Leeds General Infirmary and was influential in rekindling the interest in haemodialysis in the UK. By 1958 there were three units in the UK with dialysis facilities – Leeds, Hammersmith and Halton Air Base. Edinburgh Royal Infirmary followed with the opening of an Artificial Kidney Unit in 1958. This unit purchased a Kolff-Travenol machine which was a more advanced design incorporating a ‘twin coil’. This machine was more compact and could be pre-sterilised, reduced the resistance within the dialysis circuit and had better ultrafiltration control than the older models. However, a blood pump was required to maintain transmembrane pressure and the machine was prone to membrane rupture. The priming volume of the circuit caused problems with volume depletion and, as a result, the patients were exposed to large volumes of blood products. Despite these issues, the first dialysis

was performed successfully in Edinburgh in May 1959 and, by the end of that year, there were six haemodialysis units in the UK.

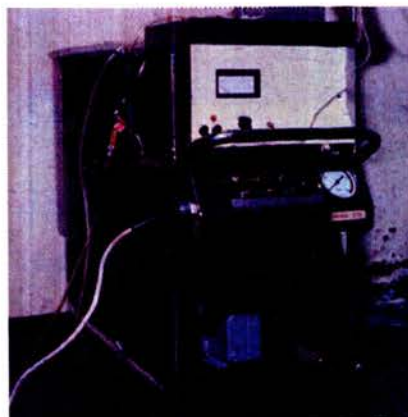


Figure 1.5. The Kolff-Travenol Dialyser

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The survival from acute renal failure was in the region of 52% in these early days, which compared favourably to the 100% mortality before dialysis was possible. The treatments were long –between eight and twelve hours. Fluid removal was not very well regulated and tended to be assessed by dialysing patients in weigh beds. The amount of fluid removal was altered manually by changing the pressure in the dialysis compartment.

The choice of which patients to dialyse was largely empirical. The patients had to be well enough to tolerate a therapy that was not without complication, so patients presenting with uraemic encephalopathy were often not treated. In the early days, the treatments tended to occur only when patients developed overt signs of uraemia and

many patients only ever received one dialysis session. The problem with dialysis disequilibrium was recognised early on and a move towards shorter more regular treatments began to occur.

1.3 The development of vascular access.

Vascular access was a big problem in the early days. There were no blood pumps on the original dialysis machines so the movement of blood depended largely on the pressure from arterial blood. Cannulae were inserted into an artery and a vein at the beginning of dialysis and these vessels were then tied off at the end of the procedure. For these reasons recurrent dialysis was rarely an option.

Haemodialysis therapy for chronic renal failure became a reality in the 1960s when Scribner, in Washington, pioneered the “Scribner shunt” manufactured by Quinton, Dillard and Scribner¹⁰.



Figure 1.6. The Scribner shunt
[Reproduced with permission from¹¹]

This device was inserted surgically with one cannula sutured into a vein and another into an artery. These were connected exteriorly with a piece of Teflon and metal connectors. This permitted recurrent access to the circulation for the first time

and the first patient to use one of these shunts survived for 11 years on haemodialysis.

Modern day vascular access saw its origins in the arterio-venous fistula described by Cimino and Brescia in 1966¹².

1.4 The Development of Chronic Dialysis –the early years– 1960s-1980s

With the advent of chronic dialysis came the need for improved hardware. The original Kolff dialysers were essentially devoid of safety features and were very demanding on time and staff resources to run. In recognition of the fact that more dialysis seemed to provide better outcomes, membranes were enlarged by the use of flat plate dialysers such as the Kiil membrane. This was made up of several layers of flat cuprophane membrane with blood passing along alternating layers of cuprophane rather than through membranous tubes. This enlarged the surface area available for dialysis and could operate at very low blood pressures. The dialysate was supplied from a large tank, chilled to prevent bacterial growth, and Kiil pioneered the use of countercurrent dialysate flow.



Figure 1.7. The Kiil flat plate dialyser

Photo by Jim Curtis
www.homedialysis.org
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The use of single pass dialysate flow became widely adopted late in the 1960s. In Seattle, Babb and co-workers designed a central water plant that was the first of its kind and supplied their outpatient dialysis unit¹³. Concentrated solutes were mixed with tap water and sodium acetate, which appeared to be superior to bicarbonate, as it did not cause precipitation of calcium and magnesium salts.

Ultrafiltration became easier to control by the application of negative pressure to the dialysate. Because the dialysate compartment was always at lower pressure than the blood compartment there was never any risk of dialysate flowing into the blood.

However, the Kiil plate dialysers were time-consuming to construct and the technicians were exposed to the risk of infection from blood contamination. A move towards the use of membranes that provided a physical separation of blood from the machine therefore occurred. Disposable flat plate dialysers and new hollow fibre membranes replaced the older Kiil plates and twin coil dialysers.

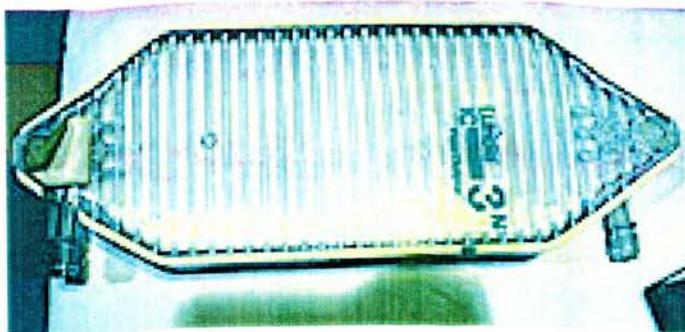


Figure 1.8. Disposable flat plate dialyser

Photo by Jim Curtis
www.homedialysis.org
[reproduced with permission]

Stewart produced the first hollow fibre membrane in 1964. Instead of the traditional flat plate or large cellulose tubes utilised in the previous membranes these new dialysers were made up of many capillary size tubes thus increasing many-fold the surface area available for dialysis.

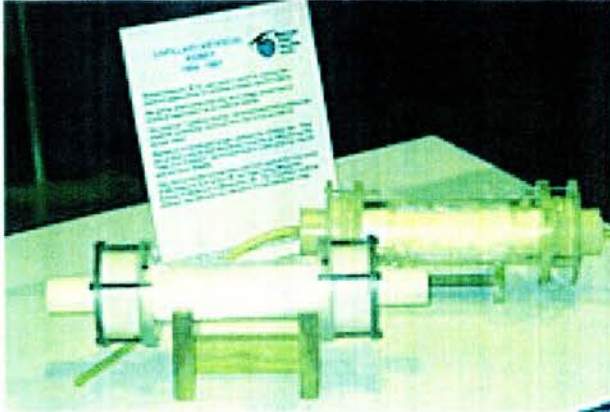


Figure1.9. Disposable hollow fibre membrane

Photo by Jim Curtis
www.homedialysis.org
[reproduced with permission]

The materials available for the construction of membranes also developed over the years. The first membranes were formed from regenerated cellulose or cellophane, which were later replaced by the thinner cuprophane. These membranes could be made extremely thin which provided excellent diffusive capacity for small molecules such as urea. The relatively small pore size and marked hydrophilicity meant they were not as good at fluid removal or middle molecule clearance. There were also problems with complement activation due to the presence of hydroxyl groups. Despite these drawbacks, these membranes are still in use in some centres today due to their low cost.

Later, modified cellulose and synthetic membranes were developed that induce less complement activation and are considered more biocompatible. Machines were improved with more advanced features such as controlled ultrafiltration and safety mechanisms such as those to detect air embolisation.



Figure 1.10. Early Cordis Dow haemodialysis machine.

Photo by Jim Curtis
www.homedialysis.org
[reproduced with permission]

However, despite these advances, new problems became increasingly apparent as the prevalence of haemodialysis as a chronic therapy increased.

1.5 Historical Complications of Dialysis

Hepatitis B

The life saving properties of dialysis therapy were well recognised by the 1970s but complications of the therapy were beginning to emerge. In the early 1970s, there was a countrywide outbreak of hepatitis in dialysis centres, during which many patients and staff were infected. The renal unit at Guy's Hospital was closed due to the size of its outbreak, and dialysis practice was altered across the country. It was recognised that the outbreak occurred as a consequence of blood contamination of the dialysis machines. In addition to providing the impetus for the development of a new generation of dialysis membranes and machines, it also highlighted the importance of the prevention of cross-contamination of machines and focussed the need for

infection control within dialysis units. When a vaccination for hepatitis B was developed in 1982, all renal units in the UK adopted a policy of immunisation.

Aluminium toxicity

Again in the 1970s it became apparent that many dialysis patients were experiencing adverse neurological outcomes. Alfray in the USA reported the existence of encephalopathy in dialysis patients and correlated it to the amount of aluminium in post-mortem studies of their brains¹⁴. At the time, the presence of excess aluminium was attributed to the use of aluminium containing phosphate binders. It became apparent however, that there was a geographical distribution of this problem with the highest incidence in the UK being in Newcastle-upon-Tyne. It was also noted that patients in these areas had a higher incidence of osteomalacia. These problems of dialysis “dementia” and bone disease were both ultimately attributed to the presence of aluminium in the water supply¹⁵. When the aluminium was removed, either by reverse osmosis or by de-ionisation of the water, the problem was reduced dramatically. A precedent was therefore set to provide purified water for the purposes of dialysis.

Acetate vs. Bicarbonate

Sodium acetate had been used instead of sodium bicarbonate in dialysis fluid through the 1960s and 70s to prevent precipitation of calcium and magnesium salts. It became apparent that some patients dialysed against acetate developed vascular instability presumably due to acetate accumulation. For this reason a move was made

in the 1980s back towards bicarbonate dialysate which was facilitated by the design of new machines with accurate proportioning systems.

1.6 The Development of Chronic Dialysis – the later years -1980s and on

The pioneers of early dialysis were less concerned with reducing morbidity as, in the early days, dialysis was considered purely as a life saving treatment applied to acute situations.

The definition of dialysis proposed by de Palma in 1971 encompassed the ideals of chronic dialysis therapy.

“Dialysis permits the patient to be fully rehabilitated, to have a satisfactory nutritional intake and a sufficient production of red blood cells, to maintain normal blood pressure values and to prevent the development of neuropathy”¹⁶.

It was clear even when de Palma detailed his objectives, that dialysis therapy was sub-optimal in achieving those goals.

Native renal function is predominately a convective process with superimposed specific absorption and excretion mechanisms. The original dialysis modalities were diffusive in nature, depending entirely on the passage on solutes down a concentration gradient across a semi-permeable membrane. The diffusive clearance of a solute is determined by the concentration gradient across the membrane and the properties of the membrane itself including the surface area, the thickness and the size of the pores. Diffusion can remove small molecules but for larger molecules, this process becomes less efficient. It was noted in the 1960s and 70s that even when patients achieved good urea clearance they still often complained of neuropathy. This observation led to the assumption that toxins that could not be removed by

conventional dialysis were present in these individuals and ultimately led to the development of the “middle molecule” theory and the development of enhanced dialysis therapies.

Membranes that are more permeable to water permit enhanced convection. For solutes smaller than the size of the membrane pores, convective removal is in direct proportion to the concentration of solute in the fluid passing across the membrane. For larger molecules, the removal is determined by the sieving coefficient, which is a function of the size of the molecule in relation to the size of the membrane pores. Convective removal is far more efficient than removal by diffusion for larger molecules.

1.7 The concept of dialysis adequacy

Wolf provided the first description of haemodialysis adequacy in 1951 although he concentrated solely on the performance of the membrane¹⁷. He studied a Kolff-Brigham dialyser in vitro and published kinetic equations to describe the removal of solute from the “blood” circulating through the machine. He described in detail the clearance of urea when the dialysate bath was fresh and introduced the concept of dialysance to describe the way in which solute was cleared when there was solute present in the dialysate. He related the kinetic parameters of the artificial kidney to the function of the native kidney and noted the different dialysance measurements for different solutes. His work was technically very advanced and can still be directly applied to haemodialysis today.

Further assessment of dialyser function was provided by Michaels in 1966 with his analysis of the mass transfer coefficient¹⁸. This gives the theoretical clearance of a dialyser at infinite blood and dialysate flows and permits the clearance of a solute at a given blood and dialysate flow to be predicted. For diffusive therapies, his equations are still applicable today but inaccuracies have entered into these equations with the increased prevalence of convective dialysis therapies. Several authors have attempted to provide correction factors to take ultrafiltration and changes in haematocrit into account, but, at best, the equations are a close approximation^{19;20}. The first publication trying to correlate dialyser function to patient outcome appeared in 1971 when Babb devised the square meter/hour theory. He stated that patients needed a minimum exposure to dialysis of 21m^2 -hours in the course of a week to prevent the development of complications²¹. The following year the same group introduced the concept of “middle molecules” in dialysis therapy²². They proposed that larger molecules that diffuse more slowly across a membrane depend on long duration of dialysis for adequate removal and that the accumulation of these so called “middle molecules” is responsible for some of the toxic effects of renal failure.

In 1973, the Federal Government in the United States took over funding of the chronic dialysis programme, which prompted research into the most cost-effective way of delivering the service.

In 1974, Gotch and Sargent for the first time put forward their recommendations for urea kinetic modelling as a way of prescribing and monitoring haemodialysis therapy. The origins of these equations will be considered in chapter 2.

This technique was used in the protocol of the National Co-operative Dialysis Study (NCDS). The results of this landmark study were published in 1983²³. It had a 2x2 factorial design with patients randomised to a low or high time averaged urea (TAC_{urea}) to examine small solute clearance, and a long or short dialysis treatment as a surrogate for middle molecule clearance. It showed that patients had a better outcome if their TAC_{urea} value was lower but failed to show a significant difference for patients dialysing for 4.5 hours compared to 3 hours. This was part of the reason that the move towards shorter hours of haemodialysis in the United States continued, but it was always apparent that the reduction of urea was not the only relevant parameter. There were patients with low TAC_{urea} who did poorly and others with a high TAC_{urea} who were very well. The study could be criticised on several counts. The study was short (24-48 weeks), there were small numbers (165), all patients were <60 and none were diabetic. The arm of the study with high TAC_{urea} and short hours was terminated early due to an excess mortality in this group. This study was however, for a long time, the only prospective study looking at outcomes on different dialysis schedules, but it remained unusual to monitor dialysis using urea kinetics. In 1985 Gotch and Sargent re-analysed the NCDS data²⁴ and for the first time could give a marker of “adequate” haemodialysis on the basis of Kt/V where K is the dialyser clearance, t is the time on dialysis and V is the distribution volume of urea. They analysed the data in terms of a step function and suggested that improvements in outcome continued up to a Kt/V of 1.0 and patients with a Kt/V <0.8 did badly. Figure 11. This was the first time that body mass (expressed as V) and therefore nutritional status was correlated to dialysis outcome. This finding was subsequently used as a marker against which to measure future achievements.

Keshaviah further analysed the data in 1993 and fitted an exponential curve to the same data. This suggested that there is continuing benefit even with a $Kt/V > 0.8^{25}$.

Figure 12.

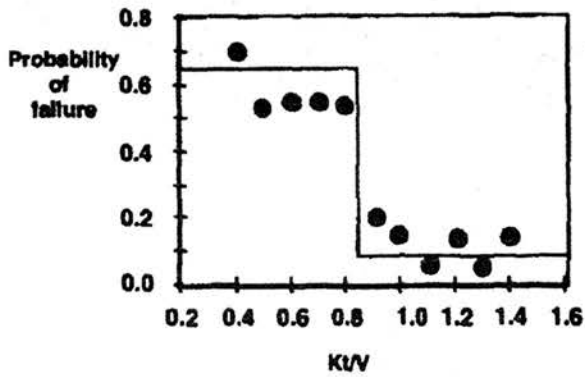


Figure 1.11. Gotch analysis of NCDS data showing step function [reproduced with permission from ²⁶]

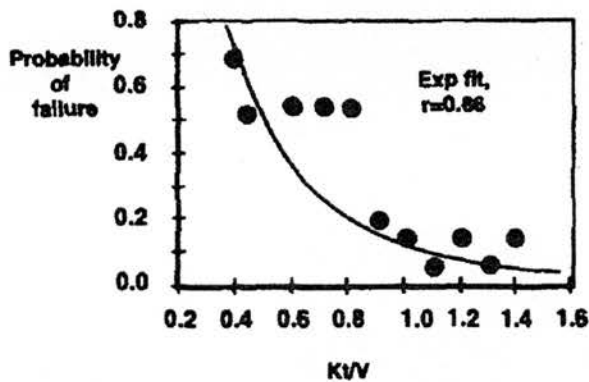


Figure 1.12. Keshaviah analysis of NCDS data showing exponential function [reproduced with permission from ²⁶]

Some of the advantages of using urea kinetic modelling to prescribe dialysis dose are

- the facility to measure dialysis efficiency by comparing the prescribed to delivered dialysis dose
- the correlation with markers of nutritional status
- the ability to individualise dialysis treatments based on body mass.

Some of the disadvantages are

- the necessity to understand the mathematical concepts underlying the modelling process
- the need to rely on the potentially inaccurate measurements of dialyser clearance (K)
- the errors introduced when an anthropometric volume is utilised in the initial prescription

Because of these complexities, several other measures of dialysis adequacy have been proposed.

In 1991 Lowrie and Lew advocated the use of the urea reduction ratio (URR) to simplify the monitoring of dialysis adequacy²⁷. A URR of 63% gives essentially the same amount of dialysis as a single pool Kt/V of 1.2 and the two parameters are often quoted together. The URR gives no information about nutritional status and no information about solute clearance other than for urea. It takes no account of solute clearance by ultrafiltration and does not recognise the contribution of residual renal function. It is not possible to accurately alter the dialysis prescription if the URR is sub-optimal and there is no way of comparing prescribed to delivered dose of dialysis. Despite these drawbacks, the URR has been widely used due to its simplicity. Many of the errors that are encountered when using the URR result in the dose of dialysis delivered being higher than would be predicted by formal urea kinetic modelling so the patient is relatively protected.

How much is enough?

At present, there is a general consensus in the guidelines about the amount of dialysis that should be prescribed. K-DOQI (2006) recommends a minimum single pool Kt/V of 1.2, with a target of 1.4²⁸; the UK guidelines recommend an equilibrated Kt/V of 1.2 (spKt/V 1.3)²⁹, with similar recommendations from the European Best Practice guidelines (2002)³⁰.

There are many studies that suggest that increasing the delivered dialysis dose above the spKt/V of 1.2-1.4 currently recommended is beneficial. The Japanese Registry has demonstrated a reduction in mortality with Kt/V measurements up to 1.8³¹. The experience from Tassin where the spKt/V averages 1.67 demonstrates improved outcomes with higher Kt/V³². The benefit of higher Kt/V is not universally acknowledged, as there seems to be a point at which increasing the Kt/V further does not improve mortality. This may relate to the use of V as the denominator in the Kt/V calculation which could represent a confounding factor due to artificially low estimates of V in malnourished patients. The V in the original Gotch and Sargent Kt/V equation is calculated from three blood samples (pre-post-pre dialysis) and gives a dynamic V that represents the catabolic activity of an individual. Due to the complexity of measuring V, an anthropometric measure of V such as the Watson volume is frequently utilised³³, but this is a static V that gives only an estimate of solute distribution volume. The Watson formula is based on a historical set of data that may not be accurate when applied to the highly co-morbid, ethnically diverse dialysis population that now exists. If a measure of catabolic activity, rather than a static volume was used as the denominator in the dialysis prescription, a different set

of adequacy values would be generated, with the net effect of providing proportionally less dialysis in small individuals compared to their larger peers.

Despite many studies and much anecdotal evidence of improved outcomes with higher Kt/V, the optimal dose of dialysis is still debated and there is resistance to increasing the current targets for dialysis adequacy.

In an effort to clarify the optimal dose of haemodialysis the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) commissioned the Haemodialysis [HEMO] study, the results of which were published in 2002³⁴. This study recruited 1846 prevalent dialysis patients to a 2x2 prospective randomised study of low dose vs. high dose dialysis (to assess small solute clearance) and low-flux vs. high-flux membranes (to assess middle molecule clearance) and followed them for a mean of 2.84 years. No significant difference was found in the incidence of hospitalisation or in all cause mortality between the dose groups. However, women in the high dose group (achieved equilibrated Kt/V 1.53) had a significantly lower mortality than those in the standard dose group (achieved equilibrated Kt/V 1.16), $p=0.02$. Even though differences in body size could not be implicated by the authors of this report³⁵, the suspicion of a denominator effect remains.

In terms of middle molecule clearance, no significant difference could be demonstrated between the outcomes in the high-flux and low-flux groups as a whole. In subgroup analysis, there was a 20% reduction in cardiovascular mortality in the high dose group, $p=0.04$. In patients who had dialysed for in excess of 3.7 years before inclusion in the trial, a 36% reduction in mortality on high-flux dialysis

($p=0.001$) was seen, particularly if they had been treated with low-flux membranes before the trial began³⁶. Additionally, in post-hoc analysis it was shown that the pre-dialysis level of beta2-microglobulin was a predictor of mortality regardless of the flux of the membrane. This gives currency to the argument that improved middle molecule clearance confers long term survival advantage³⁷.

While the HEMO study did not show current dialysis practice to be detrimental, it also did not show any adverse outcomes in the patients receiving higher dialysis doses or using high-flux membranes. It has fuelled debate about whether Kt/V or URR are the correct dosing parameters and whether they should be viewed with caution in certain sub-groups of patients. The HEMO study has rekindled interest in the use of more frequent dialysis as a potential means of improving outcomes.

1.8 The Development of New Dialysis Techniques

i) High-flux versus high-efficiency therapy

The flux of a membrane refers to the ultrafiltration coefficient or hydraulic permeability of the membrane. Traditional cellulose membranes are considered to be low-flux as the maximum ultrafiltration rate is approximately 5-6 ml/hr/mmHg/m². Newer synthetic membranes are generally high-flux with ultrafiltration coefficients in excess of 20ml/hr/mmHg/m². It is vital to use volumetric control with these membranes, as there is potential for excessive ultrafiltration.

The term efficiency refers to the enhanced diffusive clearance of solute brought about by an increase in blood and dialysate flows. High-flux membranes with a large

surface area tend to be used in high efficiency dialysis but it should be noted that high efficiency dialysis could be achieved with a low-flux membrane.

ii) *The Development of Haemodiafiltration*

Native renal function is mainly a convective process tempered by specific absorption mechanisms. The ideal renal replacement therapy should therefore mimic this as closely as possible. This has led to the development of the technique of haemodiafiltration, which permits good solute clearance across the spectrum of metabolic size by a combination of diffusion and convection. For small molecules the most important factor is diffusive clearance with a 40% increase when blood flow increases from 300 to 500ml/min, with only minor increases seen when the membrane surface area or convection are increased. For middle molecules, however, it is the increase in convection that influences solute removal most³⁸.

Where HDF is used there are two practical options to be considered which have different implications for solute removal.

In predilutional HDF, extra fluid is added before the dialyser and removed by ultrafiltration across the dialyser. Pre-dilution HDF is better for middle molecule clearance but small molecule clearance is reduced because of haemodilution. The huge volumes of infusate involved limit the use of pre-dilution techniques but this is less of a consideration with on-line production of fluid.

In post-infusion HDF, fluid is removed by ultrafiltration across the dialyser and is then resubstituted in the venous bubble trap post-dialyser. Figure 13.

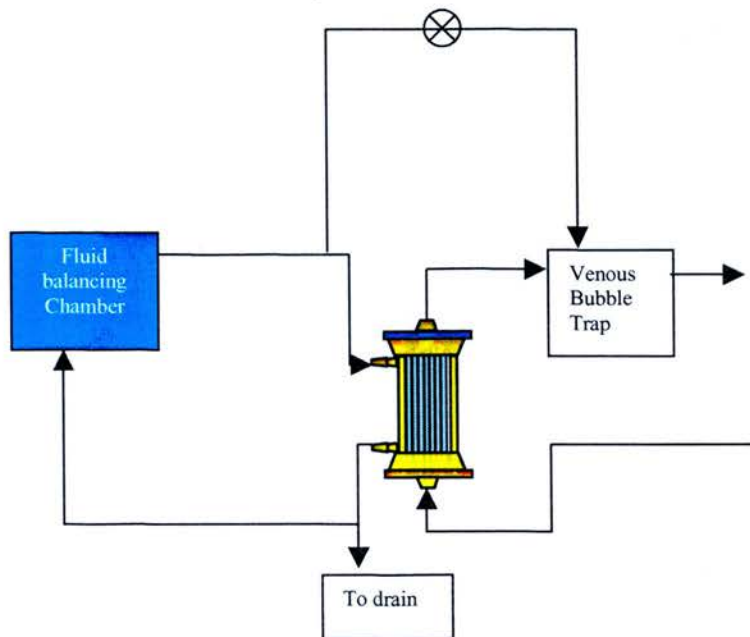


Figure 1.13. Schematic of post-dilution HDF therapy

Post dilution HDF is the technique most commonly used in practice. It has been demonstrated that urea clearance in post-dilutional HDF is equivalent to that seen in high-flux haemodialysis. The increase in convection is most important for middle molecules with improved clearances up to a post-dilution substitution volume of 100ml/min. Infusion rates of 100ml/hr permit beta2-microglobulin removal twice that of high-flux dialysis. Further increases show no further benefit possibly due to the effect of increased haematocrit. The fraction of plasma flow that can be diverted for convective purposes is around 35%. This equates to a post-dilution infusion volume of 60-100ml/min³⁸.

To maximise the benefits of the pre and post infusion techniques some have advocated a mixture of the two approaches. A mid-dilution membrane has been designed to take advantage of pre and post-dilution HDF features. There is a double core so blood flows through the dialyser twice with infusion fluid being added at the distal end. The first pass through the membrane is thus in post-dilution mode and the second pass is in pre-dilution mode. Minor reductions in urea clearance but a further significant increase in beta2-microglobulin clearance is achieved when compared to post-dilutional HDF³⁹.

While there are yet no randomised, controlled studies that prove that HDF has a beneficial effect on morbidity or mortality, there are equally no studies that prove it is detrimental. There are many anecdotal reports of improved outcomes. Given the growing acceptance of the technique and abating concerns relating to cost and safety, the technique is likely to become much more prevalent in the coming years. At present only 1-1.5% of patients in the UK receive this therapy vs. about 14 % in Italy and a much higher proportion in Japan. The technique has not been licensed in the United States due to FDA concerns about the infusion of dialysate directly into the blood stream.

With the growing acceptance of the technique of haemodiafiltration comes a responsibility to prove that it is a superior technique as, theoretically, it may favourably influence some of the complications experienced by long-term dialysis patients.

1.9 Complications of Modern Dialysis Therapies

i) Cardiovascular Mortality

The substantial increase in morbidity and mortality seen in dialysis patients can largely be attributed to a very significant increase in cardiovascular risk. There is now undisputed evidence that the presence of renal failure causes a disproportionate increase in the risk of cardiovascular death with 40-50% of deaths in dialysis patients being directly attributed to a cardiovascular event. The widely quoted study performed by Foley et al demonstrates that dialysis patients at the age of 25-34 have a similar cardiovascular risk to normal controls at the age of >85 and a 100-fold risk when compared to control subjects of the same age⁴⁰, Figure 14.

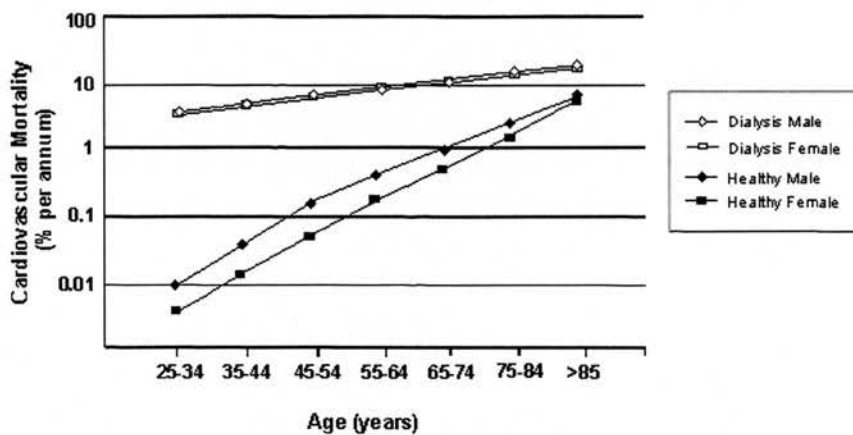


Figure 1.14 Cardiovascular mortality (death due to arrhythmia, cardiac arrest, myocardial infarction, atherosclerotic disease and pulmonary oedema) in US dialysis patients.

[reproduced with permission from⁴⁰]

While dialysis patients share many of the same traditional risk factors for cardiovascular disease such as smoking, hypercholesterolaemia, hypertension and diabetes, with the general population, some additional influences are unique to the dialysis population. In patients without renal dysfunction, most cardiac morbidity arises from atherosclerotic coronary artery disease. The aetiology in renal dysfunction is different, with the calcification arising in the medial layers of small and medium sized blood vessels leading to increased arterial stiffness. One of the main problems with addressing cardiovascular risk in the dialysis population is the lack of good randomised controlled trials of interventions in this group. Care needs to be taken when extrapolating the results of prevention trials carried out in the general population, as the risk factors for cardiovascular disease in patients with renal failure on dialysis are clearly different from the general population. For example, it is undisputed that lowering cholesterol in the general population improves cardiovascular outcomes but studies looking at the effects of lowering cholesterol in dialysis patients have been largely negative⁴¹.

Two of the non-traditional risk factors that are considered to be of most importance in dialysis patients are the impact of altered bone metabolism and the presence of chronic inflammation.

a) *Divalent Ion Metabolism*

Data from the USRDS identified an elevated serum phosphate and calcium x phosphate product as an independent risk factor for mortality⁴². Excess cardiovascular mortality has been confirmed in many subsequent studies^{43;44} and

there is increasing evidence that altered divalent ion metabolism predisposes to increased premature coronary vessel calcification^{45;46}.

At present, there are no prospective randomised controlled trials that demonstrate improved cardiovascular outcomes with improved control of mineral metabolism. HDF removes phosphate more efficiently than HD but the actual change in serum phosphate levels probably relates more to internal phosphate transfer within cells and body compartments. Although the phosphate concentration may be reduced rapidly in the early part of HD this may be offset by intra and interdialytic rebound.

There have been a number of observational studies looking at the effect of using calcium containing phosphate binders and non-calcium containing phosphate binders which would suggest a reduction in coronary calcification when non-calcium containing compounds are used⁴⁷. The Dialysis Clinical Outcomes Revisited Study (DCOR) did not demonstrate improved mortality in a group treated with non-calcium containing phosphate binders although they were able to demonstrate improved survival in a post-hoc analysis of patients receiving the non-calcium containing binder who were over 65 years of age, or had taken the drug for more than two years (unpublished data).

b) Chronic Inflammation

The role of inflammation in increasing cardiovascular risk is incompletely understood. It has been demonstrated that elevations in CRP are associated with increased cardiovascular risk in both the general population and in dialysis patients⁴⁸⁻⁵⁰. In dialysis patients the biocompatibility of the dialysis circuit and the quality of the water supply have come under scrutiny as these are potential sources of chronic exposure to pro-inflammatory stimuli.

Membranes

Traditional cellulose membranes, which were the first hollow fibre membranes to be developed, cause marked complement activation during their use. This bioincompatibility is attributed to the presence of hydroxyl groups on the membrane. In an attempt to improve on this, modified cellulose membranes were developed that replaced the hydroxyl groups with acetate. These certainly caused less complement activation but enhanced hydraulic permeability of newer membranes permitted back-diffusion of dialysate into the blood stream. Technical advances have seen the development of synthetic membranes which have the same benefits of biocompatibility but have an asymmetric membrane structure. This ensures a hydrophobic layer that limits the potential for back-diffusion of potential pyrogens into the blood stream. There is an increasing body of evidence that the use of newer high-flux membranes is beneficial in terms of mortality^{36,51-54}

Water Quality

There is a potential for bacteria and endotoxin to be present in the water supply and the exposure of dialysis patients to large volumes of water (approximately 500 litres per week), together with the use of high-flux dialysers that permit back-filtration of fluid, makes the quality of water of paramount importance. In haemodiafiltration, the problem with back-filtration does not occur but some of the dialysate is infused directly into the blood stream emphasising that even minor contamination of the water supply can have a negative impact on the patient. Theoretically, the use of ultrapure water should reduce the level of inflammation in dialysis patients but there is no conclusive evidence that this reduces cardiovascular mortality.

In addition to the problems with microbiological purity, there are also potential issues with chemical contamination of the water supply. The problem with aluminium contamination as detailed previously is only one of a number of issues with the water supply. In the presence of chloramines profound haemolysis or erythropoietin resistance can occur⁵⁵, excess calcium or magnesium can cause a “hard-water syndrome” where the patient suffers nausea, vomiting and muscular cramps, and copper can cause headaches, hepatic damage or haemolysis. The American Association of Medical Instrumentation (AAMI) publish guidelines on the acceptable levels of water contaminants⁵⁶, Table 1. These standards are accepted by the UK Renal Association who recommend that chemical quality is assessed every 3 months.

Table 1.1. AAMI Standards for Haemodialysis Water

Solute	ppm
Total chlorine	0.1
Chloramine	0.1
Nitrate	2
Chlorides	50
Sulphate	50
Fluoride	0.2
Sodium	50
Potassium	2
Calcium	2
Magnesium	2
Copper	0.1
Zinc	0.1
Silver	0.005
Lead	0.005
Mercury	0.001
Aluminium	0.01
Ammonium	0.2

ii) Dialysis related amyloid

Patients on long-term dialysis develop amyloidosis that has beta2-microglobulin as an integral component. This has a predilection for peri-articular deposition and leads to disabling arthropathies and classically carpal tunnel syndrome. There are also reports of visceral deposition although this is much less frequent. The prevalence depends on the diagnostic method employed but is generally accepted to be 20-30% at 2-3 years and >90% after greater than 7 years of dialysis⁵⁷. Elevated circulating levels of beta2-microglobulin are a pre-requisite for the development of dialysis related amyloid and it is clear that, because beta2-microglobulin is a “middle molecule”, it is poorly cleared by predominately diffusive low-flux therapies. Convective therapies such as HDF have been shown to enhance removal⁵⁸, but

whether improved removal of $\beta_2\text{m}$ translates into a lower incidence of dialysis related amyloidosis is uncertain. There is some evidence that the use of high-flux membranes may delay the onset of dialysis related amyloid with several cross-sectional and longitudinal studies suggesting that the incidence of carpal tunnel syndrome is lower than in those treated with convective modalities of dialysis^{54;59-61}.

The pathophysiology of $\text{A}\beta_2\text{m}$ is incompletely understood although there is increasing evidence that complex polymerisation of the beta2-microglobulin molecule with collagen and glycosaminoglycans occurs. At a more advanced stage an interaction of the Maillard type with advanced glycosylation end products (AGEs) occurs which seems to render the deposits much more resistant to proteolytic degradation. At this stage there appears to be a marked influx of inflammatory cells, which may explain the onset of symptoms.

The best strategies for preventing and treating $\text{A}\beta_2\text{m}$ have not yet been fully established. At present, the only form of renal replacement therapy that provides clear symptomatic benefit is transplantation. Although it is clear that haemodialysis is sub-optimal in removing beta2-microglobulin, the kinetics of $\beta_2\text{m}$ removal in dialysis patients have not been fully elicited. The factors limiting beta2-microglobulin removal in haemodialysis warrant further study, as there is an increasing cohort of patients who are destined to receive dialysis for many years as their only form of renal replacement therapy.

1.10 Conclusions

The nephrology community has clearly come a long way from the pioneering work of Abel, Haas and Kolff and the advances in available technology are a testament to the ingenuity of the scientific and engineering communities that have been involved in dialysis from the outset. While much is still unknown about the precise nature of uraemic toxins and why certain substances accumulate, it is becoming ever clearer that advances in dialysis techniques are required to reduce morbidity and mortality for the large cohort of patients destined to spend long periods of time on haemodialysis.

Chapter 2

Mathematical Modelling of the Haemodialysis Process - Origins and Application to Modern Practice

Mathematical Modelling of the Haemodialysis Process

Origins and Application to Modern Practice

2.1 Background

The concept of mass balance is integral to the understanding of mathematical modelling. In order to derive a mathematical model for any system it is first necessary to understand all the factors that contribute to the system in terms of inputs and outputs. A pictorial representation of the model being considered should be constructed before any mathematics are applied. It is often easiest to start with a very simplified version of the model being considered and add more complex factors as the model is developed. This is the approach I have used both in my initial review of the urea kinetic model and in the subsequent development of the models for phosphate and beta2-microglobulin kinetics.

2.2 Single pool urea kinetic model

The human body in the simplest terms can be considered as a single pool of fluid. The concentration of solutes throughout this pool is considered uniform. Assuming that no solute is generated that would increase the concentration, and no water is removed or added, the solute concentration at the end of a period of dialysis can be calculated if the removal rate is known. Conventional dialysis occurs by a process of diffusion such that the removal rate is dependant on the concentration gradient across the dialysis membrane. The pictorial representation of this is shown overleaf.

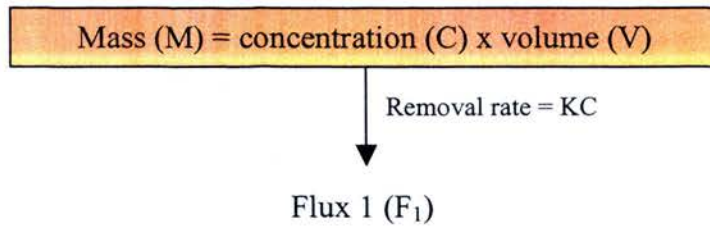


Figure 2.1. Schematic representation of single pool kinetics

Assuming that the concentration of the solute in the dialysate is zero, the mass balance equation can be written accordingly.

$$\frac{dM}{dt} = -F_1 = -KC \quad (1)$$

where M is the mass, K is the removal rate and C is the concentration in the body fluid.

To predict the concentration at a given time this equation requires to be differentiated according to the equations detailed below.

$$\frac{dM}{dt} = \frac{dVC}{dt} = -KC \quad (2)$$

$$V \frac{dC}{dt} + C \frac{dV}{dt} = -KC \quad (3)$$

But V is constant so

$$V \frac{dC}{dt} = -KC \quad (4)$$

$$\frac{1}{C} \frac{dC}{dt} = -\frac{K}{V} \quad (5)$$

integrate both sides with respect to t

$$\int \frac{1}{C} \frac{dC}{dt} = \int -\frac{K}{V} \quad (6)$$

$$\ln C = -\frac{Kt}{V} + A \quad (7) \quad \text{where } A \text{ is the constant of integration.}$$

take inverse logs

$$C = e^{-\frac{Kt}{V}} \cdot e^A \quad (8)$$

when $t=0$

$$C = e^A = C_0 \quad (9)$$

Therefore

$$\boxed{C_t = C_0 \cdot e^{-\frac{Kt}{V}}} \quad (10)$$

This is the equation of a first order differential equation and demonstrates the origin of the dimensionless parameter Kt/V . When this is plotted graphically the exponential decline in solute concentration can be appreciated. The slope of the line is determined by the clearance characteristics of the membrane utilised and the size of the patient as assessed by their body “volume”. As the body is being considered as a single pool the intracellular concentration is assumed to be identical to the extracellular concentration.

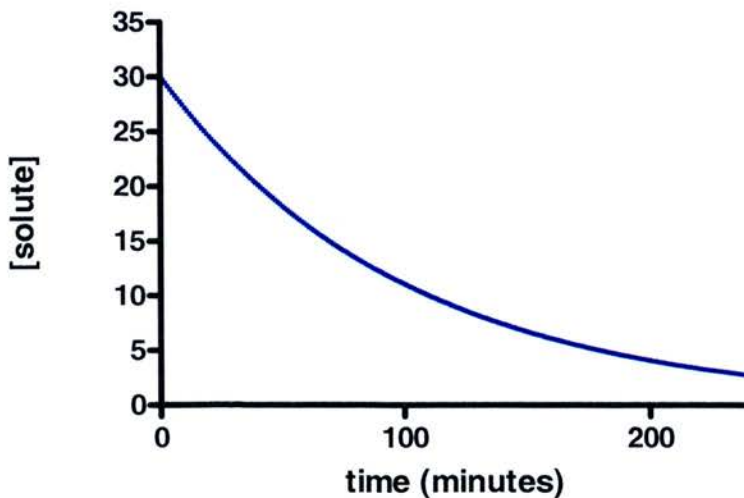


Figure 2.2. Graphical representation of single pool kinetics

This most simplistic mass balance equation can be applied to the dialysis process but has clear limitations. The inherent problems are

1. The volume of the system may not be constant
2. The patient may still have significant residual renal function
3. There may be an ongoing generation of solute during the dialysis process and in the interdialytic period
4. The distribution of solutes within the body is not constant and more than one pool of fluid is involved. In addition to the removal by the dialysis process the solute concentration in the extracellular space is changing because of intracellular to extracellular solute shifts
5. The effect of ultrafiltration on haematocrit and the resultant effect on solute clearance across the haemodialyser may need to be taken into account
6. The levels of solute in the blood may vary with time of day, posture or concomitant drug therapy

These different issues will be addressed in turn.

2.3 Single pool model with variable volume

Once again the starting point is a pictorial representation of the system which in this case is identical to Figure 1.

However in this set of equations the effect of changing volume needs to be taken into account.

As for the single pool model a mass balance equation can be written such that

$$\frac{dM}{dt} = -F_1 = -KC \quad (11)$$

$$V \frac{dC}{dt} + C \frac{dV}{dt} = -KC \quad (12)$$

but the change in volume is effected by the ultrafiltration rate, Q_u so

$$V_t = V_o - Q_u \cdot t \quad (13)$$

so

$$\frac{dV}{dt} = -Q_u \quad (14)$$

and

$$V_o - Q_u \cdot t \frac{dC}{dt} - C \cdot Q_u = -KC \quad (15)$$

rearrange

$$\frac{dC}{dt} = -\frac{(K + Q_u)C}{V_o - Q_u \cdot t} \quad (16)$$

separating the terms

$$\frac{dC}{-(K + Q_u)C} = \frac{dt}{V_o - Q_u \cdot t} \quad (17)$$

K and Q_u are constants = K_{total}

$$\int \frac{dC}{-K_{total} \cdot C} = \int \frac{dt}{V_o - Q_u \cdot t} \quad (18)$$

substitute variables

$$-K_{total} \cdot C = u \quad (19)$$

$$V_o - Q_u \cdot t = z \quad (20)$$

$$\frac{du}{dC} = -K_{total} \quad (21)$$

$$\frac{dz}{dt} = -Q_u \quad (22)$$

$$-\frac{1}{K_{total}} \int \frac{1}{u} du = -\frac{1}{Q_u} \int \frac{1}{z} dz \quad (23)$$

$$-\frac{1}{K_{total}} \ln u = -\frac{1}{Q_u} \ln z + A \quad (24)$$

$$-\frac{1}{K_{total}} \ln(-K_{total} \cdot C) = -\frac{1}{Q_u} \ln(V_0 - Q_u \cdot t) + A \quad (25)$$

$$\ln(-K_{total} \cdot C) = \frac{K_{total}}{Q_u} \ln(V_0 - Q_u \cdot t) + A_1 \quad (26)$$

$$-K_{total} \cdot C = (V_0 - Q_u \cdot t)^{\frac{K_{total}}{Q_u}} \cdot A_2 \quad (27)$$

$$A_2 = \frac{(-K_{total} \cdot C)}{(V_0 - Q_u \cdot t)^{\frac{K_{total}}{Q_u}}} \quad (28)$$

at initial conditions when t=0

$$A_2 = \frac{(-K_{total} \cdot C_0)}{(V_0)^{\frac{K_{total}}{Q_u}}} \quad (29)$$

$$-K_{total} \cdot C = (V_0 - Q_u \cdot t)^{\frac{K_{total}}{Q_u}} \cdot \frac{(-K_{total} \cdot C_0)}{(V_0)^{\frac{K_{total}}{Q_u}}} \quad (30)$$

$$-K_{total} \cdot C = \left[\frac{(V_0 - Q_u \cdot t)}{(V_0)} \right]^{\frac{K_{total}}{Q_u}} \cdot (-K_{total} \cdot C_0) \quad (31)$$

$$C = C_0 \cdot \left[\frac{V_0 - Q_u \cdot t}{V_0} \right]^{\frac{K_{total}}{Q_u}} \quad (32)$$

but

$$K_{total} = K + Q_u$$

$$\boxed{C = C_0 \cdot \left[\frac{V_0 - Q_u \cdot t}{V_0} \right]^{\frac{K+Q_u}{Q_u}}} \quad (33)$$

This equation now describes the situation of varying volume during dialysis therapy. There is still no movement of solute between different body compartments as the assumption currently is that the fluid within the body all contains solute at identical concentration.

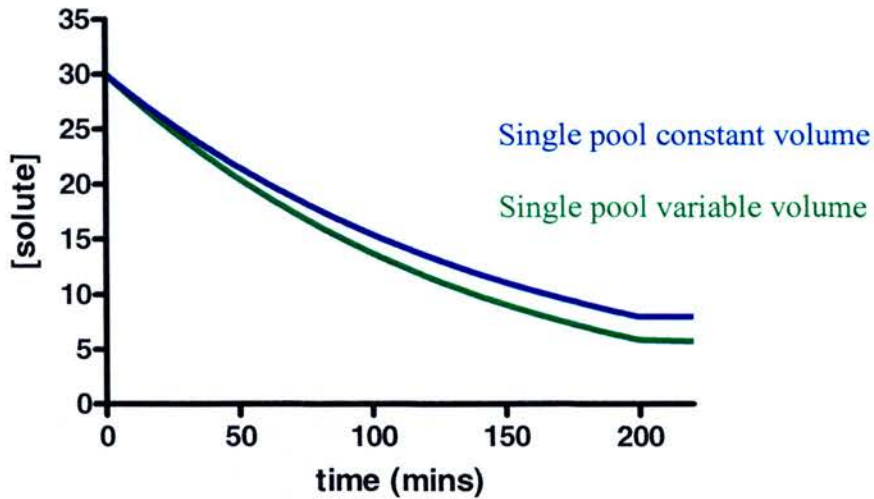


Figure 2.3. Graphical representation of single pool variable volume kinetics.

$V=30$ litres in both models. $Q_u=25$ ml/min in variable volume model.

The different lines on the graph compare the expected solute concentrations in the constant volume and the variable volume models. As would be expected in the situation where volume is changing the solute clearance is higher due to the additional removal of solute by convection.

2.4 Single pool model with variable volume and residual renal function

Where there is residual renal function the renal clearance of solute can be considered alongside the dialyser clearance of solute such that $K=K_d+K_r$

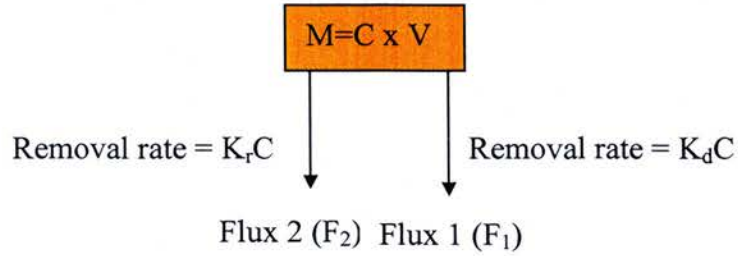


Figure 2.4 Schematic representation of single pool kinetics in the presence of residual renal function

so the equation becomes

$$C = C_0 \cdot \left[\frac{V_o - Q_u \cdot t}{V_o} \right]^{\frac{K_d + K_r + Q_u}{Q_u}} \quad (34)$$

For the purposes of modelling it is assumed that, during dialysis, residual renal clearance can be arithmetically summed with dialyser clearance to give total effective clearance.

2.5 Single pool model with variable volume and residual renal function and interdialytic generation

The final situation that needs to be considered when dealing with the single pool model is the situation that arises when there is ongoing generation of solute, G . Between dialysis treatments there is ongoing generation of solute from the ingestion of food and normal metabolic processes.

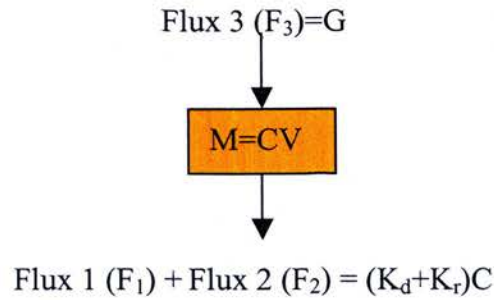


Figure 2.5. Schematic representation of single pool kinetics with urea generation.

As before the differential equations for this system can be written.

$$\frac{dM}{dt} = F_3 - (F_1 + F_2) = G - (K_d + K_r) \cdot C \quad (35)$$

$$V \frac{dC}{dt} + C \frac{dV}{dt} = G - KC \quad (36)$$

$$V_0 - Q_u t \frac{dC}{dt} + C Q_u = G - KC \quad (37)$$

$$\frac{dC}{dt} = \frac{G - C(K_d + K_r + Q_u)}{V_0 - Q_u t} \quad (38)$$

separating the terms

$$\frac{dC}{G - K_{total}C} = \frac{dt}{V_0 - Q_u t} \quad (39)$$

$$\int \frac{dC}{G - K_{total}C} = \int \frac{dt}{V_0 - Q_u t} \quad (40)$$

substituting terms

$$-\frac{1}{K_{total}} \int \frac{1}{u} du = -\frac{1}{Q_u} \int \frac{1}{z} dz \quad (41)$$

$$-\frac{1}{K_{total}} \ln u = -\frac{1}{Q_u} \ln z + A \quad (42)$$

$$-\frac{1}{K_{total}} \ln(G - K_{total}C) = -\frac{1}{Q_u} \ln(V_0 - Q_u t) + A_1 \quad (43)$$

$$\ln(G - K_{total}C) = \frac{K_{total}}{Q_u} \ln(V_0 - Q_u t) + A_1 \quad (44)$$

$$G - K_{total}C = (V_0 - Q_u t)^{\frac{K_{total}}{Q_u}} \cdot A_2 \quad (45)$$

$$A_2 = \frac{G - K_{total}C}{(V_0 - Q_u t)^{\frac{K_{total}}{Q_u}}} \quad (46)$$

Initial conditions when t=0

$$A_2 = \frac{G - K_{total}C_0}{(V_0)^{\frac{K_{total}}{Q_u}}} \quad (47)$$

$$G - K_{total}C = (V_0 - Q_u t)^{\frac{K_{total}}{Q_u}} \cdot \frac{G - K_{total}C_0}{V_0^{\frac{K_{total}}{Q_u}}} \quad (48)$$

$$G - K_{total}C = \left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_{total}}{Q_u}} \cdot G - K_{total}C_0 \quad (49)$$

$$G - K_{total}C = G \left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_{total}}{Q_u}} - K_{total}C_0 \left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_{total}}{Q_u}} \quad (50)$$

$$-K_{total}C = -G + G\left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_{total}}{Q_u}} - K_{total}C_o\left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_{total}}{Q_u}} \quad (51)$$

$$K_{total}C = G - G\left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_{total}}{Q_u}} + K_{total}C_o\left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_{total}}{Q_u}} \quad (52)$$

$$C = \frac{G}{K_{total}} - \frac{G}{K_{total}}\left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_{total}}{Q_u}} + C_o\left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_{total}}{Q_u}} \quad (53)$$

$$C = \frac{G}{K_{total}}\left[1 - \left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_{total}}{Q_u}}\right] + C_o\left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_{total}}{Q_u}} \quad (54)$$

$$C = C_o\left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_d + K_r + Q_u}{Q_u}} + \frac{G}{K_d + K_r + Q_u}\left[1 - \left(\frac{V_0 - Q_u t}{V_0}\right)^{\frac{K_d + K_r + Q_u}{Q_u}}\right] \quad (55)$$

This equation fully describes the single pool model in the presence of residual renal function, a variable volume and urea generation.

2.6 The two pool model and the introduction of modelling software

In all single pool equations, an assumption has been made that there is no transfer of solute between the intracellular and extracellular compartments during the dialysis process. While this may be true for some large solutes, it is clearly not the case for small solutes such as urea and that move freely between body compartments. In reality urea is in equilibrium such that under initial conditions it is equally distributed between the intracellular and extracellular compartments. Once the dialysis process

begins, solute is removed from the extracellular compartment according to the removal equations detailed above. At this point, a process of passive diffusion between the intracellular and extracellular compartments begins. The transfer of solute is a bi-directional process with a rate determined by the mass transfer coefficient for that solute, K_{ie} .

The mass balance for these equations is represented pictorially below.

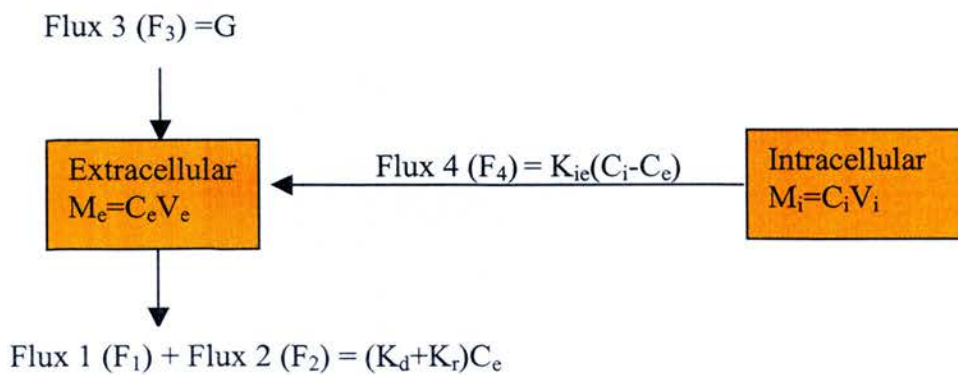


Figure 2.6. Schematic representation of two-pool kinetics

In the two-pool model the predicted extracellular concentration of solute falls more rapidly as the theoretical volume of distribution is lower. This is tempered by solute influx from the intracellular space. Assuming that dialyser clearance is greater than intracellular to extracellular mass transfer, the predicted solute removal during dialysis is greater as shown in Figure 7.

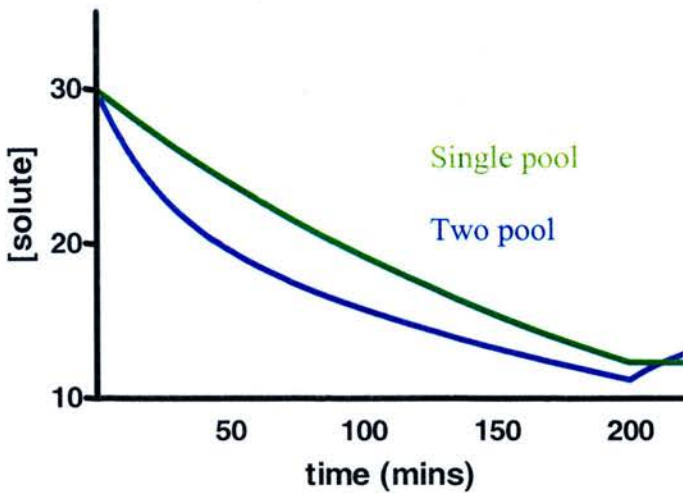


Figure 2.7. Comparison of concentration profiles predicted by single pool and two-pool kinetics.

The concentrations in the intracellular and extracellular compartments will drop at different rates during dialysis due to resistance to diffusion across the cell membrane. When the dialysis process stops there is a differential between the intracellular and extracellular solute concentrations. The removal of solute from the extracellular space has ceased but the intracellular to extracellular transfer of solute will continue until a new equilibrium is reached.

The period during which the re-equilibrium is occurring is known as the rebound period.

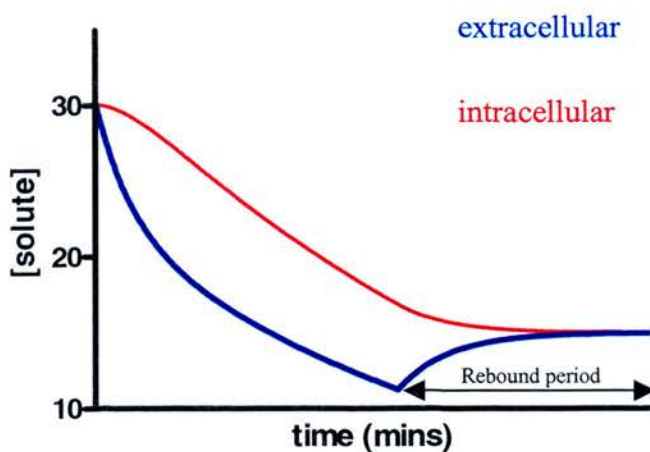


Figure 2.8. Graphical representation of intracellular and extracellular concentration profiles during dialysis showing the rebound period after cessation of dialysis.

The presence of the rebound period makes it difficult to provide an accurate model of solute kinetics that can be easily utilised in clinical practice. Ideally, the model should predict the solute concentration at the end of the rebound period. If the pre and post dialysis solute concentrations alone are used, the prediction is invalidated, as solute clearance will be overestimated. Several different correction formulae have been devised to circumvent the problem resulting in the concept of the equilibrated Kt/V (eKt/V), which aims to predict the solute concentration at the end of the rebound period from samples taken in the immediate post-dialysis period.

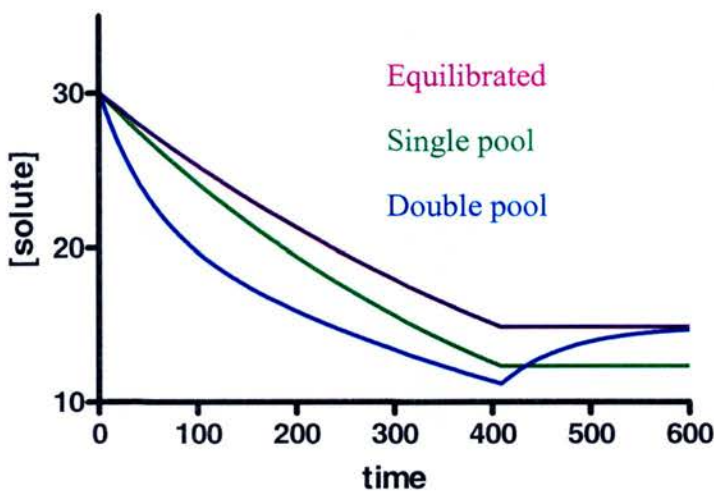


Figure 2.9. Graphical representation of theoretical concentration profiles in single pool, double pool and equilibrated kinetic models

The commonly used formulae for predicting the equilibrated urea are the Daugirdas and Schneditz rate equation⁶², the Smye method⁶³ and the Tattersall equation⁶⁴. The methodology in each is different but, in common, each uses a blood sample taken in the immediate post dialysis period to predict the urea concentration when

re-equilibrium is largely complete. When adequacy of dialysis is referred to in the work presented here, the Tattersall equation has been used.

$$e \frac{Kt}{V} = sp \frac{Kt}{V} \cdot \frac{t}{t+tp} \quad (56) \quad \text{and} \quad C_{eq} = C_0 \cdot \left(\frac{C_t}{C_0} \right)^{\frac{t}{t+tp}} \quad (57)$$

Clearly, these complexities make the interpretation of solute kinetics difficult to apply at the bedside. Once two pools are involved, it becomes complex to solve the equations analytically as separate equations have to be written to explain the changes in the solute concentrations in the intracellular and extracellular compartments.

For the intracellular compartment

$$\frac{dM_i}{dt} = -F_4 = -K_{ie}(C_i - C_e) \quad (58)$$

and for the extracellular compartment

$$\frac{dM_e}{dt} = F_4 + F_3 - (F_1 + F_2) = K_{ie}(C_i - C_e) + G - (C_e K_{total}) \quad (59)$$

For the intracellular space the change in volume is considered to be negligible so

$$V_i \frac{dC_i}{dt} = -K_{ie}(C_i - C_e) \quad (60)$$

$$\frac{dC_i}{dt} = \frac{-K_{ie}(C_i - C_e)}{V_i} \quad (61)$$

For the extracellular space

$$V_e \frac{dC_e}{dt} + C_e \frac{dV_e}{dt} = K_{ie}(C_i - C_e) - (C_e K_{total}) \quad (62)$$

$$(V_{e(0)} - Q_u t) \frac{dC_e}{dt} + C_e Q_u = K_{ie}(C_i - C_e) - (C_e K) \quad (63)$$

$$\frac{dC_e}{dt} = \frac{K_{ie}(C_i - C_e) - C_e(K + Q_u)}{V_{e(0)} - Q_u t} \quad (64)$$

The presence of two varying concentration parameters makes this difficult to solve analytically and it is necessary to introduce computer simulation software. The software used throughout the studies described in this thesis is VisSim, (Visual solutions Inc). This programme allows the equations to be solved iteratively by the Runge Kutta method. Details of the modelling technique are given in Chapter 3 on general methodology.

2.7 The effect of rising haematocrit on solute clearance

The fifth potential problem identified when considering solute clearance is the effect of rising haematocrit. In the modern era where haemoglobin levels tend to run almost in the normal range, patients can have a haematocrit of 35% or even higher at the beginning of dialysis. The effect of ultrafiltration particularly in therapies such as post-dilutional HDF is to raise the haematocrit. While the clearance of urea is not

thought to be adversely affected by this^{65;66}, the effect on larger molecules has not been extensively investigated. The study presented in chapter four aims to clarify the effect of haematocrit on a range of solutes of different molecular size. Both high-flux dialysis and haemodiafiltration have been assessed to identify whether there is a difference between the two treatments. This study is presented as part of the general methodology section as the results impact on the other studies presented in the thesis.

2.8 The influence of varying solute levels in the interdialytic period on solute clearance during dialysis

The sixth identified problem with solute kinetics would arise if the concentration of the solute in question had an inherent biological rhythm. It is well recognised that many solutes exhibit a diurnal variation in their concentration but, at present, no allowance is made for this when assessing solute concentration in dialysis patients. In the context of haemodialysis, this is potentially most significant for phosphate and its regulating hormones. It has been repeatedly demonstrated that phosphate exhibits diurnal variation in normal individuals^{67;68}, but it has never been convincingly demonstrated whether this diurnal variation is maintained in end-stage renal failure and it is certainly not taken into account. The study described in chapter 5 assesses the diurnal variation in patients with stage 5 Chronic Kidney Disease and compares this to age and sex matched controls. The study was designed to give information about the diurnal variation in phosphate in patients with advanced renal impairment that could be extrapolated to patients on haemodialysis.

Section 2

Methodology

Chapter 3

General Methodology

General Methodology

Each of the studies in this thesis has some elements of methodology that are unique to that study and these aspects of methodology are discussed within each chapter. Some of the methods and statistical analyses are common to all chapters and are discussed here.

3.1 Setting

The majority of the studies described in the thesis were carried at the Lister Hospital, a 500 bed District General Hospital in Stevenage. The dialysis unit serves as a tertiary referral centre for a population of 1.3 million and at the time of the studies, there were approximately 300 patients receiving haemodialysis therapy. Around 120 of these patients were dialysed at the Lister Hospital itself, with the remainder being dialysed at satellite units in Luton and St Albans.

In the studies of beta2-microglobulin additional studies were carried out at the Royal Free Hospital and its satellite unit at Barnet Hospital. The Royal Free Hospital is a teaching hospital in north London that catered for a dialysis population of approximately 300 at the time of the study.

3.2 Dialysis techniques

Dialysis treatments were mainly carried out using Fresenius 2008 or 4008 dialysis machines. In the Lister Hospital, either haemodiafiltration or high-efficiency high-flux dialysis was delivered utilising biocompatible membranes (HF80, ARH9, 18GWS, Polyflux, KF201, PAN11). At the Royal Free Hospital and Barnet Hospitals

until 6 months before the studies patients dialysed using low-flux membranes (F6, F8, GFS 16+). At the time of the study, all patients were dialysing using high-flux membranes (ARH9, HF60LS, HF80LS) although some of these were utilised in a low-efficiency configuration termed “intermediate-flux haemodialysis”, IFHD. All the water utilised was ultrapure and conformed to Renal Association standards (see page 48). In all centres, a central water plant supplied the water.

The standard composition of dialysate used unless there was a clinical indication to do otherwise was K^+ 2.0mmol/l, glucose 5.5mmol/l, HCO_3^- 35mmol/l, Ca^{2+} 1.25mmol/l. If there was a clinical need one of the other dialysate compositions detailed in table 1 was used.

Table 3.1. Dialysate compositions used in studies

Dialysate	Potassium mmol/l	Calcium mmol/l	Glucose mmol/l
1	2	1.25	5.5
2	2	1.35	5.5
3	1	1	5.5
4	2	1	5.5
5	3	1.5	0
6	1	1.35	5.5
7	3	1.75	0

Dialysate flow was 800ml/min in the patients receiving high-efficiency high-flux dialysis or haemodiafiltration and 500ml/min in those receiving low-efficiency intermediate-flux dialysis.

The blood pump speed was set to the highest tolerated blood flow for the vascular access employed, and the HDF fraction, where utilised, was calculated from 35% of the plasma flow and reinfused into the venous bubble trap in post-dilution mode. Dialysate collections were made in their entirety by diverting the drain fluid into a 250-litre container that was placed on a weigh scale. The volume of the collected dialysate was considered equivalent to its weight and concentrations of solutes were measured by sampling in triplicate from this tank after thorough mixing.

At the time of these studies being undertaken, there was a policy at the Lister Hospital of reusing dialysis membranes. Membranes were treated with peracetic acid (Renalin, Minntec Inc, USA) and tested for membrane integrity before each dialysis session. After 2002 this practice was abandoned due to a change in medical device legislation. Disposable membranes were used throughout at the Royal Free Hospital sites.

At the Lister Hospital, all patients received dialysis according to a modelled prescription. Monthly measurements of urea kinetics were made utilising a three sample (pre-post-pre) technique. Residual renal function was taken into account by measuring the volume of urine passed in a two-day dialysis break and assessing the concentration of urea in this fluid. The target equilibrated Kt/V for each dialysis session was 1.2 and the predicted dialysis time was calculated from rearrangement of the equation Kt/V , where K is the dialyser clearance predicted by the manufactures literature and V is the Watson volume. The efficiency of dialysis was then monitored by measuring the delivered Kt/V , and alterations made in the prescription to keep the dialysis dose on target.

Patients at the Royal Free Hospital all received four hours of dialysis if this achieved a URR of 65% or four and a half hours if dialysis adequacy fell below this target. Monitoring of dialysis adequacy was carried out on a monthly basis at all centres.

3.3 Blood and dialysate sampling technique during dialysis

When blood and dialysate samples were taken during dialysis the following protocol was used.

1. Needle the fistula
2. Take a sample from the "A" fistula needle.
3. Start the dialysate collection.
4. Start dialysis and record exact time.
5. Take blood samples from the "A" and "V" sample ports at the times detailed recording the exact time each sample is taken.
6. At end of treatment, stop dialysis. Start stopwatch. Put machine into bypass.
7. Terminate dialysate collection. Reconnect dialysate drain line to drain.
8. After 90 seconds of no blood flow restart blood pump at 150ml/min. Cancel alarms if necessary
9. At two minutes on stopwatch, take further samples from "A" port.
10. Finish dialysis as usual.
11. Record weight of dialysate.
12. Stir fluid using paddle for 2 minutes ensuring bottom layers well mixed and collect samples in triplicate.

Method for taking blood from fistula needle

Withdraw 10ml blood from fistula needle and keep in syringe. Take 2ml sample into sample tube. Re-inject 10ml sample. Flush with 5ml saline.

Method for taking blood from sample ports on dialysis

Clean sample port with sterile swab. Withdraw 2ml using needle and sample tube.

Do not slow blood pump for sampling.

The post-dialysis blood sample was taken 2 minutes after the end of dialysis using a slow-flow method. The following equation was utilised to convert the urea concentration to one that could be used in the calculation of an equilibrated Kt/V^{64} .

$$C_{eq} = C_0 \cdot \left(\frac{C_t}{C_0} \right)^{\frac{t}{t+35}}$$

Recirculation was assessed by saline dilution techniques either via an integral module on the dialysis machine in the Royal Free units or by a Transonics ultrasound device at the Lister Unit.

Method to measure haematocrit

Obtain blood from “A” and “V” sample ports as above and transfer into three separate heparinised capillary tubes. Analyse using the microhaematocrit centrifuge, Hawksley, England. Insert capillary tubes into centrifuge and spin for 3 minutes at 5000rpm. Read results manually on microcentrifuge reader and take average of three values.

3.4 Laboratory measurement of solutes

Measurement of Extracellular Phosphate

The serial nature of the blood sampling in this work presented a challenge for the processing of the blood samples. There was concern that, by leaving the samples until the end of the study the results of the earlier samples may be affected due to haemolysis and release of inorganic phosphate from the cells. It was also noted early on that if samples were spun immediately there was a tendency for the supernatant to clot, which prevented the sample being analysed successfully. This was assumed to be the effect of heparin utilised in the dialysis circuit. It was not known whether keeping the samples at room temperature or at 4°C would influence the results.

A series of studies were conducted to determine

1. Whether leaving samples to sit before centrifugation was detrimental.
2. If refrigerating samples improves stability.
3. To determine the stability of phosphate measurements over longer periods of time and to investigate if storage temperature has any influence on this.

The results of these analyses revealed that fibrin clot developed for up to 120 minutes post-dialysis, which is presumably the length of time before the effect of heparin has completely worn off. It was apparent that prolonged delays in centrifugation caused the phosphate concentration to fall significantly and there was a tendency for samples stored at room temperature for in excess of four hours to have higher values although not significantly. Once the samples were centrifuged, the

phosphate concentrations were stable for up to 24 hours. The standard method of processing all samples for analysis of phosphate was therefore to leave samples at room temperature for 60 minutes before centrifugation. All samples were stored at 4°C after centrifugation and processed in a single run at the end of each study.

Two different autoanalysers were utilised in the course of this work. This was unavoidable due to a refurbishment of the laboratory. The QA for the phosphate assay on the two analysers was very similar. Table 2

Table 3.2 QA Measurements of Autoanalysers

Beckman LX20			Olympus AU600		
Mean (mmol)	SD	CV%	Mean (mmol)	SD	CV
0.54	0.03	5.7	0.50	0.04	7.1
1.11	0.05	4.1	1.12	0.04	3.5
1.85	0.03	1.7	2.03	0.05	2.7

Measurement of Intracellular Phosphate

The method of Challa et al⁶⁹ was used to measure plasma and whole blood phosphate concentration. Red cell phosphate concentration was assumed to be the difference between these two values. Details of the techniques used are given in Chapter 8.

Beta2-microglobulin measurement

At the outset of these studies the beta2-microglobulin assays at the Lister Hospital were carried out by a nephelometric technique on a Beckman Array analyser. A similar method was utilised at the Royal Free Hospital using a Beckman Image analyser. The technique was time consuming as samples had to be pre-diluted to 1:6 and very operator dependant due to this manual processing of samples. It was also an expensive technique. There were problems at the lower end of the analytical range with the limit of sensitivity being 1mg/l. I felt that this might cause problems when analysing dialysate samples. For these reasons I investigated other potential methods of assaying the samples.

Other automated techniques that could have been utilised in the lab were an automated microparticle enzyme immunoassay on the Abbott Asxym analyser but this proved to be cost prohibitive.

Radioimmunoassay lacked sensitivity at the lower end of the analytical range.

ELISA kits were commercially available with sensitivity down to 0.1mg/l and it appeared that a comparable degree of sensitivity could be achieved with a luminometric technique.

I undertook a comparison of the nephelometric technique (lab standard at the time of these studies) with the luminometric and ELISA techniques.

The laboratory did not own a luminometer but a desktop luminometer was loaned by Cambridge Life Science to facilitate the experiment. Reagents were purchased from the same company. ELISA kits were purchased from IBL Hamburg. Samples were

run concurrently on the Beckman Array (the current standard), on the Berthold Luminometer and on ELISA plates.

Luminometer

The initial studies using the luminometer were acceptable with a satisfactory standard curve achieved. (Figures 1,2). All samples were run in duplicate and the mean value utilised. When control samples were run, the intrassay coefficient of variation was 2.6%.

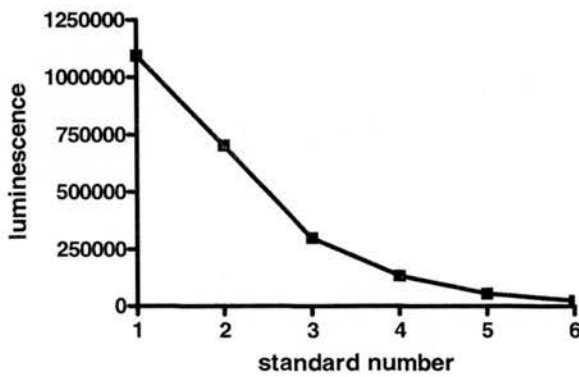


Figure 3.1. Standard curve of beta2-microglobulin concentrations obtained with luminometer

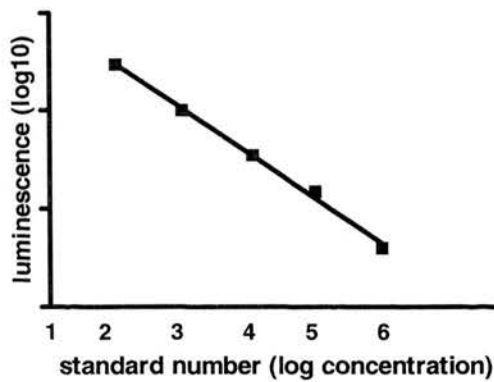


Figure 3.2. Standard curve of beta2-microglobulin concentrations obtained with luminometer (log transformation)

When the results were compared with the Beckman Array analyser a close correlation within the normal range was found although there was a correction factor of 1.71 and a poorer correlation at higher values. Figure 3.

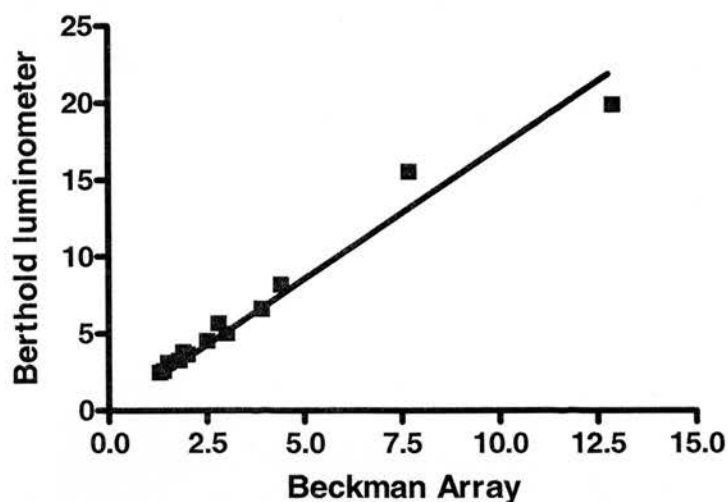


Figure 3.3 Correlation between luminometer and Beckman Array

The luminometric technique was very time-consuming and, additionally, the reagents were not entirely reliable with several batches giving ambiguous results. For these reasons the luminometric technique was abandoned.

ELISA

The ELISA technique was set up to run on an automated analyser that had recently been obtained by the immunology lab at the Lister Hospital. Unfortunately, this malfunctioned repeatedly making it impossible to continue with this method.

At the time of these studies, the autoanalysers in the biochemistry laboratory were being upgraded and the Beckman LX20 analyser was replaced with an Olympus

AU600. This facilitated the use of a turbidimetric method of measuring beta2-microglobulin, which ran in a fully automated manner on this machine. The correlation between the results from the Beckman Array and the Olympus autoanalyser were very close (figure 4, $r^2=0.97$) within the range of values of interest. The laboratory have now adopted this technique as their standard and have adjusted the normal ranges accordingly with the application of a correction factor of 1.2.

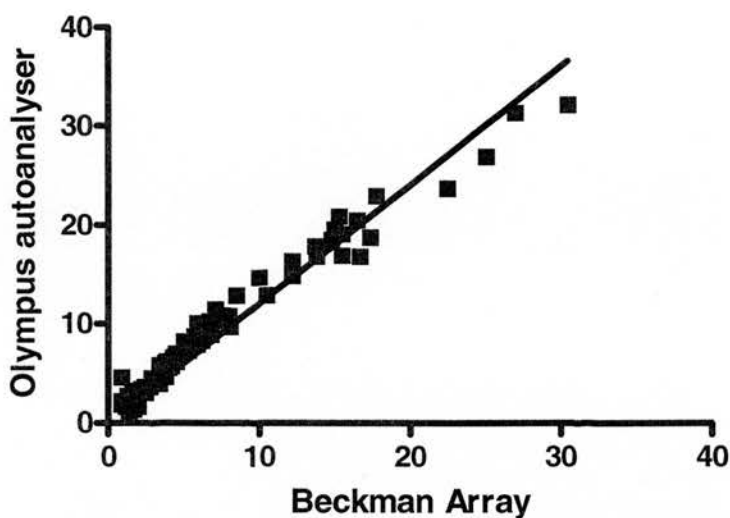


Figure 3.4. Correlation between Beckman Array and Olympus Autoanalyser for Beta2-microglobulin

The normal range with this assay is 0-3.0mg/l and the average interassay co-efficient of variation was 10.05%.

Measurement of Myoglobin

When I began this work the laboratory at the Lister Hospital did not routinely carry out estimates of myoglobin concentration. I introduced a turbidimetric technique than ran on the Olympus autoanalyser and this has continued to be used as an in-house assay. The normal range was quoted as 0-85 μ g/l with an average interassay coefficient of variation of 10.1%.

3.5 Statistics

Statistical methods employed were parametric and non-parametric as appropriate including Students t-test, Mann-Whitney U-test, Kruskal-Wallis test, Friedman's test and Bonferroni and Dunn's post-hoc analyses to compare multiple samples. For the work on diurnal variation, harmonic periodic regression analysis was used and cross-correlation analysis was employed. All statistical analyses were carried out using Minitab version 14. GraphPad Prism version 4 and Sigma Plot 2000 were used for graphical representations.

In all statistical tests a p value of <0.05 was deemed significant.

3.6 Biological Modelling Course

A structured program of mathematical tuition was devised by Dr Paul Chamney, which took the form of regular tutorials. The programme is detailed below. Through this program, the concepts of dialysis adequacy and mathematical modelling of the dialysis process were explored and this permitted me to take the modelling studies forward independently.

Main Concepts

- Course intended to provide introduction to basic ideas behind modelling
- Applied maths utilised where possible with explanation of key physical concepts underpinning mathematical theory
- Special emphasis on techniques relevant to dialysis
- Topics generally applicable to pharmacological systems
- Use of VisSim
- Development of data analysis techniques with limited statistics

Details of the content of the individual modules covered during this aspect of my research are given in Table 3.

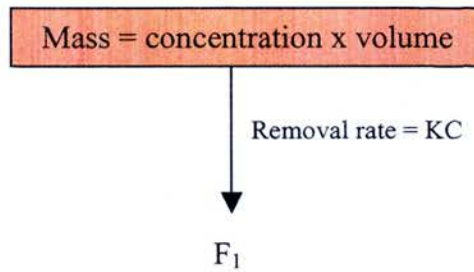
Table 3.3. Biological Modelling Course followed during Research

Module 1	Mass balance principles	Concentration, mass, volume, flux, Kirchoffs laws, definition of clearance, effective clearance, dialyser circuits, accumulation & depletion
Module 2	Functions, graphs, slopes and areas	Plotting standard functions. Slopes, areas, offsets, equation of straight lines, first ideas behind calculus, intercept, quadratics, hyperbolas, asymptotes, logarithmic, inverse square, even powers.
Module 3	Basic Algebra	Indices & logs. Linear factors, quadratics, solutions, logarithmic relationships
Module 4	Simultaneous equations	Solving simultaneous equations in 2 dimensions and 3 dimensions. Vectors and matrices for solutions of linear equations.
Module 5	Basic differential calculus	Finding derivatives of simple functions, chain rule, product and quotient rules, mass balance, accumulation and storage elements, lag effect, partial differentiation
Module 6	Basic integral calculus	Euler & Simpson rules, basic integrals. Velocity – time graphs, constant of integration, initial conditions, geometrical problems.
Module 7	Solution of ordinary first order differential equation	Use of integration to solve 1 st order differential equations, derivation of the single pool UKM
Module 8	Further mass balance equations of physical systems	Derivation of differential equations of physical systems. Transient effects and steady state conditions
Module 9	Approximation	Finding roots of equations. Newton Raphson techniques
Module 10	Optimisation	Finding parameters in general expressions, mean squared error
Module 11	Rates of change	Handling problems with several variables that change with time
Module 12	Control theory	Principles of feedback control, gain, stability
Module 13	Statistics	SD, CV, SEM, variance, p-values, R values, correlation

3.7 Modelling Theory

All the modelling of solute kinetics undertaken in this thesis utilised computerised modelling software, VisSim (Visual Solutions Inc.). When constructing the models the first step was to understand the system under investigation and to draw a schematic diagram of the solute fluxes involved. Next, the mass balance equations and basic differential equations required to describe the system were written. The equations were then imported as 'blocks' and 'connectors' into the modelling software.

For example in the simplest case of single pool kinetics with constant volume the graphical representation is



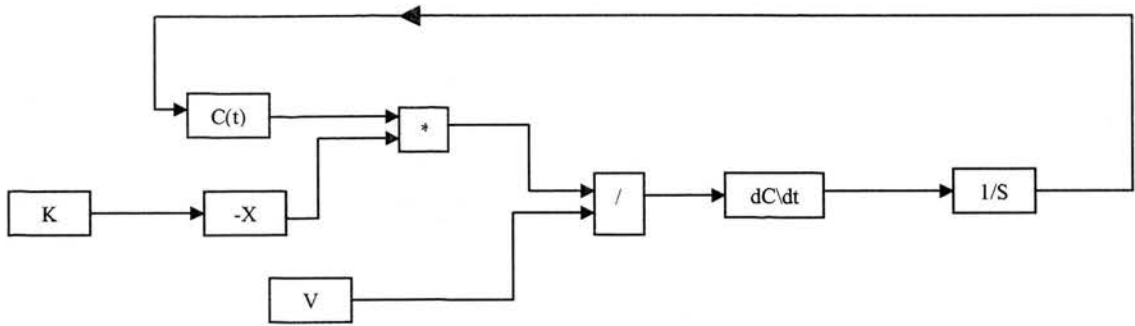
The mass balance equation is

$$dM = -F_1$$

the differential equation is

$$V \frac{dC}{dt} = -KC$$

and the wiring diagram is



When the simulation is run, the resultant output can be plotted as a graph or observed as a data series and predicts the time course of data that is expected from a particular set of differential equations.

The raw data collected during experiments is introduced as an input to the software and the observed time course of solute clearance can be visualised and compared to different theoretical models.

The use of such modelling software allows models to be constructed based on physiological principles. Assumptions can be made about parameters that can not be measured, to determine whether the proposed mechanism is in agreement with the observed data.

For each of the models the standard deviation between the data and the model can be calculated via the expression

$$\sqrt{\frac{1}{N} \sum_{i=0}^N (C_{e_Data}(i) - C_{e_Model}(i))^2}$$

This provides an objective measure of the degree of fit between the raw data and the proposed model over the whole treatment period.

The use of this software allows very complex models to be constructed without the need for all parameters to be measured. It facilitates understanding of biological systems and, while the precise physiological mechanisms often can not be proven, hypotheses can be suggested from the observed time course of the data permitting further directed research. It allows models to be graphically represented in a manner that is easily understood without the need to interpret complex mathematical equations. This modelling technique has been utilised in the studies examining phosphate and beta2-microglobulin kinetics, but the same process could theoretically be applied to any solute that can be physically measured during the dialysis process.

3.8 Research Protocols and Ethics

All the studies presented in this body of work have been approved by the Ethics Committees of the East and North Herts NHS Trust and have received approval from the Research and Development Committee of that organisation. In additional Ethical Approval was granted by the Royal Free & Hampstead NHS Trust for the studies in Chapters 10 & 11 and the Ethics Committee of Roche Pharmaceuticals approved the study in Chapter 5.

All patients involved in the studies gave written informed consent and all studies adhered to the Declaration of Helsinki.

Section 3

Haematocrit and Solute Clearance

Chapter 4

The Effect of High Haematocrit on the Efficiency of High-Flux Dialysis Therapies.

The Effect of High Haematocrit on the Efficiency of High-Flux Dialysis Therapies.

4.1 Background

Standard low-flux haemodialysis is entirely a diffusive process where solutes are cleared from the blood according to the concentration difference across the dialyser membrane, the size of the membrane pores and the thickness of the membrane. Ultrafiltration rates are limited to the volume required to maintain euvolaemia, which is on average 1-2 litres. The newer high-flux therapies introduce an element of convection to the dialysis process. All solutes that can pass through the pores on the membrane are removed dependant on the volume of fluid that passes through the membrane and their sieving co-efficient. The high ultrafiltration co-efficient of the membranes may lead to marked haemoconcentration during the dialysis process. This is particularly the case in haemodiafiltration (HDF) where up to 35% of the dialysed plasma volume is ultrafiltered during a treatment. This can be equivalent to 20-30 litres of ultrafiltrate that is replaced by infusion of physiological replacement fluid into the venous bubble trap after the dialysis membrane, Figure 1.

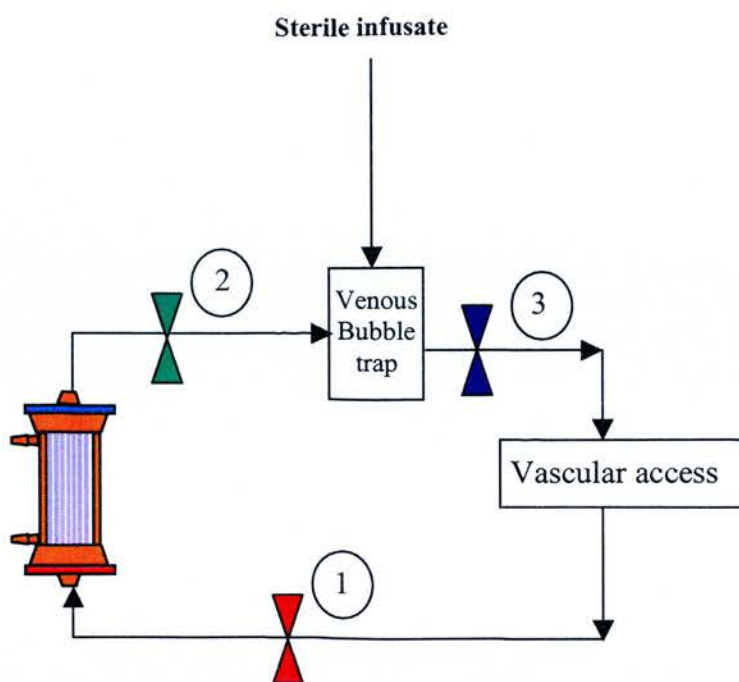


Figure 4.1. Schematic representation of dialysis circuit showing sampling ports

Patients on erythropoetin therapy maintain a near to normal haemoglobin and haematocrit (hct) and this potentially has a significant impact on the clearance of solute during the dialysis process. This may be relevant if the increased haematocrit at the end of dialysis makes the process less efficient. The concept of dialysis adequacy is based on the measured clearance of urea, a small molecule distributed in an essentially uniform manner throughout body water compartments. New concepts of adequacy also focus on the removal of larger molecules such as beta2-microglobulin (β_2m) that diffuse less freely. While changes in haematocrit are not thought to be of major significance in the clearance of low molecular weight substances during dialysis^{65,66}, the impact on higher molecular weight substances has

not been assessed. The majority of previous work has measured haematocrit in low-flux (LFHD) or high-flux dialysis (HFHD). The specific effect on solute clearance across the range of molecular size in haemodiafiltration (HDF) has not been extensively investigated.

The clearance of the dialysis membranes quoted in the product literature assumes a constant haematocrit throughout dialysis. Hence, while this may give an accurate estimation of clearance at the beginning of a treatment, there may be inaccuracies by the end. Additionally, the clearances in the product literature tend to be quoted in terms of pure diffusive clearance and take no account of the contribution of convection, which, for larger solutes, is a major component of total clearance.

In order to extend the concept of dialysis adequacy, particularly in relation to haemodiafiltration, it is necessary to fully understand the impact of high ultrafiltration rates on haematocrit and dialyser solute clearance during the haemodialysis process.

This study was designed to ascertain the degree of haemoconcentration caused by ultrafiltration during high-flux haemodialysis and haemodiafiltration and to study the effect of this haemoconcentration on solute clearance.

4.2 Subjects and Methods

Twelve patients were studied with six patients undergoing high-flux haemodialysis and six patients undergoing on-line post-dilutional haemodiafiltration. The patients were stable on haemodialysis for a minimum of three months, and had stable vascular access. Any patient with a haematocrit >35% was eligible for inclusion in the study. The baseline characteristics of the subjects are detailed in Table 1.

Table 4.1. Baseline characteristic of patients.

	HDF	HD	
Age (years)	63.5±16.5	67.0±9.2	p=ns
M:F	3:3	3:3	
Q _b (ml/min)	350.0±77.4	366.7±98.3	p=ns
Q _d (ml/min)	800±0	800±0	p=ns
T _d (min)	208.0±41.3	186.8±70.8	p=ns
UF (ml)	2166.7±1555.2	1750±1143.2	p=ns
Q _u (ml/min)	10.3±6.7	8.8±3.4	p=ns
Kt/V	1.5±0.4	1.2±0.2	p=0.04
KRU (ml/min)	0.4±1.0	0.7±0.7	p=ns
Q _{HDF} (ml/min)	75.0±18.7	0	p<0.001

Where Q_b=blood flow, Q_d=dialysate flow, T_d=total dialysis duration, UF=total ultrafiltration volume, Q_u=ultrafiltration rate, Kt/V=most recently recorded dialysis adequacy, KRU=residual renal clearance of urea and Q_{HDF}=rate of post-dialyser infusion of fluid.

Measurement of haematocrit

Three simultaneous blood samples were taken in Sarstedt monovette tubes every 30 minutes from the arterial sample port before the dialyser membrane, from a sample port immediately after the dialysis membrane and from the venous sample port after the sterile infusate had been re-infused. (Sample ports 1,2 &3, Figure 1). In this way, the maximum haematocrit reached within the dialysis membrane during haemodiafiltration, and the effective haematocrit to which the patient is exposed was measured. It is not possible to directly measure the maximal haematocrit reached within the dialyser membrane during high-flux haemodialysis due to the changing pressure profile within the membrane, Figure 2. In the first part of the membrane, there is ultrafiltration that exceeds the desired weight loss and this is then compensated for by backfiltration in the latter part of the membrane. The maximal haematocrit will therefore be achieved at the point at which these two mechanisms crossover.

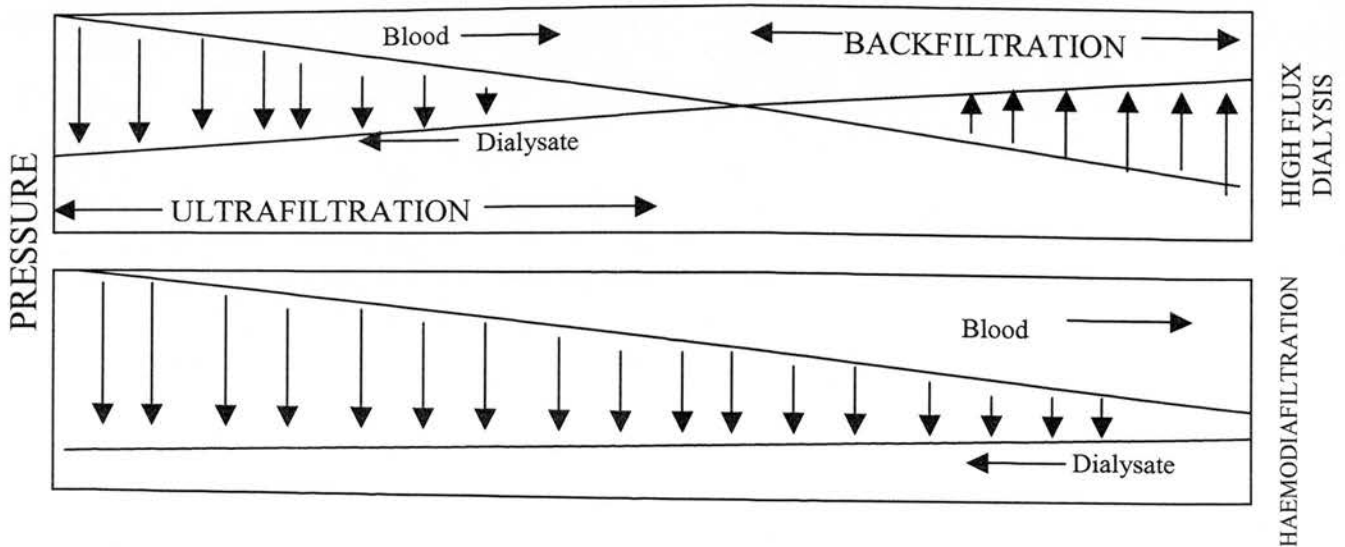


Figure 4.2 Backfiltration seen in high flux dialysis is not seen with on-line haemodiafiltration due to different pressure profiles

The haematocrit was measured in triplicate using a microhaematocrit centrifuge (Hawksley, England).

Measurement of solute clearance

Blood sample analysis for urea and phosphate was carried out using a Beckman LX20 autoanalyzer in the pathology lab at the Lister Hospital. Beta2-microglobulin (11.8kD) and myoglobin (17kD) concentrations were assayed in-house using an immunoturbidimetric method on an Olympus AU600 autoanalyser.

The total dialyser clearance was measured in two ways. The first method used separate equations to describe the individual components of diffusion and convection while the second method used an equation that incorporated an element of interaction between the two types of clearance.

Method 1

The diffusive clearance K_{diff} of urea, phosphate, myoglobin and β_2m across the dialysis membrane was calculated from the concentrations measured at the arterial and venous sample ports according to the equation

$$K_{diff} = \frac{Qb(Cb_i - Cb_o)}{Cb_i} \quad \text{[Equation 1]}$$

where Qb =blood flow rate; Cb_i = concentration of solute in blood at inlet (sample port 1); Cb_o = concentration of solute in blood at outlet (sample port 3).

An assumption is made at this point that pure diffusion is occurring and no ultrafiltration is taking place.

This does not reflect the true situation, as blood is not a homogenous fluid. There are red cells suspended in the plasma so the distribution of solute has to be considered the product of the aqueous fraction x the blood flow rate. In addition, the water content of blood can be considered to be in two compartments, the plasma water and the intracellular red cell water. To factor this into the equation the following calculations are needed.

$$\text{Plasma water flow rate} = Qb\left(1 - \frac{Hct}{100}\right)F_p \quad [\text{Equation 2}]$$

where F_p = plasma fraction

$$\text{Red cell water flow rate} = Qb\left(\frac{Hct}{100}\right)F_r \quad [\text{Equation 3}]$$

where F_r = red cell fraction

$$\text{Total blood water flow rate} = Qb\left[F_p - \frac{Hct}{100}(F_p - F_r)\right] \quad [\text{Equation 4}]$$

If there is no transfer across the red cell membrane, the effective flow rate is equal to the plasma flow rate. However, if there is transfer across the red cell membrane during haemodialysis, this needs to be taken into consideration. The factor γ

represents the fraction of red cell water involved in the dialysis process. If the red cell membrane is completely impermeable to the solute $\gamma = \text{zero}$ and if the red cell membrane is completely permeable to the solute $\gamma = 1$. γ has been measured at 1.11 for urea due to a reversibly bound pool of urea within the red cell and for phosphate is essentially zero⁷⁰. It was also assumed to be zero in the calculations for myoglobin and $\beta 2$ -microglobulin.

The equation for effective blood flow rate then becomes

$$Qb(\text{effective}) = Q_b \left[Fp - \frac{Hct}{100} (Fp - \gamma Fr) \right] \quad [\text{Equation 5}]$$

This needs to be substituted back into the original equation to accurately assess the diffusive clearance of a solute.

$$K_{diff} = \frac{Qb(\text{effective})(Cb_i - Cb_o)}{Cb_i} \quad [\text{Equation 6}]$$

Convective clearance of solute K_{uf} is calculated from the formula

$$K_{uf} = Q_u \left(\frac{Cb_o}{Cb_i} \right) \quad [\text{Equation 7}]$$

where Q_u is the ultrafiltration rate in ml/min.

When there is no diffusion occurring this simplifies to

$$K_{uf} = Q_u \quad [\text{Equation 8}]$$

For each solute in question calculations were made using these independent equations and the total clearance estimated from

$$K_{total} = \frac{Qb(effective)(Cb_i - Cb_o)}{Cb_i} + Q_u \left(\frac{Cb_o}{Cb_i} \right) \quad \text{[Equation 9]}$$

The results from these calculations are likely to be an over-estimation of true clearance, as, in high-flux therapies and particularly in haemodiafiltration, the interaction between simultaneous diffusion and convection needs to be taken into account. Total clearance is usually lower than the sum of the two component parts.

Method 2

Many different equations can be utilised to describe the combined effect of diffusive and convective clearance. Ficheux et al have validated a linear model to predict the effect of convection on urea clearance in HDF but this can only be applied to small molecules⁷¹. The equation proposed by Villarroel¹⁹, although an over-simplification, takes into account the sieving coefficients of larger molecules.

$$K_{total} = Kd_0 + T_r Q_u \quad \text{[Equation 10]}$$

Where Kd_0 =diffusive clearance in the absence of ultrafiltration (equivalent to equation 6) and $T_r Q_u$ =convective clearance. Q_u =ultrafiltration rate in ml/min and T_r =transmittance coefficient.

T_r can be calculated experimentally from the equation

$$T_r = S \left(1 - \frac{Kd_o}{Qb_i} \right) \quad \text{[Equation 11]}$$

Where S is the sieving coefficient, calculated under conditions of pure convection from

$$S = \frac{C_F}{C_{b_i}} \quad \text{[Equation 12]}$$

where C_F =concentration of solute in the dialysate and C_{b_i} =concentration of solute at the dialyser inlet.

It was not possible to calculate these values during this study so values quoted in the literature were used. It was assumed that $S=1$ for urea and phosphate, $S=0.65$ for beta2-microglobulin and $S=0.33$ for myoglobin⁷². It was assumed that S remained constant during dialysis.

It is clear from equations 10 and 11 that convective clearance has an inter-relationship with diffusive clearance. By calculating clearance using Method 2, it is probable that a more accurate representation of total clearance can be obtained which is of more interest and clinical relevance.

4.3 Data analysis

GraphPad Prism version 4.00 for Windows, (GraphPad Software, San Diego California USA) was used for all graphical data and non-parametric statistical analysis.

The comparison of solute clearance between HD and HDF calculated using method 1 was carried out using the Mann-Whitney test. The difference between the total clearance measurements obtained by methods 1 and 2 was compared using the Mann-Whitney test.

The change in haematocrit in the two groups was compared using the repeated measures Friedman test with Dunn's post test multiple comparisons for intra-individual measurements, and with the Mann-Whitney test for inter-group analysis.

The relationship between haematocrit and clearance was examined in two ways. Initially serial mean clearance values were examined using the repeated measures Friedman test with Dunn's post test multiple comparisons to determine whether a significant change in clearance occurred during dialysis. Next the haematocrit and solute clearance at 30 minutes and at the end of dialysis were examined to calculate the percentage change in these parameters for each solute in the two groups with comparisons conducted using the Mann-Whitney test.

Since the duration of the dialysis sessions varied from 140 to 250 minutes, clearance values at 30, 60, 90, and 120 minutes together with the final clearance value for each session were compared.

In all analyses a p value <0.05 was deemed significant.

4.4 Results

Haematocrit

There was a significant rise in arterial haematocrit during dialysis in all subjects, Figure 3.

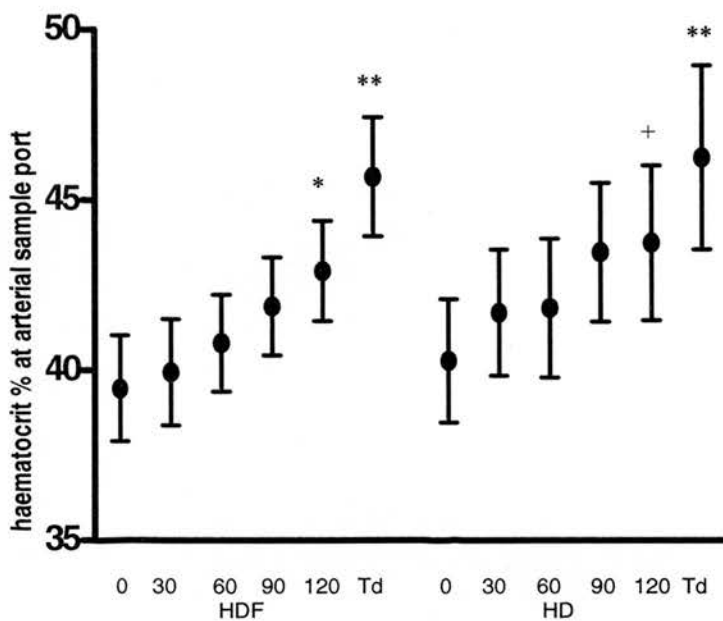


Figure 4.3 Rise in arterial haematocrit during dialysis in HD and HDF. *denotes significance when compared to value at t=30. *=p<0.05, +=p<0.01, **=p<0.001. Error bars represent SEM.

There was no significant difference between the arterial haematocrit in the two groups either at the beginning or at the end of treatment. There was a significant difference between the arterial haematocrit (sample port 1) and the post-dialyser haematocrit before re-infusion of fluid (sample port 2) within each group. The

haematocrit post-dialyser at the end of the dialysis treatment was higher in HDF when compared to HD although in absolute terms this failed to reach significance,

Table 2.

Table 4.2. Comparison of haematocrit before and at the end of dialysis and at different sample ports in the two treatment groups. *denotes significance compared to arterial baseline values

	HDF	HFHD	p
Initial arterial hct %	40.3±4.4	40.3±3.6	ns
Final arterial hct %	46.2±6.6*	46.4±4.4*	ns
Post-dialyser hct %	53.7±9.3*	48.4±4.5*	ns
Venous hct %	48.7±6.2*	48.4±4.2*	ns

When the percentage change in haematocrit across the dialyser was examined at all time points, the rise was significantly higher in HDF. After re-infusion of replacement fluid, this difference was no longer apparent, Figure 4.

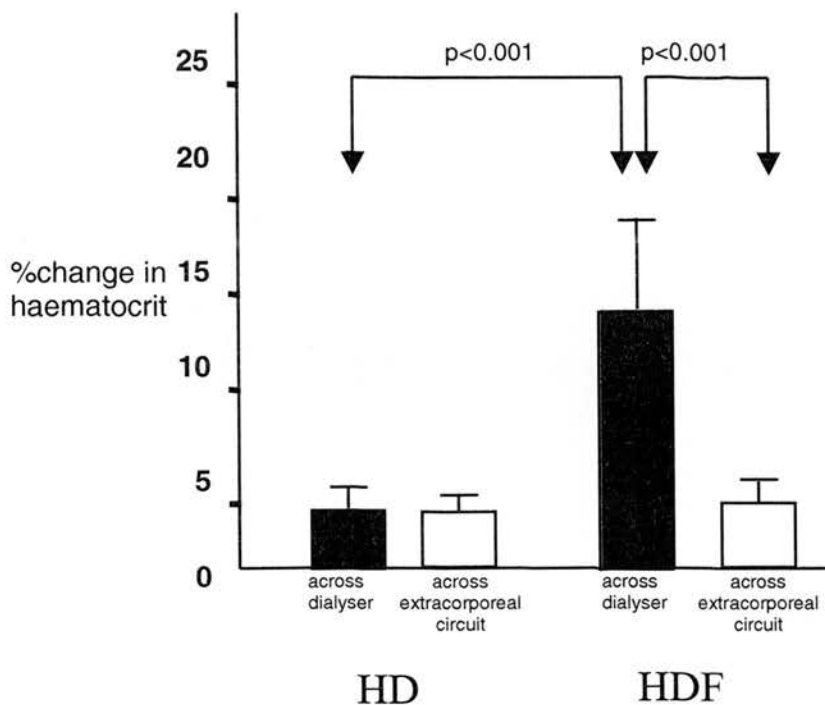


Figure 4.4 Percentage change in haematocrit within dialyser (sample port 2-sample port 1) and in extracorporeal circuit (sample port 3-sample port 1) in HD and HDF. Error bars represent SEM.

Solute clearance measurements.

Method 1

The clearance of urea and phosphate when calculated according to equation 9 is comparable in HD and HDF. The higher total clearance of the middle molecules β_2m and myoglobin in HDF is as a result of the increase in convective transport, Table 3.

Table 4.3 Comparison of average total clearance values (ml/min) in HDF and HD using method 1

	HDF	HD	p
urea	258.3±51.1	236.6±52.0	0.11
phosphate	149.3±35.3	151.4±26.8	0.64
β2microglobulin	59.3±33.4	45.2±19.7	0.05
myoglobin	33.1±44.7	13.4±20.1	0.03

Figure 5 shows total clearance data obtained by method 1 for all four solutes. There was no detectable change in total clearance over time for urea, β 2m or myoglobin in the HD and HDF groups either when considered separately or together.

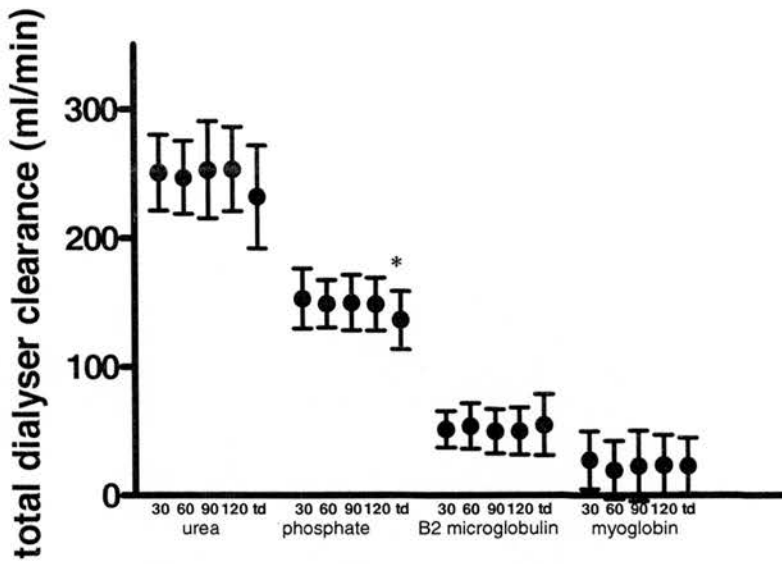


Figure 4.5 Total dialyser clearance obtained by method 1 for solutes at five time intervals during dialysis in HD and HDF groups combined. *denotes $p < 0.001$. Error bars represent 95% CI.

When analysed using the separate equations for diffusion and convection it appeared that, in HD, total clearance of phosphate at the end-point of dialysis was significantly lower than when first measured at 30 minutes, Figure 6; $p < 0.001$.

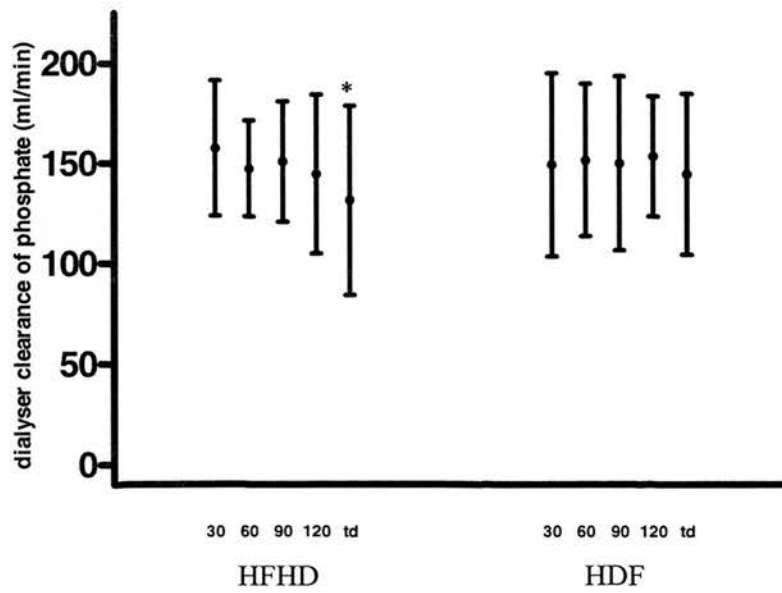


Figure 4.6 Total clearance of phosphate in HD and HDF obtained by method 1. *denotes $p < 0.001$. Error bars represent 95% CI.

In terms of potential phosphate removal this equates to a 1.47 ± 0.75 mmol decrease.

The total measured mass removal for the treatments was 20.89 ± 9.38 mmol. This reduction in phosphate clearance was not seen in HDF but was replicated in the group as a whole.

Method 2

Total clearance measurements using the combined equation of the second method are shown in Figure 7. There were no significant changes in clearance values during dialysis for any of the solutes during HD, HDF or in the group as a whole.

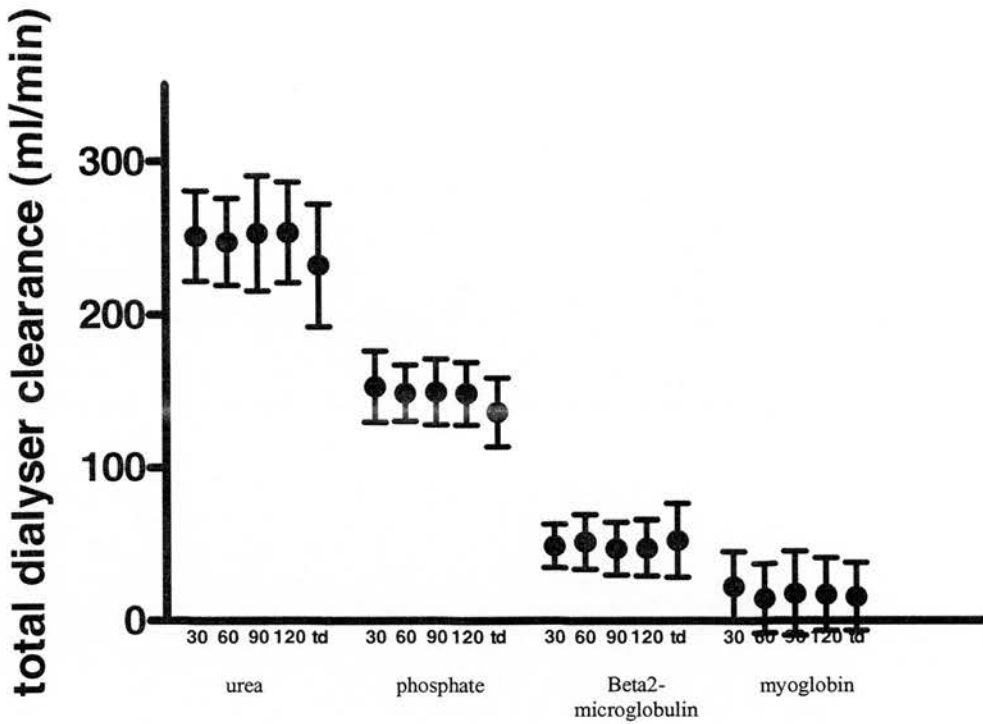


Figure 4.7 Total dialyser clearance obtained by method 2 for solutes at five time intervals during dialysis in HD and HDF groups combined. Error bars represent 95% CI.

Comparison of different methods of calculating total clearance in HD and HDF

When calculating the clearance of small solutes (urea and phosphate) with a sieving coefficient of approximately 1 there is no difference in the clearance measurements obtained with the two methods of calculation.

For larger solutes with sieving coefficients less than unity, the use of the combined equation in method 2 resulted in lower calculated levels of clearance, Table 4.

The total clearance of beta2-microglobulin and myoglobin, although lower when calculated using method 2, was not significantly different to the value obtained with method 1.

Table 4.4 Comparison of solute clearances (ml/min) calculated by methods 1 and 2

		Method 1	Method 2	p
HD	urea	236.6±52.0	236.6±52.0	ns
	phosphate	151.4±26.8	151.4±26.8	ns
	β2microglobulin	45.2±19.7	42.8±19.6	ns
	myoglobin	13.4±20.1	7.9±20.0	ns
HDF	urea	258.3±51.1	258.3±51.1	ns
	phosphate	149.3±35.3	149.3±35.3	ns
	β2microglobulin	59.3±33.4	56.7±33.7	ns
	myoglobin	33.1±44.7	27.2±45.3	ns

Percentage change in clearance method

There was no difference between HD and HDF treatments in terms of the percentage rise in arterial hct (12% vs. 13%; $p=0.669$). The percentage change in clearance was measured using clearance values taken at 30 minutes and those at the end of dialysis. There were no statistically significant differences between HD and HDF with respect to the percentage change in total clearance (measured by either method) of any solute.

4.5 Discussion

Haematocrit

The finding of a significant increase in arterial haematocrit was expected and occurred in all haemodialysis sessions studied. The haematocrit immediately after the dialyser was higher in HDF. The increase in haematocrit had no impact on the patient per se, as re-infusion of ultrapure dialysate into the venous bubble trap corrected haematocrit before blood returned to the patient.

The structure of the membrane is of critical importance in the presence of high haematocrit. The blood flow and wall shear rates are lowest in the peripheral fibres of a membrane where the dialysate flow is highest⁷³ and, unless blood flow

rates are increased to compensate, rises in blood viscosity are likely to exacerbate this problem⁷⁴, Figure 8.

The high fluid flux across the dialysis membrane in HDF could presumably act to improve the velocity of blood through the peripheral fibres making HDF less prone to the potential reduction in efficiency. Advances in the technology of membrane structure aim to reduce this phenomenon both in terms of making blood distribution more homogenous and also by optimising dialysate flow⁷⁴.

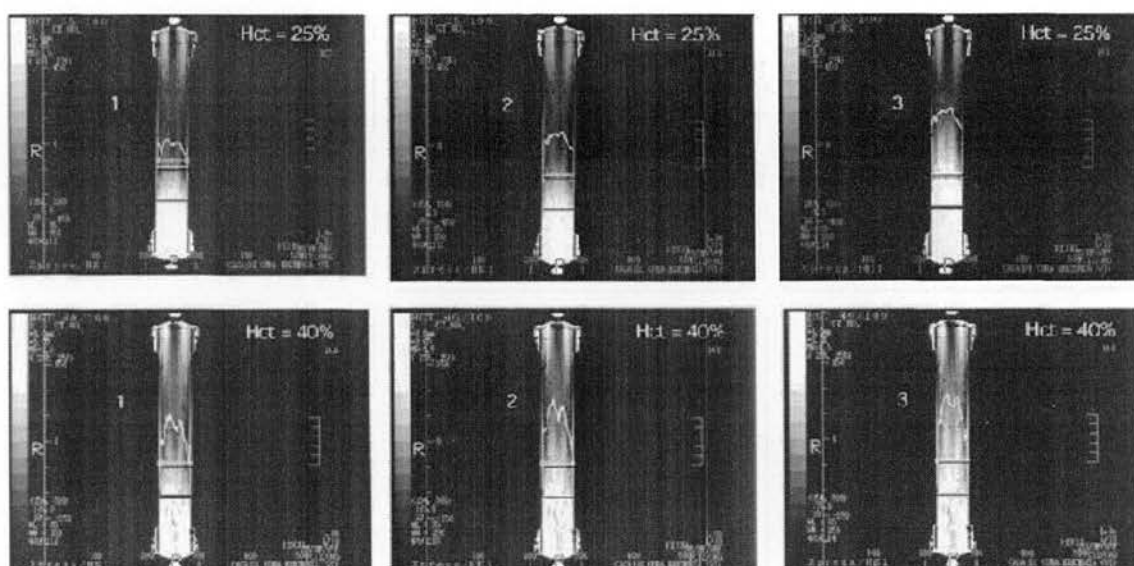


Figure 4.8. Effect of rising haematocrit on blood flow within the dialysis membrane. Typical parabolic distribution of blood flow in the fibre bundle documented by dye injection in the blood compartment and image reconstruction by a helical scanner. The increase in hematocrit further enhances the discrepancy between central and peripheral fibres in terms of relative flow velocity. [Reproduced with permission from⁷⁴]

Solute clearance

It was hypothesised that a rising haematocrit would adversely affect intra-dialyser haemodynamics and solute clearance. The results suggest that total clearance is not significantly influenced by rising haematocrit regardless of how clearance is measured.

The occurrence of simultaneous diffusion and convection within the dialysis membrane in high-flux therapies makes an accurate description of the individual components difficult. In method 1, an attempt was made to understand which component of clearance was dominant for each solute and to assess how this might be influenced by rising haematocrit. The use of method 2 however gives a more accurate representation of the impact of rising haematocrit on total solute clearance.

It is expected that the total clearance should be less than the sum of its constituent parts, as diffusion and convection have influences on each other during the course of dialysis that may affect the efficiency of each individual process.

As diffusion occurs, the concentration of a solute in the blood compartment falls and, as the degree of convection relies on solute concentration, this may be adversely affected.

Conversely, convective removal of solute may reduce the potential for diffusive removal due to a build up of a protein layer on the surface of the membrane that can increase the resistance to diffusion.

The comparison of the two methods of calculating solute clearance demonstrates that there is no difference when calculating small solute removal. The tendency for ultrafiltration to cause a build-up of a protein layer at the membrane surface could theoretically lead to a reduction in the diffusive clearance. Jaffrin et al have demonstrated in vitro that this is offset by the increase in solute concentration in this protein layer, with the two effects cancelling each other out⁷². Their results are applicable to urea and myoglobin and presumably also hold true for phosphate and beta2-microglobulin.

The finding that beta2-microglobulin and myoglobin removal is lower when calculated by method 2 was expected. The convective removal calculated using method 1 assumes a sieving coefficient of 1 for all solutes and is thought to be an over-estimate due a failure to take the relatively low sieving coefficients for larger molecules into account. It should be noted that the clearances quoted are theoretical clearances assuming that the sieving coefficient does not change throughout dialysis.

In high-flux haemodialysis treatments, when clearance is measured by method 1, there is a significant reduction in phosphate clearance towards the end of dialysis, but, as this is not seen when clearance is measured by method 2, it may be largely artefactual. Indeed, whether there are any clinical sequelae from a 7% reduction in potential mass removal of phosphate is debatable.

4.6 Conclusions

This study shows that, in the course of high-flux dialysis treatments, despite marked haemoconcentration, there is an insignificant change in the total solute clearance of urea, phosphate, beta2-microglobulin and myoglobin. It also demonstrates the effect of taking into account the interaction between diffusive and convective modalities of solute removal when assessing predominately convective therapies.

It should be noted that this study was conducted in the era of widespread erythropoetin use and all the patients had a haematocrit >35% at the beginning of dialysis. It is entirely possible that if patients with lower haematocrit were examined, the influence of a rising haematocrit would be greater.

Section 4

Phosphate Kinetics in Dialysis

Chapter 5

Assessment of Diurnal Variation in Plasma Phosphate and Regulatory Hormones in Advanced Renal Disease

Assessment of Diurnal Variation in Plasma Phosphate and Regulatory Hormones in Advanced Renal Disease.

5.1 Background

Hyperphosphataemia is present in a large proportion of patients with advanced renal failure and current treatment schedules provide inadequate control. An elevated calcium-phosphate product has been linked to the excess cardiovascular mortality experienced by patients with chronic renal failure⁴², and hyperphosphataemia plays a central role in the development of renal bone disease⁷⁵. In subjects with normal renal function there is a marked diurnal variation of plasma inorganic phosphate levels⁶⁷, but it is not known if this diurnal variation exists in the presence of advanced renal impairment. This makes the available data on the pathological role of hyperphosphataemia in the pre-dialysis phase difficult to interpret. Once patients are established on dialysis it becomes increasingly difficult to interpret phosphate levels due to the complex nature of intradialytic phosphate kinetics and the widespread use of vitamin D supplementation and phosphate binders.

There are three existing studies of diurnal phosphate variation in dialysis patients that give conflicting results. The first study measured phosphate concentration in nine dialysis patients on a non-dialysis day and demonstrated diurnal variation in six patients⁷⁶. The second study was carried out during incidental hospitalization of dialysis patients and failed to demonstrate diurnal variation⁷⁷. Both studies could have been complicated by ongoing rebound of phosphate in the post-dialysis period. The third study attempted to detect whether the timing of dialysis could account for differences in pre-dialysis phosphate concentration. Higher phosphate concentrations

were found in patients dialysed during the evening shift, which is consistent with the notion of diurnal variation but not proof of this⁷⁸.

The normal circadian rhythms of PTH⁷⁹ and cortisol^{80;81} are thought to be maintained in advanced renal disease, but there is very limited data in the published literature.

Under normal circumstances cortisol exerts a phosphaturic effect due to inhibition of the Na-Pi co-transporter in the proximal tubule⁸². Cortisol deficient states tend to lead to hyperphosphataemia possibly as a direct result of changes in tubular reabsorption of phosphate⁸³. There is no information available on the status of this relationship in advanced renal disease.

Variations in growth hormone (GH) are known to be very dependant on the sleep-wake cycle and in normal individuals demonstrate only a weak circadian rhythm. It is not known whether the same relationship between GH and sleep-wake cycles is maintained in advanced renal disease. Elevated GH levels are often seen in renal failure partly because of decreased renal clearance⁸⁴ and partly due to disturbances in the hypothalamic-hypophyseal axes⁸⁵. Whether this has an impact on the normal ability of GH to enhance tubular reabsorption of phosphate is not known.

Many patients with advanced renal disease exhibit impaired glucose tolerance as a result of either insulin resistance or abnormalities in insulin excretion or degradation⁸⁶⁻⁸⁸. The ability of insulin to stimulate cellular uptake mechanisms appears however to be intact in renal failure⁸⁹, and may impact on plasma phosphate concentrations.

This study was designed to investigate the nature of the diurnal variation in plasma inorganic phosphate levels in subjects with normal renal function and to study the

effect of advanced renal failure on this. Due to the complexity of phosphate kinetics in haemodialysis patients, it was decided to study subjects who were within weeks of starting dialysis rather than to study patients already receiving haemodialysis. The study additionally aimed to investigate whether there were any abnormalities in the concentrations of hormones known to regulate phosphate handling, and whether there were abnormalities in calcium homeostasis, acid-base status or phosphate excretion.

5.2 Subjects and methods

Eight subjects were studied, four subjects with normal renal function (eGFR 68-117 ml/min) and four subjects with stage 5 chronic kidney disease (eGFR 9-12 ml/min).

The eGFR was calculated from the MDRD 4 formula⁹⁰.

$$\text{eGFR} = 186 * (\text{creatinine})^{-1.154} * (\text{age})^{-0.203} * (0.742 \text{ if female}) * (1.210 \text{ if African American})$$

Patients were matched with controls of similar age, sex and body mass index, Table 1. Patients with stage 5 chronic kidney disease were recruited from the Lister Hospital pre-dialysis clinics. The controls with normal renal function were recruited from Roche Pharmaceuticals volunteer panel.

Subjects were allowed to continue with their usual drug regime with the exception of

the cessation of a beta-blocker in one patient and withdrawal of calcium supplements in another, changes that were made one week prior to the study. No subject received phosphate binders or vitamin D supplementation at any time.

Table 5.1 Demographic data for all subjects

CKD					Normal				
Subject	Age (years)	Gender	BMI $\frac{\text{height}}{\text{weight}^2}$	eGFR (ml/min)	Subject	Age (years)	Gender	BMI $\frac{\text{height}}{\text{weight}^2}$	eGFR (ml/min)
1	61	M	27.3	10	5	60	M	26	83
2	34	M	20	12	6	34	M	22.5	117
3	59	M	22.3	9	7	60	M	24.8	70
4	63	F	27.3	10	8	63	F	32.3	68

Participants were admitted to the Roche Clinical Pharmacology Unit (CPU) at Welwyn Garden City. The subjects all received equal portions of the same food during the study with meals being served at 08:00, 13:00, and 18:00 and snacks at 16:00 and 21:00. Free access to water was allowed at any time. One of the subjects had a diagnosis of coeliac disease and both he and his matched control received a gluten free diet for the 24-hour period. Blood pressure, pulse and haemoglobin were measured at two hourly intervals during the study to ensure safety.

An indwelling cannula was placed in the antecubital fossa and was kept patent using saline flushes between samples. Blood was sampled hourly for plasma phosphate (PO_4^{2-}), plasma total calcium (Ca^{2+}), bicarbonate (HCO_3^-) and glucose, and two hourly for insulin, growth hormone (GH), parathyroid hormone (PTH), and cortisol. To ensure that changes in phosphate were not the result of haemolysis or haemoconcentration/dilution, potassium and albumin were measured at each time point.

A 2.5 ml venous blood sample was stored on ice after collection until centrifuged at 4°C . Plasma samples were immediately stored, in an upright position at 4°C until processing, and serum for hormone assays was stored at -20°C . Samples for plasma phosphate, calcium, bicarbonate, potassium, albumin and glucose were analysed in the Lister Hospital biochemistry department using standard technique on an Olympus AU600 auto-analyser. Hormonal assays were carried out at the Hammersmith Hospital.

Six four-hourly aliquots of urine were collected with the total urine produced by subjects during each sampling interval collected in polyethylene containers. The volume of all specimens was measured and recorded at the end of each collection interval. An aliquot of 10 ml was transferred to a polypropylene tube for each collection interval and stored at 4°C until analysed for urea and phosphate concentrations.

The phosphate clearance was calculated from

$$PO_4 \text{ clearance} = \frac{UV}{PO_4 * t}$$

Where U=measured concentration of phosphate in urine, V is volume of urine collected, PO_4 is the average plasma phosphate and t is the duration of the collection interval.

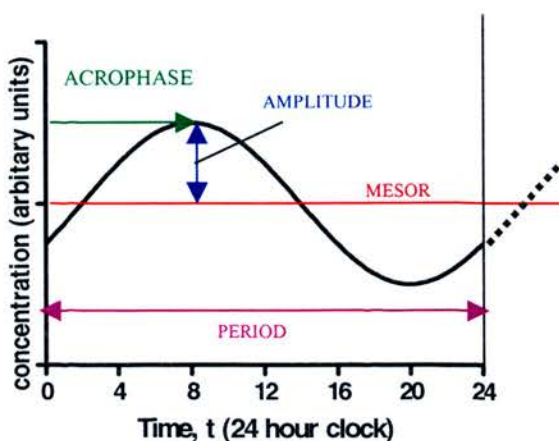
5.3 Data manipulation and Statistical Analysis

For the purposes of identifying peaks and troughs, data were smoothed by the method of weighted averages using the nine closest neighbours but, for all further analysis, the raw data series were used.

Rhythm biometry

Graph Pad Prism software version 4.00 for Windows, San Diego, California, USA, was used to fit a harmonic periodic regression equation to the data using the formula.

$$Concentration(t) = MESOR + \sum_{i=1}^n A * \cos \frac{2\pi * (t - ACROPHASE_i)}{P_i}$$



where MESOR (acronym for Midline Estimating Statistic Of Rhythm) is the mean of the oscillation, n is the number of harmonic components, A is the amplitude of the maximum oscillatory distance from MESOR, ACROPHASE is the temporal location of the maximal crest with respect to midnight and P is the oscillatory period.

Harmonic periodic regression utilizes the least squares method to find the best fitting sinusoidal wave form giving the smallest value for the sum of the squared residuals. The basic circadian period of 24 hours was used as the baseline pattern. The superimposed harmonics each has a period equal to a fraction of the basic rhythm (12, 8 or 6 hours). For each combination of harmonic waveforms, the goodness-of-fit was estimated by R^2 and the statistical significance was derived from the F-value comparing the resultant waveform to one of zero amplitude.

Cross Correlation

Relationships between parameters were assessed using cross correlation analysis utilizing Minitab version 14, (Minitab Inc). The discrete time point data was interpolated to provide equally spaced measurements using the Runge Kutta 2nd order method in VisSim version 4.5, (Visual Solutions Inc.). The time series were compared at concurrent time points to detect any correlation. If this was not present, lag periods of up to six hours were introduced to assess whether the correlation improved.

The significance values were calculated from

$$\frac{2}{\sqrt{24-lag}} \text{ for } \alpha=0.05 \text{ and } \frac{3}{\sqrt{24-lag}} \text{ for } \alpha=0.01.$$

In all analyses statistical significance was inferred when $p < 0.05$. All values are quoted with 95% CI unless otherwise stated.

5.4 Results

Descriptive

For all parameters measured, with the exception of potassium and insulin, there were significant differences between the two groups, Table 2.

Table 5.2 Relationship between average values for all parameters in CKD and normal renal function

	Normal	CKD	p
PO ₄ ²⁻ (mmol/l)	1.15 ± 0.05	1.63 ± 0.07	<0.001
K ⁺ (mmol/l)	4.0 ± 0.1	3.9 ± 0.0	ns
HCO ₃ ⁻ (mmol/l)	24.7 ± 0.3	17.7 ± 0.4	<0.001
Ca ²⁺ (mmol/l)	2.33 ± 0.01	2.26 ± 0.07	0.04
Glucose (mmol/l)	5.8 ± 0.2	6.2 ± 0.3	0.009
Albumin (mg/dl)	37.5 ± 0.5	33.6 ± 0.7	<0.001
PTH (pmol/l)	4.4 ± 0.2	38.3 ± 6.0	<0.001
Cortisol (nmol/l)	303.6 ± 38.8	371.4 ± 42.7	0.02
GH (mIU/l)	10.4 ± 3.3	27.3 ± 1.7	<0.001
Insulin (uU/ml)	16.9 ± 3.9	19.7 ± 4.6	ns
PO ₄ excretion (mmol/24hrs)	31.4 ± 5.8	15.7 ± 7.1	0.01

Phosphate

The mean phosphate concentration over 24 hours in the subjects with stage 5 CKD was 1.63 ± 0.07 mmol/l and in normal renal function was 1.15 ± 0.05 mmol/l, $p < 0.001$. When the average plasma phosphate value for CKD was compared to the average value for normal renal function at each time point, values were higher although this did not always reach significance, Figure 1.

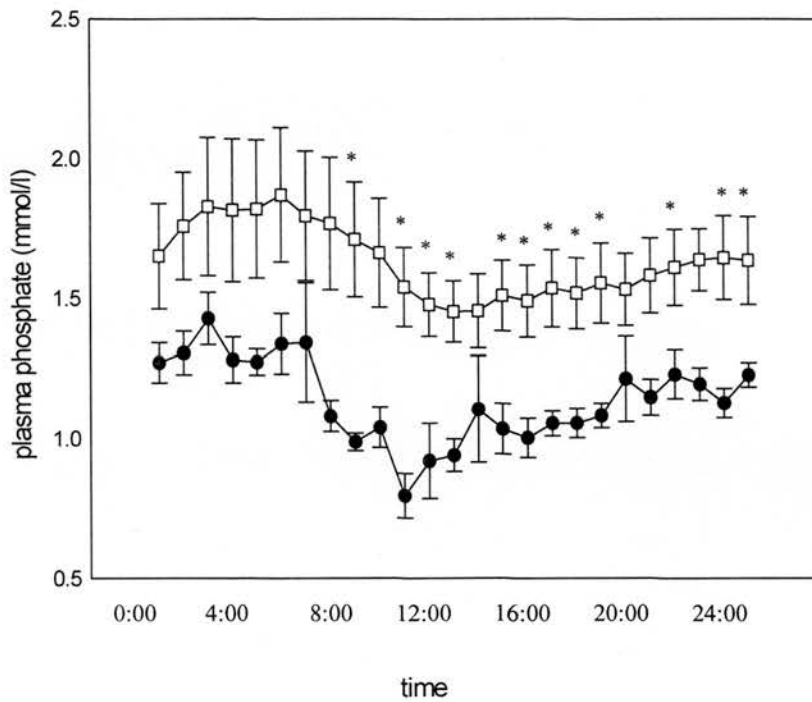


Figure 5.1. Average plasma phosphate concentration (\pm SEM) in normal renal function \bullet and CKD \square . * $p < 0.05$.

The dietary questionnaires filled out by our subjects did not reveal any individual to have excessive phosphate intake. During the study, ingestion of food could not explain the marked variability seen in plasma phosphate. Figure 2.

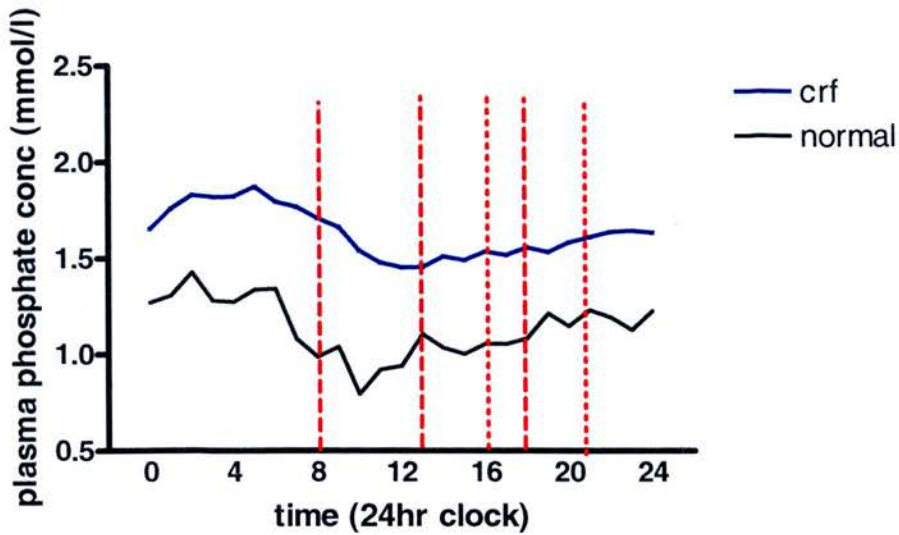


Figure 5.2. 24-hour variation in plasma phosphate with times of meals marked.
 ----- main meals, snacks.

Marked variability in plasma phosphate was seen in all eight subjects studied. In subjects with normal renal function, the variation from the mean was 56% (range 28-85%) and in subjects with CKD, the variation was 17% (range 8-31%). There were no corresponding changes in albumin or potassium to indicate sampling error.

In all subjects, there was a peak in phosphate concentration between 02:00 and 06:00. In two of the subjects, there was an additional peak between 20:00 and 21:00, and again between 12:00 and 13:00.

A nadir in phosphate concentration was seen in all subjects between 08:00 and 12:00. In two subjects, there was a further trough between 14:00 and 15:00. There is an

assumed third trough in these two patients occurring around midnight but this cannot be adequately seen due to its occurrence at the extreme of the data series, Figures 3,4.

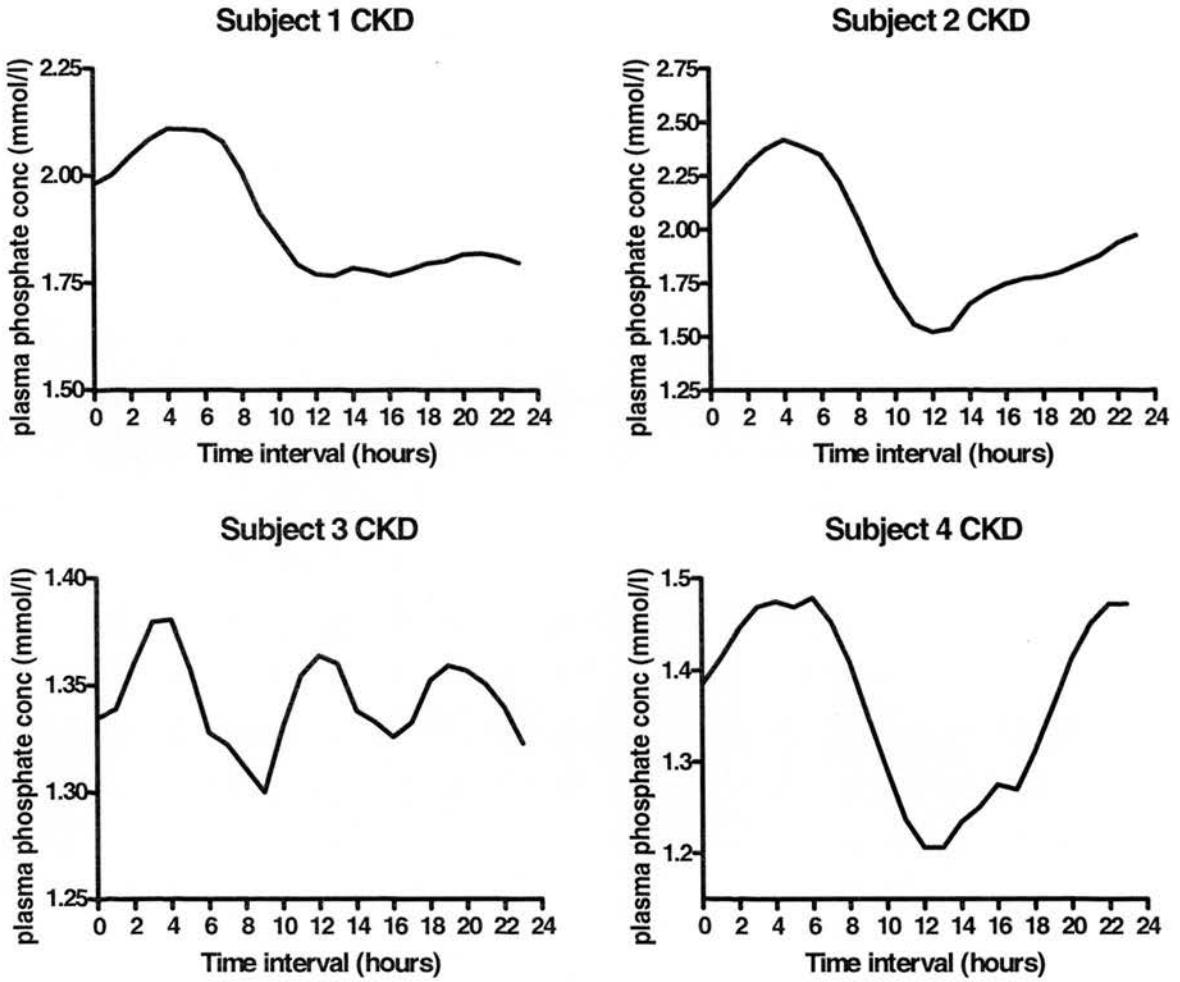


Figure 5.3. 24-hour variation in plasma phosphate in subjects with stage 5 CKD. Data smoothed using weighted averages of nine closest neighbours.

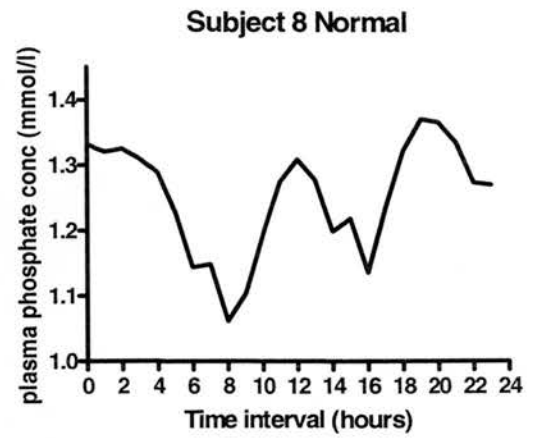
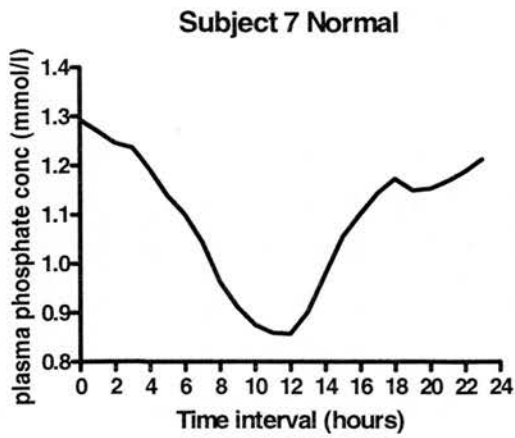
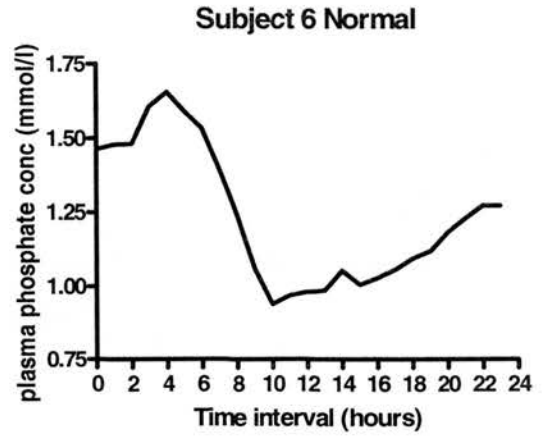
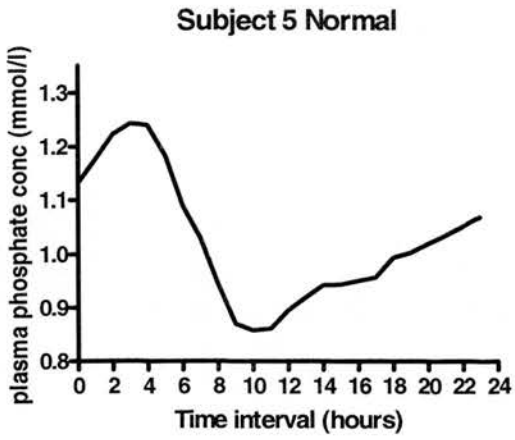


Figure 5.4. 24-hour variation in plasma phosphate in subjects with normal renal function. Data smoothed using weighted averages of nine closest neighbours.

Phosphate Rhythm biometry Table 3

All eight subjects exhibited both circadian and ultradian variation in phosphate concentrations. The additive effects of four harmonic components with periods 24,12,8 and 6 hours could adequately explain each 24-hour profile, ($p<0.001$). The contribution of the four harmonics to the overall waveform in one patient is shown in figures 5 and 6.

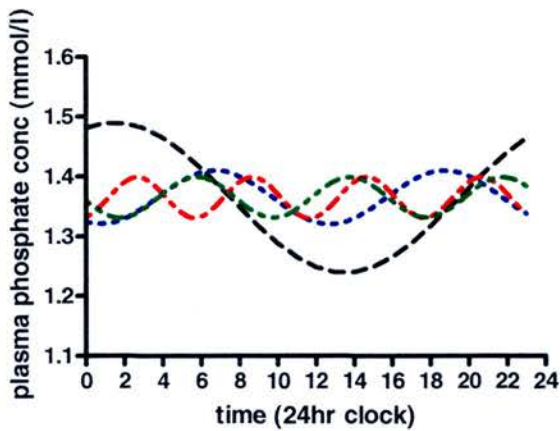


Figure 5.5. Four harmonic components used in cosinor analysis of rhythm.

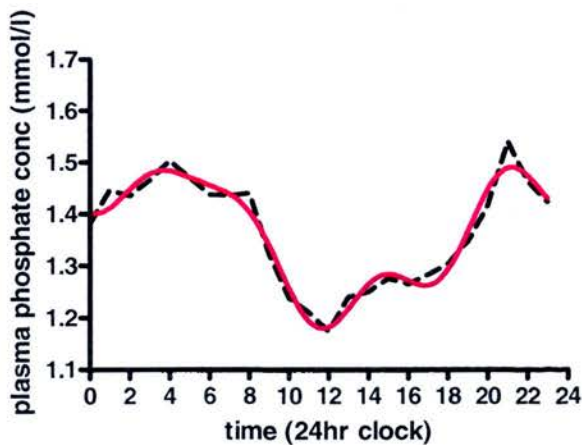


Figure 5.6 Resultant curve when four harmonics are combined compared to raw data set ----- $R^2=0.96$ $p<0.001$

In six patients the amplitude of the 24hr waveform accounted for most of the variation, whilst in the other two subjects (one CKD and one control) the major influence came from the 8-hour waveform. The MESOR in the CKD patients was significantly higher than the control subjects ($p < 0.05$) but the amplitude of the major oscillatory component did not differ. The acrophase of the main oscillatory component in all subjects occurred between 23:40 and 04:40 accounting for the peak in phosphate concentration in the early hours of the morning. The two subjects who demonstrated three peaks in phosphate concentration were the two in whom the 8 hour oscillatory waveform was dominant.

Table 5.3 Summary of rhythm biometry for phosphate

Subject	Status	Overall p	Mesor (mmol)	24hr Harmonic		12hr Harmonic		8hrs Harmonic		6hrs Harmonic		24+12+8+6hrs harmonics R ²				
				Amplitude (mmol)	Acrophase (hours)	R ²	Amplitude (mmol)	Acrophase (hours)	R ²	Amplitude (mmol)	Acrophase (hours)		R ²	Amplitude (mmol)	Acrophase (hours)	R ²
1	CKD	<0.001	1.90±0.00	0.17±0.01	4.66±0.15	0.80	0.07±0.01	5.23±0.19	0.13	0.01±0.01	6.67±1.51	0.001	0.04±0.01	1.92±0.15	0.05	0.98
2	CKD	<0.001	1.93±0.01	0.37±0.02	2.49±0.20	0.75	0.19±0.02	4.45±0.20	0.20	0.04±0.02	7.41±0.45	0.02	0.04±0.02	1.41±0.53	0.007	0.97
3	CKD	<0.001	1.34±0.01	0.01±0.01	23.66±3.79	0.03	0.01±0.01	2.76±1.53	0.05	0.03±0.01	4.00±0.40	0.34	0.01±0.01	0.00±0.64	0.08	0.50
4	CKD	<0.001	1.36±0.00	0.13±0.01	1.52±0.23	0.75	0.04±0.01	6.75±0.33	0.09	0.03±0.01	5.78±0.41	0.05	0.03±0.01	2.68±0.21	0.06	0.96
5	Normal	<0.001	1.02±0.01	0.15±0.01	1.71±0.32	0.65	0.09±0.01	3.87±0.28	0.21	0.03±0.01	4.75±0.57	0.03	0.04±0.01	1.47±0.34	0.04	0.93
6	Normal	<0.001	1.22±0.03	0.30±0.04	2.00±0.48	0.62	0.14±0.04	3.81±0.52	0.13	0.09±0.04	5.17±0.52	0.06	0.08±0.04	0.16±0.45	0.04	0.85
7	Normal	<0.001	1.09±0.01	0.18±0.01	23.66±0.28	0.81	0.07±0.01	4.30±0.38	0.11	0.03±0.01	0.42±0.60	0.01	0.01±0.01	4.73±1.17	0.01	0.94
8	Normal	<0.001	1.25±0.02	0.09±0.02	21.08±1.11	0.21	0.04±0.02	0.88±1.37	0.03	0.11±0.02	3.43±0.35	0.33	0.08±0.02	0.25±0.32	0.16	0.73

Calcium

Only one of the subjects with CKD had plasma total calcium consistently within the normal range. Two patients were hypocalcaemic and one was persistently hypercalcaemic. Despite this, they all showed variability in their calcium concentration with a 5-7% difference in the peak and trough calcium levels. The variability in subjects with normal renal function was more pronounced 7-21% but this was not significantly different from the variability in CKD.

Calcium Rhythm biometry

The pattern of calcium oscillation could be explained by periodic harmonic regression taking into account three oscillations of period 24, 12 and 8 hours, Table 4.

Table 5.4 Summary of rhythm biometry for calcium

Subject	Status	Overall p	Mesor (mmol/l)	24hrs Harmonic			12hr Harmonic			8hr harmonic			3 Harmonics 24hrs+12hrs+8hrs	
				Amplitude (mmol/l)	Acrophase (hours)	R ²	Amplitude (mmol/l)	Acrophase (hours)	R ²	Amplitude (mmol/l)	Acrophase (hours)	R ²	Amplitude (mmol/l)	Acrophase (hours)
1	CKD	<0.001	2.39±0.00	0.05±0.00	15.11±0.37	0.68	0.02±0.00	2.104±0.648	0.08	0.02±0.00	2.86±0.26	0.15	0.89	
2	CKD	<0.001	2.70±0.00	0.04±0.01	9.26±0.73	0.60	0.01±0.01	0.316±1.596	0.03	0.00±0.01	4.30±2.76	0.00	0.63	
3	CKD	<0.001	1.84±0.00	0.04±0.00	13.57±0.32	0.73	0.02±0.00	2.397±0.396	0.11	0.01±0.00	2.38±0.33	0.08	0.92	
4	CKD	<0.001	2.09±0.00	0.01±0.00	1.56±2.40	0.06	0.02±0.05	5.056±0.481	0.40	0.01±0.00	8.67±0.60	0.12	0.58	
5	Normal	<0.001	2.32±0.01	0.04±0.01	1.94±0.94	0.36	0.02±0.01	5.665±1.292	0.04	0.03±0.01	6.95±0.39	0.23	0.63	
6	Normal	<0.001	2.27±0.01	0.02±0.01	23.39±1.95	0.11	0.02±0.01	1.338±1.029	0.09	0.04±0.01	0.43±0.38	0.32	0.52	
7	Normal	<0.001	2.36±0.00	0.02±0.01	0.94±0.95	0.26	0.02±0.01	3.902±0.532	0.21	0.02±0.01	0.77±0.33	0.35	0.72	
8	Normal	<0.001	2.36±0.01	0.01±0.01	19.44±2.49	0.08	0.03±0.01	2.195±0.631	0.32	0.00±0.01	7.95±2.18	0.01	0.41	

There were differences between subjects with renal impairment and those with normal renal function and between male and female patients. The small numbers of subjects studied means that little can be concluded with certainty but the observations are interesting.

Male

The 24-hour component of the periodic regression model in normal male subjects showed an acrophase at 00:45 but in male subjects with CKD this was seen at 12:38. For this component of the model, the peaks and troughs are therefore at opposite sides of the 24-hour cycle. As this is a highly influential component of the model, it results in major differences in the overall oscillations seen. Male subjects with normal renal function have a trough calcium concentration at around midday while male subjects with CKD show a peak at this time, Figure 7.

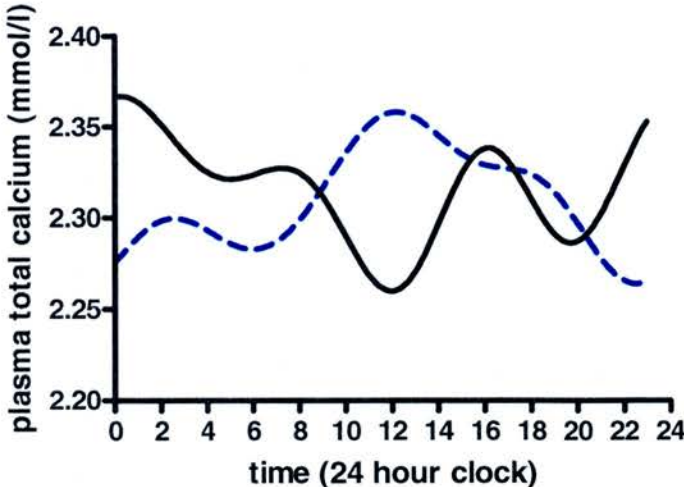


Figure 5.7. Calcium variation in 3 normal male patients — and 3 CKD male patients -----.

Female

In the female patients, the 24-hour component of the model was less influential with most variation coming from the 12-hour component. There was a three-hour difference in acrophase of the 12 hour harmonic, which is reflected, in a 3-hour difference in the occurrence of peak and trough calcium concentration in the two female patients, Figure 8.

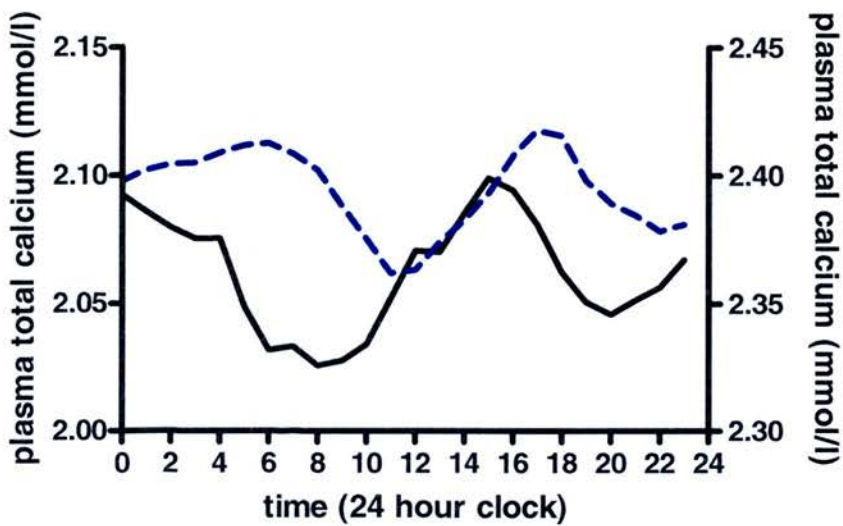


Figure 5.8. Calcium variation in one normal female subject — and one female with CKD ---

Bicarbonate

Despite two of the CKD subjects receiving oral bicarbonate supplementation, the bicarbonate levels in the CKD group were significantly lower over the 24 hours. (17.7 ± 0.6 mmol/l vs. 24.7 ± 0.4 mmol/l). Bicarbonate levels were seen to follow a circadian pattern with an acrophase of 16:34 and amplitude of 1.04 in the subjects with normal renal function and an acrophase of 01:57 and amplitude of 1.75 in subjects with CKD. Bicarbonate is therefore varying over a wider range and is high during the night in CKD. In contrary bicarbonate is varying over a much narrower range and is high during the day in normal renal function, Figure 9.

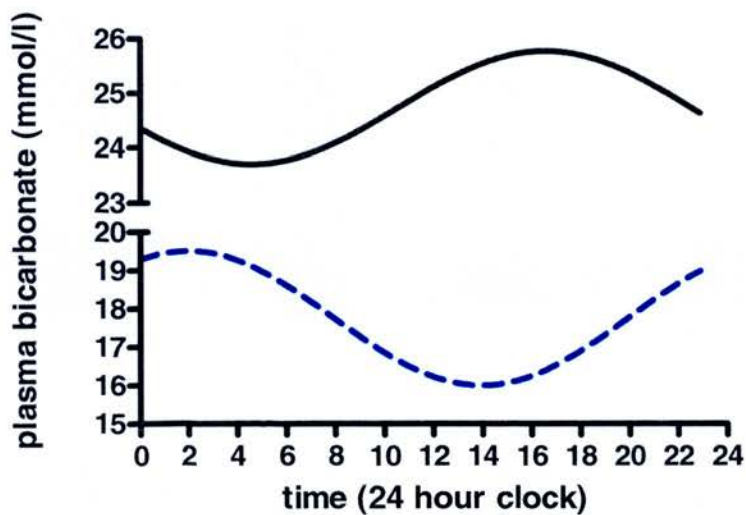


Figure 5.9. Plasma bicarbonate in normal — and CKD -----

PTH

The subjects with CKD all had significant hyperparathyroidism with an average PTH over 24hrs of 38.3 ± 6.0 pmol/l compared to the normal subjects 4.4 ± 0.2 pmol/l. In subject 2, this was associated with an elevated plasma total calcium while in the other three CKD subjects the plasma calcium was normal or low. The amplitude of variation in the subjects with CKD was much higher than in those with normal renal function with a variation from the mean of 42% in normal subjects and 100% in CKD.

PTH Rhythm biometry Table 5

Seven of the eight subjects showed three distinct peaks in the PTH level while the eighth showed four. The variation showed a circadian element with an acrophase at night (range 17:32 - 06:55). All subjects showed a 12-hour periodic variation with an acrophase during the night (range 22:53 - 08:22). Seven subjects showed an additional harmonic with period 8 hours while in the eighth subject this period was 6 hours. The additive effect of these waveforms was a peak in PTH concentration in the early hours of the morning that was of greater amplitude in the subjects with CKD. This was followed by a nadir between 08:00 and 10:00. A further peak occurred in all subjects between 18:00 and 21:00 followed by a trough between 23:00 and 01:00. Smaller fluctuations during the day that varied between subjects could be explained by the lesser harmonic periods.

Table 5.5 Summary of rhythm biometry for PTH

Subject	Status	Overall p	Mesor (pmol/l)	24hrs Harmonic		12hr Harmonic		8hr harmonic		3 Harmonics 24hrs+12hrs+8hrs			
				Amplitude (pmol/l)	Acrophase (hours)	R ²	Amplitude (pmol/l)	Acrophase (hours)	R ²	Amplitude (pmol/l)	Acrophase (hours)	R ²	R ²
1	CKD	<0.001	9.35±0.28	0.24±0.39	6.92±6.342	0.004	2.84±0.39	4.30±0.26	0.66	1.27±0.39	2.29±0.39	0.13	0.79
2	CKD	<0.001	36.98±0.55	4.59±0.78	22.36±0.65	0.32	4.77±0.78	4.38±0.65	0.34	3.55±0.78	2.86±0.28	0.18	0.84
3	CKD	<0.001	47.25±0.46	7.61±0.65	17.75±0.32	0.75	2.72±0.32	22.89±0.45	0.09	2.28±0.65	7.57±0.36	0.07	0.91
4	CKD	<0.001	60.85±1.04	13.0±1.47	20.87±0.43	0.56	1.84±1.48	4.52±1.53	0.01	9.65±1.48	3.60±0.19	0.31	0.88
5	Normal	<0.001	4.41±0.11	1.34±0.15	19.23±0.43	0.65	0.44±0.15	1.08±0.64	0.07	0.64±0.15	2.54±0.30	0.14	0.86
6	Normal	<0.001	3.94±0.01	0.30±0.11	2.29±1.36	0.20	0.13±0.11	7.05±1.66	0.03	0.40±0.11	6.37±0.25	0.35	0.58
7	Normal	<0.001	4.69±0.09	0.69±0.12	17.54±0.67	0.50	0.21±0.12	8.37±1.12	0.05	0.43±0.12	3.11±0.36	0.19	0.74
8	Normal	<0.001	4.39±0.04	0.52±0.05	0.11±0.37	0.66	0.30±0.05	7.10±0.32	0.21	0.09±0.05	7.37±0.68	0.03	0.90

6 hour period

Insulin and glucose

No difference was seen in insulin levels between the two groups, but plasma glucose was significantly higher in CKD; $p < 0.05$. Insulin levels began to rise in all individuals immediately after ingestion of food and peaked within one hour. Levels decayed back to baseline over several hours. Parallel changes in plasma glucose were seen in all subjects. No diurnal rhythm was detected for insulin or glucose.

Growth hormone

All four subjects with CKD had GH levels above the normal range. Notably one of the male patients with normal renal function also had elevated GH levels with no clinically apparent effect at the time of the study. Three of the subjects with CKD and two with normal renal function including the subject with elevated GH levels showed the expected rise in GH after the onset of sleep. The other three subjects (2 normal, 1 CKD) showed a peak at 08:00. In five subjects (3 normal, 2 CKD) the pattern of GH fluctuation showed an additional peak at 18:00-19:00. Trough concentrations were seen at around 10:00 in all subjects. It was not possible to fit the GH data by periodic harmonic regression due to the apparent pulsatile nature of hormone release.

Cortisol

The mean cortisol level over 24hrs as assessed by both the average value (371.4 ± 42.7 nmol/l vs. 303.6 ± 38.8 nmol/l) and the MESOR (358.5 ± 48.6 nmol/l vs. 286.5 ± 29.2 nmol/l) was higher in CKD than in normal subjects. Both groups exhibited circadian rhythm with an acrophase of 08:40 in the subjects with normal renal function and 10:36 in those with CKD. The morning peak in cortisol was of the same amplitude (452 nmol/l in normal subjects vs. 450 nmol/l in CKD). Subjects additionally showed ultradian variation in cortisol levels with two subjects in each group exhibiting eight-hour periodicity and two exhibiting twelve-hour periodicity.

Phosphate excretion

The urinary phosphate clearance rate differed between the two groups resulting in the mass of phosphate removed being significantly higher in normal renal function (31.4 mmol vs. 15.7 mmol, $p=0.01$). The volume of urine passed was not significantly different $p=0.66$.

In three subjects with CKD, phosphate excretion was low during the night then increased around 10:00 before falling to low levels again in the afternoon and evening. The fourth subject with CKD and all the subjects with normal renal function showed the same pattern but, in addition, had a further rise in phosphate excretion in the afternoon around 15:00.

Cross-correlation analysis

The cross-correlation analysis was used to highlight differences between normal renal function and CKD but was not intended to prove cause and effect relationships.

Phosphate/Calcium

In patients with normal renal function there was a concurrent rise in calcium and phosphate at time zero $r=0.61$, $p<0.05$. In CKD the association was significant but negative $r=-0.52$; $p<0.05$. There was further correlation when the calcium series was lagged by five hours which was negative in normal renal function and positive in CKD, Figure 10.

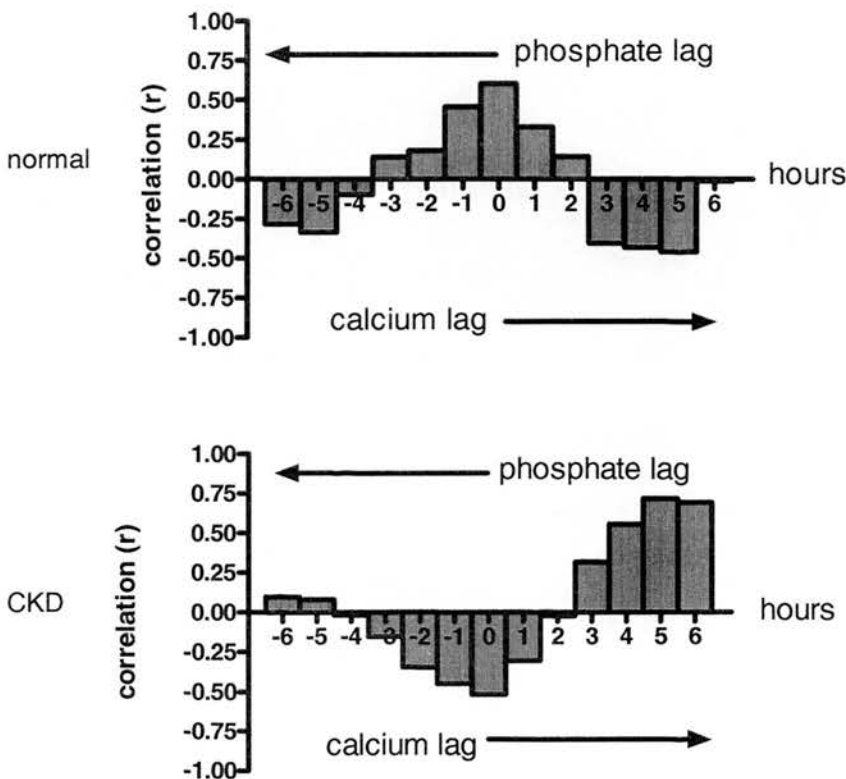


Figure 5.10 Phosphate/Calcium cross-correlation analysis in normal renal function and CKD

Phosphate /PTH

The same pattern of bi-directional correlation is seen in CKD and in normal subjects. In normal renal function the closest correlation is seen when the PTH series is lagged by five hours ($r=-0.61$ $p<0.05$) and there is positive correlation when the phosphate series is lagged by one hour ($r=0.44$ $p<0.05$). A very similar pattern is seen in CKD with maximal correlation when the PTH series is lagged by 4 hours ($r=-0.72$ $p<0.01$) and further correlation when the phosphate series is lagged by 2 hours ($r=-0.48$ $p<0.05$). This indicates that reciprocal changes occur in PTH and phosphate concentrations that are bi-directional in nature, Figure 11.

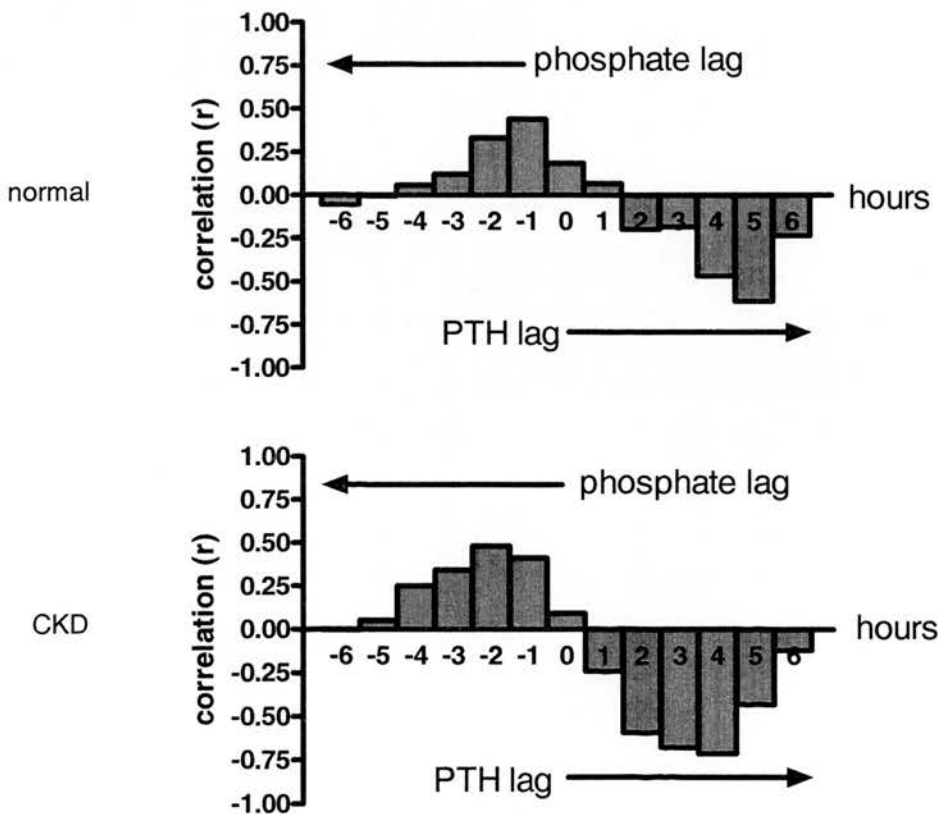


Figure 5.11 Phosphate/PTH cross-correlation analysis in normal renal function and CKD

Phosphate/cortisol

Essentially the same pattern was seen in CKD and normal renal function. When the phosphate series was lagged by 1-2 hours there was a negative association between cortisol and phosphate ($r=-0.69$ $p<0.01$ normal; $r=-0.71$ $p<0.01$ CKD). When the cortisol series was lagged there was positive correlation at three hours $r=0.63$ $p<0.05$ normal; $r=0.58$ $p<0.05$ CKD). This indicates that a rise in phosphate precedes a fall in cortisol by 1-2 hours with a corresponding fall in phosphate three hours later, Figure 12.

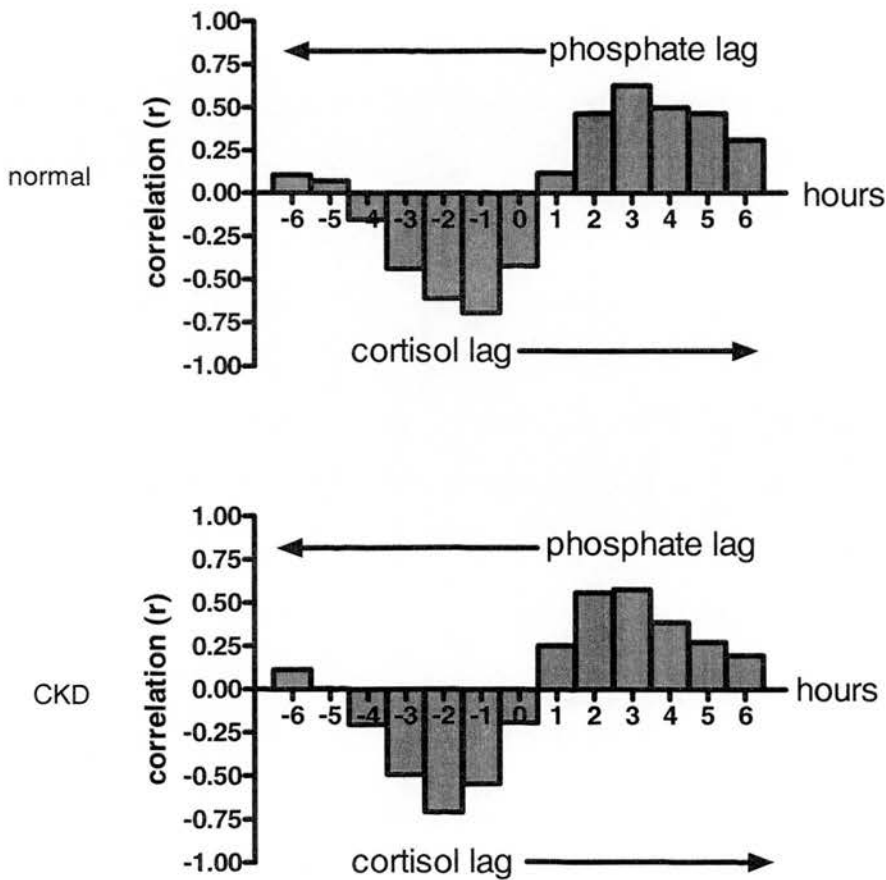


Figure 5.12 Phosphate/cortisol cross-correlation analysis in normal renal function and CKD

PO₄/Bicarbonate

In normal subjects, there is a negative association between bicarbonate and phosphate levels when the bicarbonate series is lagged by two hours ($r=-0.73$ $p<0.01$) and a positive association when the phosphate series is lagged by 3 hours ($r=0.54$ $p<0.05$). In CKD bicarbonate and phosphate change in the same direction with closest correlation when the phosphate series is lagged by 1 hour ($r=0.76$ $p<0.01$), and further correlation when the bicarbonate series is lagged by five hours ($r=-0.48$ $p<0.05$). The patterns of correlation are therefore similar but phase shifted by 2-3 hours.

Phosphate/insulin

In CKD significant negative correlation is seen at concurrent time points with an improvement in the correlation when the insulin series is lagged by 1 hour $r=-0.60$ $p<0.05$. In normal renal function significant negative correlation is seen when the phosphate series is lagged by five hours $r=-0.67$ $p<0.05$

No consistent relationships were seen in CKD in the cross-correlations of phosphate with glucose, albumin, or GH. In normal renal function, there is significant correlation between phosphate and GH when the GH series is lagged by one hour.

In summary, opposite relationships are seen between phosphate and calcium in normal renal function and CKD, Figure 10, while the relationships between

phosphate and PTH and phosphate and cortisol appear to be very similar, Figures 11,12. For bicarbonate and insulin, the cross-correlations are different in the two groups and may represent a phase shift in the relationship. Changes in phosphate and growth hormone are significantly correlated in normal renal function but not in CKD.

5.5 Discussion

Circadian rhythm in phosphate concentration in normal subjects is detailed in several publications^{67;68;91;92} but has not been well documented in CKD. Our data confirms the presence of marked circadian and ultradian variation in plasma phosphate in all subjects studied, and shows that variation in calcium, bicarbonate, PTH, cortisol and urinary phosphate excretion is also present in CKD.

It was somewhat surprising to find that ingestion of food had a limited effect on plasma phosphate levels in these subjects, but this is consistent with the one previous study that attempted to address this question⁷⁷. The influence of the diurnal variation in hour-to-hour changes appears to be far greater than the impact of dietary intake, which presumably has a more long-term effect on total body phosphate. Without further study, it is impossible to say what the effect of phosphate binders on diurnal variation might be.

In a previous study of glomerular filtration rate in normal individuals a circadian rhythm was shown with lowest levels during the night⁹³, corresponding to the lowest levels of phosphate excretion in this study. While GFR was not measured as part of this study, it could be speculated that the diurnal variation in phosphate is explained by alterations in glomerular filtration rate. However, this cannot be the entire

explanation, as there is insufficient total excretion of phosphate during the 24-hour period to account for the very marked changes in plasma phosphate concentration.

The presence of diurnal variation in both PTH and cortisol and the existence of similar cross-correlation with phosphate in both study groups makes these hormones likely candidates in the determination of diurnal variation. The absence of correlation with GH in CKD suggests that this hormone is perhaps less important. Both PTH and cortisol⁸² are known to be phosphaturic but the higher prevailing hormone levels in the presence of lower phosphate excretion in CKD implies a degree of target organ unresponsiveness. Additional mechanisms operating either at cellular level or via effects on bone reabsorption are likely to be important in the maintenance of the diurnal rhythm.

Acid-base balance is clearly abnormal in CKD. In normal subjects plasma bicarbonate is higher during the day when presumably the generation of metabolic waste and therefore potential for acidosis is highest. It is possible that compensatory buffer mechanisms fail in CKD, resulting in the observed acidosis during the day.

The different patterns in the diurnal variation in calcium between the subjects with CKD and normal renal function are reflected in the different relationship seen between calcium and phosphate in the two groups. As it is the ionized calcium that is biologically active, the observed differences in the acrophase of the circadian rhythm data for calcium may be as a result of changes in ionized calcium due to acidosis.

The marked differences in the diurnal variation in bicarbonate concentration and in calcium homeostasis between the two study groups in the presence of very similar

patterns for the diurnal variation in phosphate, suggests that calcium and acid-base status are not immediately implicated in the diurnal variation in phosphate.

A difference in calcium homeostasis in males and females has been previously described⁹⁴ and a loss of the nocturnal rise in PO_4 and PTH is used as a possible explanation for the increased bone loss experienced by post-menopausal females⁹⁵. This loss of variation was not demonstrated in the two post-menopausal women, (subjects 4 and 8) in this study, but they did appear to exhibit different calcium kinetics from the men. If this finding were confirmed in a larger data set it may be representative of differences in bone metabolism.

While no definitive explanation can be given for the observed diurnal variation in phosphate, it is very clear that it is maintained in all subjects studied. The conflicting results from the three previous studies looking at diurnal variation in haemodialysis patients may have arisen for a number of reasons. The results of the study that demonstrated diurnal variation in six out of nine subjects are in partial agreement with these results⁷⁶. The data from three of the six patients was fitted to a combined linear and sinus function but no function was proposed for the other three. The linear component of that model may be appropriate in the presence of anuria but the level of residual function was not detailed for individual patients. It is also possible that varying degrees of hyperparathyroidism or doses of phosphate binders were implicated in the different patterns seen, but again the individual detail was not published.

The second study failed to show diurnal variation in haemodialysis patients during incidental hospitalization. However, the timing of the samples may have missed significant peaks and troughs, an acknowledgement that is made by the authors themselves⁷⁷, and again no detail regarding residual renal function is available.

The third study observed that patients on afternoon and evening dialysis shifts have higher phosphate concentrations⁷⁸, a finding that would be consistent with the presence of diurnal variation, although the timing is not fully consistent with data presented here.

Secondary hyperparathyroidism is known to occur early in the course of CKD and much therapeutic focus is centered on the use of phosphate binders. In the pre-dialysis phase when deciding to commence phosphate binders or when monitoring the response to therapy, the timing of the blood sample should be considered.

Patients having venesection in the mid to late morning are more likely to have favourable plasma phosphate levels compared to those venesected very early in the morning or later in the afternoon, a factor that, at present, is not taken into account.

Differences in phosphate concentration at the beginning of a dialysis session may have implications for haemodialysis patients as higher concentration gradients across the dialysis membrane theoretically could increase mass removal of phosphate in the early part of dialysis. It is possible to render nocturnal dialysis patients, but not those on daily dialysis, normo- or hypophosphataemic⁹⁶ and, while the effect of duration of dialysis on the multi-compartmental distribution of phosphate is likely to be the most

important factor, these patients are also being dialysed at the peak of their phosphate concentration.

5.6 Conclusion

This study demonstrates the presence of diurnal variation in phosphate and regulating hormones in advanced renal failure that is similar in nature to that seen in normal renal function. Additionally it shows that there is diurnal variation in calcium and bicarbonate in a pattern that differs to that seen in normal renal function. The results presented were obtained in subjects with advanced non-oliguric renal failure. It seems reasonable to assume that while haemodialysis patients maintain a degree of residual renal function, the same diurnal variations will exist. It is unknown whether the pattern will continue to be observed in oliguric long-term haemodialysis patients.

Chapter 6

Phosphate Kinetics in Chronic Haemodialysis

Phosphate Kinetics in Chronic Haemodialysis

6.1 Background

Hyperphosphataemia is a well-recognised complication of chronic renal failure and is the source of much concern and controversy. It is becoming increasingly apparent that hyperphosphataemia per se is an independent risk factor for mortality in the dialysis population even when apparently adequate dialysis is being delivered. Chronic hyperphosphataemia is important in the pathogenesis of secondary hyperparathyroidism and its treatment with calcium containing phosphate binders may also be a factor in the progressive calcific vascular disease, which occurs in dialysed patients and is a potential contributant to the increased incidence of premature cardiovascular death in this population^{45;46}. It has been shown that a pre-dialysis serum phosphate concentration of $>2.1\text{mmol/l}$ is associated with increased mortality^{42;97}. In recognition of such factors, the British Renal Association targets a pre-haemodialysis serum phosphate of $<1.8\text{mmol/l}$ ²⁹. There is however no firm evidence that reducing pre-haemodialysis phosphate levels to this degree is beneficial, and only about 60% of British haemodialysis patients achieve this target⁹⁸.

It is useful to consider the control of phosphate under normal circumstances, as it is then easier to understand why hyperphosphataemia is such a problem in chronic renal failure.

Phosphate is one of the most widely distributed cations in the body. In total there is around 700-800g in an adult body. Most of this is in the bones with around 14% in the intracellular space. Only a small amount is present as free inorganic phosphate and this is in equilibrium with extracellular inorganic phosphate. It is this 1% of total phosphate that is measured in blood samples. At physiological pH, inorganic phosphate exists in equilibrium between the monovalent and divalent forms. HPO_4^{2-} represents 80%. The majority of phosphate is bound up in organic compounds and phosphate is involved in many vital structural and metabolic cellular processes. These include energy metabolism, mitochondrial function and delivery of oxygen to tissues via 2,3 DPG. Most importantly phosphorous is the source of high-energy bonds in ATP.

Under normal circumstances, a balance between intestinal absorption and renal excretion maintains phosphate levels. Bone resorption at steady state is in equilibrium with bone formation and thus plays a minor role in phosphate homeostasis.

Intestinal absorption is a combination of active and passive processes and is essentially unchanged in CKD, Figure 1. Intestinal uptake in normal subjects can be seen to be a combination of a fast initial phase occurring over an hour followed by a slower phase up to 7 hours. In CKD, the fast initial phase is deficient but overall phosphate uptake assessed by area under the curve is increased.

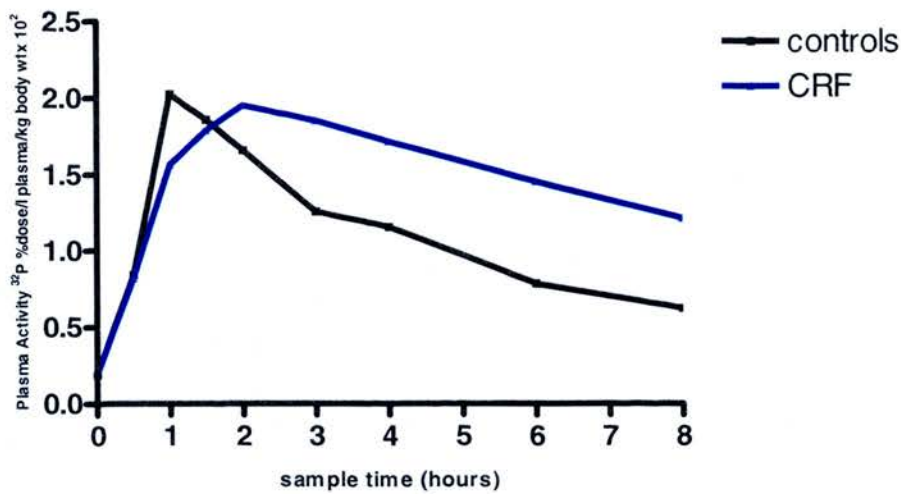


Figure 6.1 Uptake of labelled ^{32}P in controls and in CKD⁹⁹

In normal renal function, phosphate control at steady state is almost entirely under renal control with 85-90% of the filtered load being reabsorbed via a sodium-phosphate co-transporter mainly in the proximal tubule. This transporter can be influenced by changes in dietary phosphate, PTH levels, and 1,25(OH) vitamin D3.

There are many theories about the relationship between phosphate, calcium, vitamin D and PTH in chronic renal failure. The “trade-off” theory, although probably an oversimplification, suggests that in the early stages of renal disease there is a reduction in the renal excretion of phosphate. This invokes a rise in the PTH level and thus a correction of the PO_4 concentration. As the GFR decreases <20-30ml/min this mechanism is no longer sufficient and PO_4 rises while the PTH remains high. As GFR decreases further to <10ml/min hyperphosphataemia becomes virtually ubiquitous. Although this probably an oversimplification of the mechanism the observed changes in patients with CKD support the mechanism with phosphate and

PTH increasing dramatically as GFR falls with virtually no change in serum calcium levels.

There are three main ways of controlling the plasma phosphate.

1. Dietary restriction
2. Use of phosphate binders
3. Increased removal by dialysis

A normal daily Western diet contains around 25-65 mmol of phosphate¹⁰⁰. It is difficult to reduce this to less than approximately 17-35 mmol due to the need to maintain an adequate protein intake of 1.0-1.2g/kg/day. Around 40-80% of ingested phosphate is absorbed mainly in the jejunum necessitating the removal of 48-196 mmol per week. Haemodialysis typically removes 20-40mmol of phosphate per session, and CAPD removes approximately 10-12mmol/day. Thus, with current dialytic schedules virtually all dialysis patients find themselves in positive phosphate balance.

Phosphate binders in all their guises are suboptimal in effect and tolerability. As an example 1g of elemental calcium in the form of calcium carbonate is required to neutralize 1.4 mmol PO₄; thus in a patient with a daily excess of 14mmol of phosphate, 10 tablets of calcium carbonate would be required which presents both an unacceptably high calcium load and is poorly tolerated by the patient.

Research into phosphate control has primarily focussed on haemodialysis and has consistently highlighted some major observations. It is clear that the kinetics of phosphate are complex and can not be explained in terms of the traditional two-pool model as applied to urea. Secondly, it has been repeatedly demonstrated that phosphate falls very rapidly in the first 30-90 minutes of dialysis and plateaus thereafter. Thirdly, it is clear that in the post-dialysis period there is a significant and rapid rebound of phosphate such that by four hours post-dialysis phosphate levels are equivalent to the pre-dialysis level.

This study investigated intradialytic phosphate kinetics through mathematical modelling and explored the potential physiological processes involved.

6.2. Subjects And Methods

The data utilised in the mathematical modelling process were generated during a study on urea kinetics carried out by Tattersall et al at the Lister Hospital⁶⁴.

Patients

Twenty-nine stable haemodialysis patients (26 male, 3 female) were studied. Their median age was 54 years (19-81 years). They had been stable on haemodialysis for at least three months. All patients dialysed via an arteriovenous fistula and the absence of access recirculation was confirmed by a saline dilution method with a sensitivity of 5%¹⁰¹. Residual renal solute clearance (KRU) varied from 0 to 4.0 ml/min (mean 1.2 ± 0.3) but was disregarded for the purposes of the study. Patients who were

severely malnourished (normalised protein catabolic rate (nPCR) <0.7 and albumin <35 g/l) or hospitalised with infection or heart failure were excluded.

Dialysis Techniques

Haemodialysis (HD) treatments were performed using a Fresenius 2008D haemodialysis machine, bicarbonate dialysis fluid and polysulphone dialysers (Fresenius HF80 or HF60). Blood flow rates (Q_b) were 253 to 545 ml/min and dialysate flow rates (Q_d) were 500 to 800 ml/min. The same equipment was used for haemodiafiltration (HDF) but 100 to 120ml/min filtration was performed simultaneously. Replacement fluid was generated by filtration of the dialysate using the Fresenius on-line HDF system.

Study Protocol

Patients were studied during a conventional haemodialysis (mean 240.7 ± 5.3 min) and a short haemodiafiltration (mean 147.3 ± 6.5 min) on the same day of two consecutive weeks. Dialysis prescriptions were adjusted to achieve a desired two-pool Kt/V (urea) of 1.0 where K is the dialyser clearance rate, t is the duration of the dialysis session and V is the urea distribution volume or total body water volume (TBW) estimated from the Watson formula³³. The duration of dialysis required to achieve the desired Kt/V was calculated from the equation

$$t = \text{desired } Kt/V \times \left(\frac{V}{K} + tp \right)$$

where t_p compensates for urea rebound in the post-dialytic period in accordance with two-pool kinetics. In all treatments t_p was set to 35 minutes as described previously⁶⁴.

Sampling and assay techniques

Blood samples were taken from the arterial needle before the start of HD/HDF, from the arterial line at six equally spaced time intervals during HD/HDF and at the end of the treatment without slowing the blood pump. Further samples were taken at 2, 15, 30 and 60 minutes post-dialysis from the fistula needle. The washback was performed after the two-minute sample. The ultrafiltration rate (Q_u) was calculated from pre- and post-dialysis weight. Dialyser clearance was calculated approximately twenty minutes after the start of HD/HDF from dialyser inlet and outlet concentration measurements, taking haematocrit into account. Concentrations of urea, creatinine, phosphate and bicarbonate were measured.

Data Analysis

- i. Statistical Analysis. Statistical analysis was carried out using the Student t-test for paired samples and linear correlation as appropriate. A p value <0.05 denoted the presence of a statistically significant difference. Mean values are quoted with 95% confidence intervals unless otherwise stated.
- ii. Mathematical modelling. Models were implemented using VisSimTM (Visual Solutions Inc.) modelling software. The measured dialyser clearance ($K_{d\text{ meas}}$), Q_b , Q_d , dialysis time (T_d), sex, pre- and post-dialysis weights were provided as inputs to the model.

Urea Model

The two-pool model of urea kinetics was used as the starting point for the phosphate model, Figure 2.

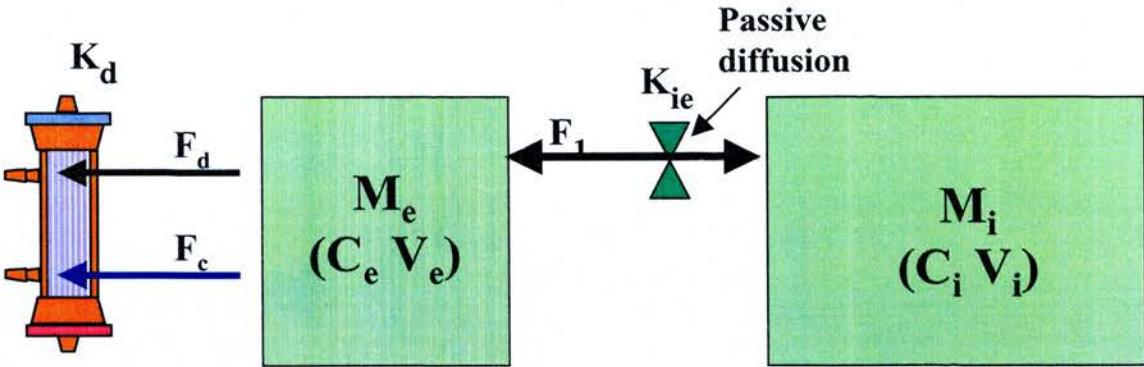


Figure 6.2. Model A. Schematic representation of two-pool kinetics. Abbreviations are: M_e , extracellular mass of solute; C_e , extracellular concentration of solute; V_e , extracellular volume; M_i , intracellular mass of solute; C_i , intracellular concentration of solute; V_i , intracellular volume.

The dialyser clearance of solute (K_d) induces diffusive flux of solute (F_d) from the extracellular space and introduces disequilibrium between the intracellular and extracellular compartments. Solute then diffuses freely down the concentration gradient (F_1) at a rate determined by the intracellular to extracellular transfer coefficient (K_{ie}). In addition, the ultrafiltration process adds convective flux of solute (F_c). Total body water volume (V) was calculated according to the Watson formula³³ with an assumed intracellular to extracellular volume ratio of 2:1. The mass transfer coefficient (K_{ie}) between the intracellular and extracellular spaces was manipulated

to achieve the best fit with the two-pool model. Where adjustments to the total body water were required, the intracellular and extracellular volumes were reduced in equal proportions, as this was considered more physiologically representative of the effects of peripheral compartmentalisation, and thus effective solute distribution volume. An element of cardiopulmonary recirculation (CPR) was included in the design of the model.

Phosphate Models

Since the kinetics of urea is more straightforward, this marker was used as the reference for establishing the compartment volumes, intracellular to extracellular ratio and cardiopulmonary recirculation effects. Phosphate was assumed to be distributed in the same spaces and subject to the same basic factors influencing urea kinetics. The initial conditions were set to the prevailing phosphate concentration at the start of simulation.

Model A

This was identical to the two-pool kinetic system as described for urea kinetics, Figure 1.

Model B1

The established elements from model A were implemented, and a regulatory mechanism involving a third pool was added. This regulatory mechanism endeavoured to maintain a pre-determined target phosphate concentration that was initially inferred by inspection of the nature of the rebound period and subsequently

optimised. Initially it was assumed that phosphate would enter the intracellular environment from an internal third pool and that the intracellular to extracellular transfer coefficient for phosphate ($K_{ic}PO_4$) would remain constant throughout the treatment. Using this variant of the model, the fit with the data was particularly poor and this approach was therefore abandoned.

Model B2

In a second approach, additional phosphate flux into the extracellular space was explored. In this model, phosphate was dialysed initially according to two-pool kinetics. A regulatory system was invoked releasing phosphate from a third pool into the extracellular space limiting the fall of phosphate levels below an intrinsic target concentration. No assumptions were made regarding the source of the additional phosphate. The phosphate generation process was assumed to be proportional to the difference between the momentary extracellular phosphate concentration and the intrinsic target concentration. The constant of proportionality or “gain” determined the magnitude of the rate of phosphate generation. Values for the intrinsic target concentration and the gain were optimised manually by direct inspection of the data and comparison of cumulative standard deviation measurements. The rate of removal by the dialysis process was matched by concomitant liberation of phosphate via the regulatory process, thus maintaining phosphate concentrations in accordance with the data. It is probable that intracellular phosphate is protected since phosphate plays a key role in all energy dependant processes within the cell. Intracellular phosphate concentrations are not readily measured so the extracellular phosphate concentration was used as a surrogate of the intracellular concentration. The mass transfer of

phosphate across the cell membrane was assumed to be a bi-directional process thus enabling the intracellular phosphate concentration to re-establish equilibrium with the extracellular space in the post-dialysis period. A schematic representation of the model is shown in figure 3. One representative treatment is shown in figure 4.

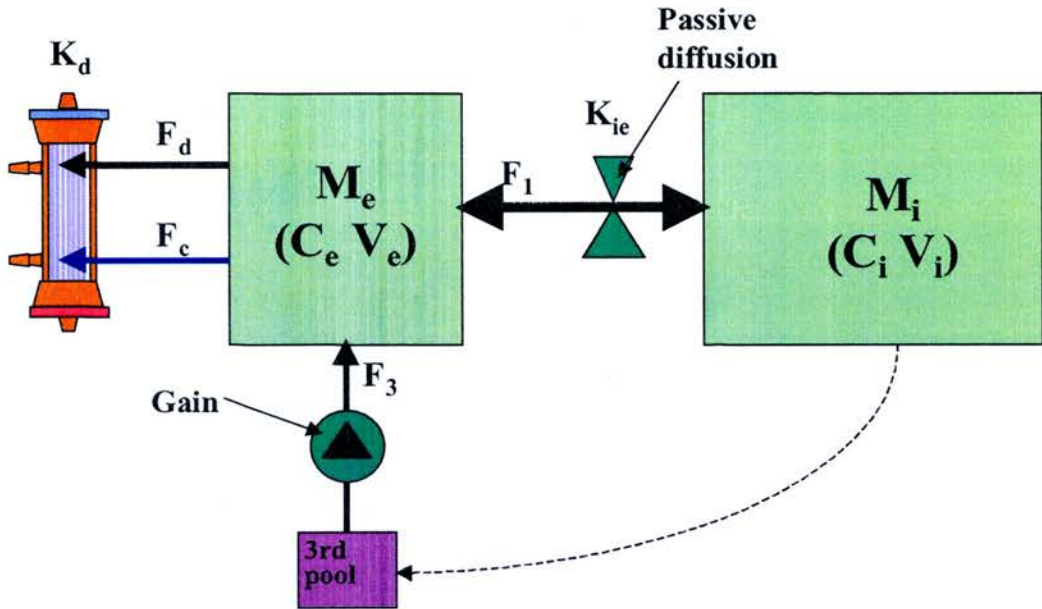


Figure 6.3. Model B. Schematic representation of three-pool kinetics. The two pool kinetic model of model A (figure 1) forms the basis of this model with additional phosphate flux (F_3) into the extracellular space from a third pool. This occurs in proportion to the phosphate error (Σ) or the difference between the momentary intracellular phosphate concentration and an intrinsic intracellular phosphate target concentration. The magnitude of phosphate generation is dependent on a constant factor termed the gain. Abbreviations are as for figure 2.

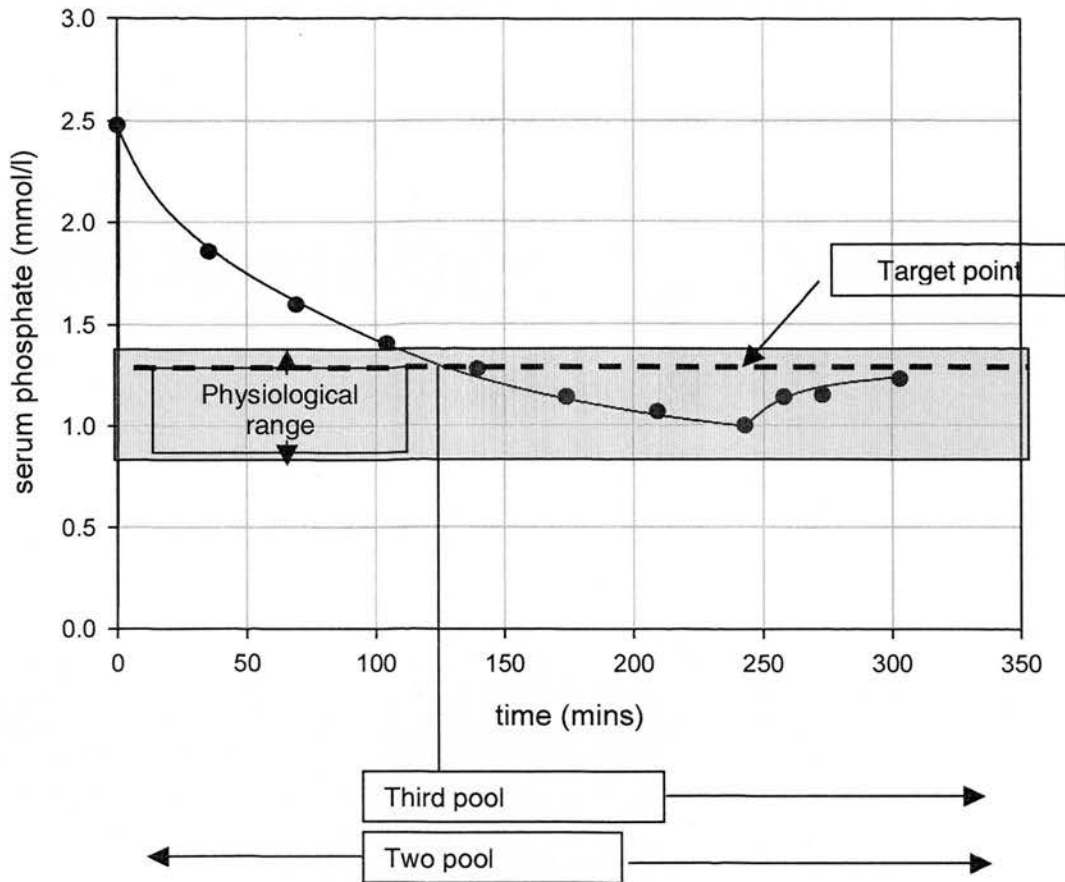


Figure 6.4. Representative treatment exhibiting features of model B. Two-pool kinetics are operational for the duration of dialysis. When the prevailing phosphate concentration falls below the target point phosphate is released from the third pool.

Model C

This model implemented all the elements of model B with the further introduction of a fourth pool and a hysteresis element. In addition to the phosphate that is released to maintain a target intracellular concentration, it was postulated that there must be a critically low intracellular phosphate concentration, below which energy dependant processes are compromised and a life-threatening situation exists. This model considers an additional mechanism that is invoked when phosphate falls below this

critical limit. Under this condition, phosphate is liberated immediately at a fixed rate F_4 , from a fourth pool contained within the intracellular environment. A more sophisticated mechanism is beyond limits of that which could be deduced from the data available.

The hysteresis element has the properties

If $C_i < C_{iCrit}$ AND F_4 off, THEN F_4 on

If $C_i > C_{iHighLimit}$ AND F_4 on THEN F_4 off

The switching point of the hysteresis element was inferred from the observed discontinuity that could be observed in the time course of phosphate variation as shown in figure 5.

When the intracellular phosphate concentration falls to the critically low level the hysteresis element results in a rapid switching on of the generation process with phosphate generated from a fourth pool local to the intracellular space. The hysteresis element ensures liberation of phosphate over a limited concentration band, which prevents oscillations about the critically low limit. Once intracellular phosphate concentrations are restored, normal proportional control applies as described for model B. The critically low limit may be encountered several times during the course of a treatment as shown in figure 6. This is particularly likely to occur when the pre-dialysis serum phosphate concentration is low. The model is represented schematically in figure 7.

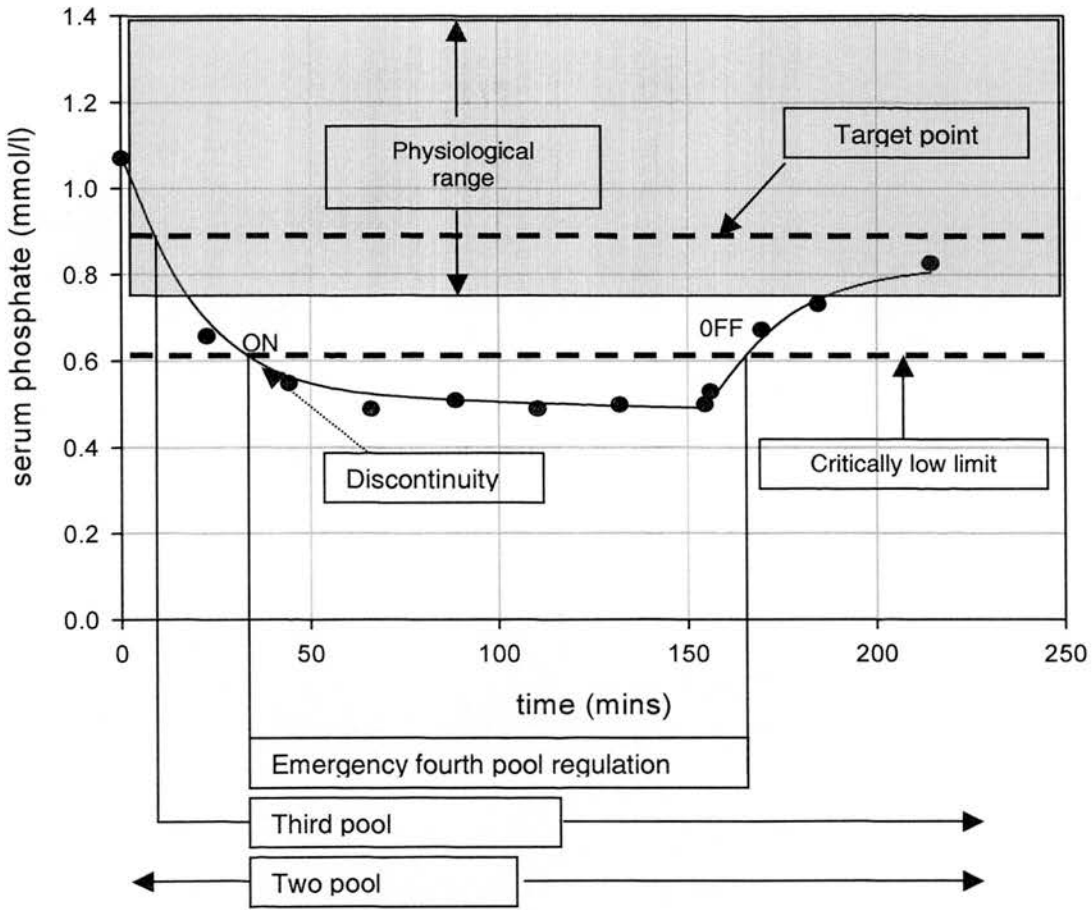


Figure 6.5. Representative treatment exhibiting features of model C. Kinetics are as for model B (figure 3) but, in addition, when the critically low limit is exceeded, fourth pool kinetics become operational. The influx from this pool switches off when a safe phosphate level has been re-established

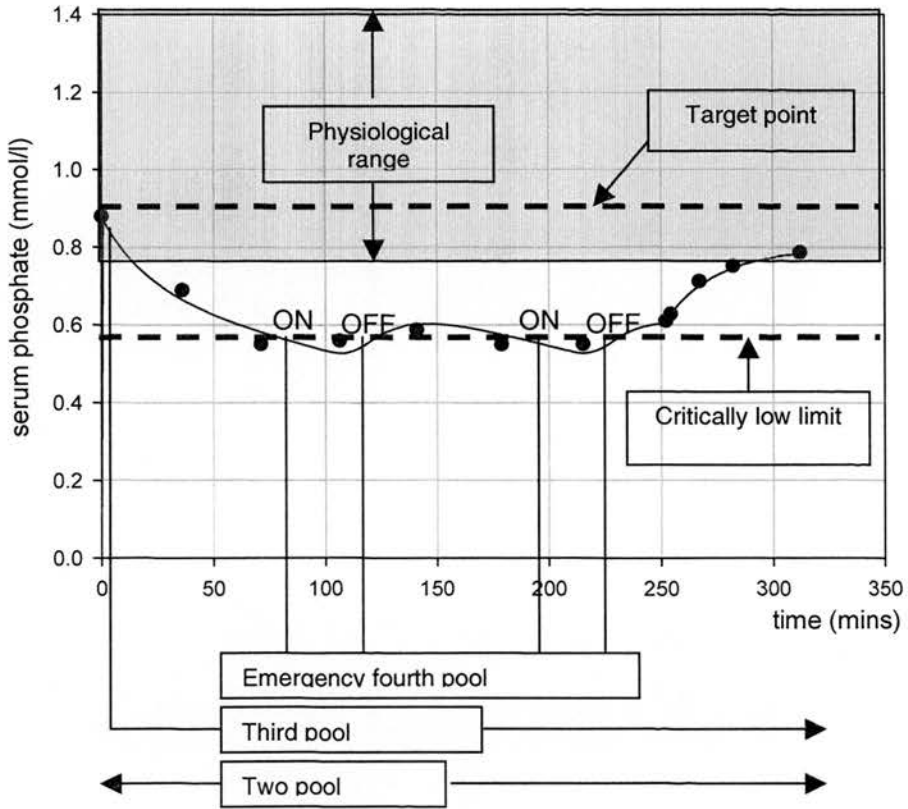


Figure 6.6. Representative treatment exhibiting features of model C with repeated activation of fourth pool kinetics.

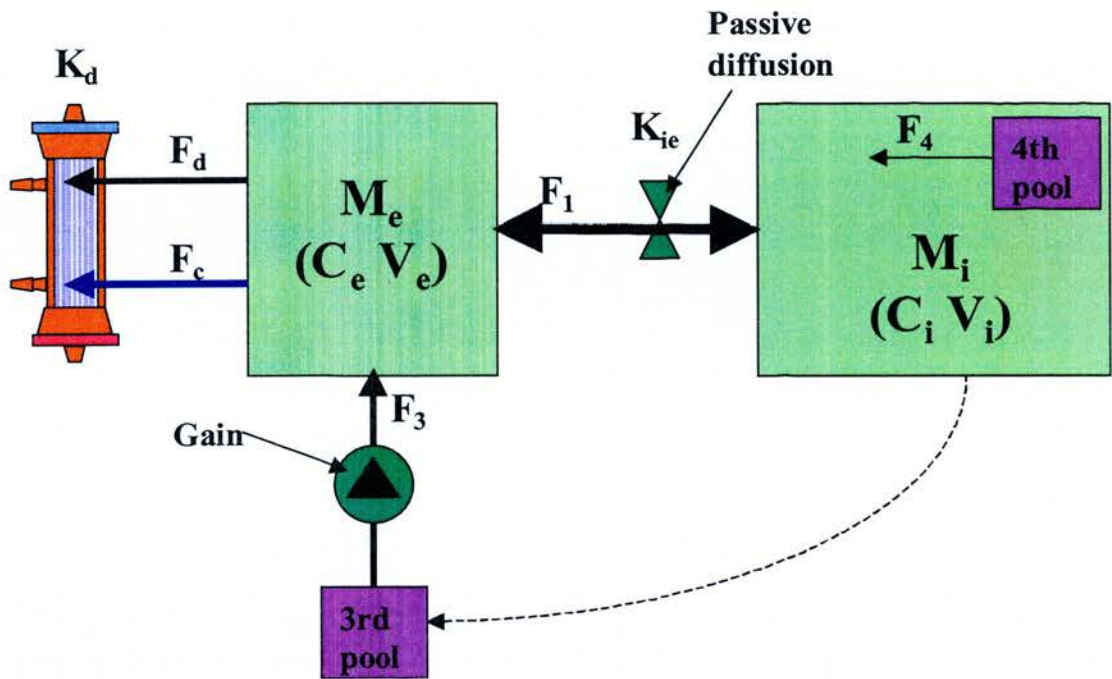


Figure 6.7. Schematic representation of Model C. Addition of hysteresis element to model B.

In addition to the proportional control mechanism shown in the three-pool model (figure 4), critically low intracellular phosphate levels trigger immediate release of phosphate from a fourth pool local to the intracellular space (F_4). This independent mechanism serves to protect the intracellular environment from dangerously low phosphate concentrations. Abbreviations are as in figures 2 and 3.

6.3 Results

Urea Kinetics

It was possible to achieve excellent fits with a two-pool model for the urea data in long dialysis, figure 8 with a median intracellular to extracellular transfer coefficient for urea (K_{ie} urea) of 750ml/min and total body water $96.6 \pm 3.2\%$ of the predicted Watson volume (V). In contrast, during short dialyses it was necessary to assume a lower effective total body water $68.0 \pm 4.5\%$ of predicted volume, and a corresponding reduction in K_{ie} urea in order to achieve the same degree of accuracy. Figure 9. The values used to compensate for cardiopulmonary recirculation (CPR) averaged $4.3 \pm 0.4\%$ in long dialysis and $5.4 \pm 0.8\%$ in short dialysis, $p < 0.01$.

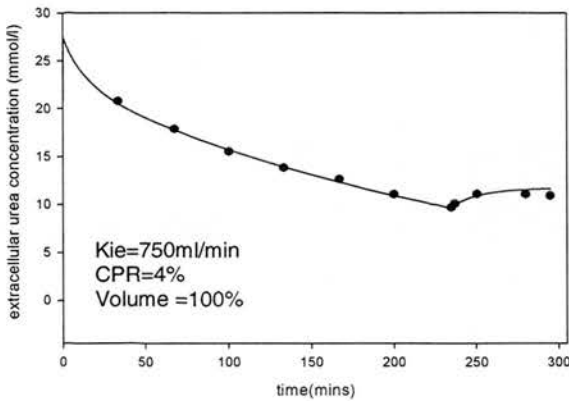


Figure 6.8. Representative data from one patient showing observed urea levels during dialysis [●] vs. urea levels predicted by two-pool model [represented by line] in long dialysis.

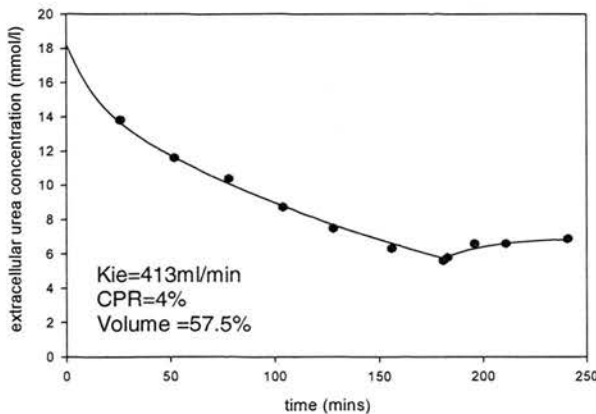


Figure 6.9. Representative data from one patient showing observed urea levels during dialysis [●] vs. urea levels predicted by two-pool model [represented by line] in short dialysis.

Allowing for cardiopulmonary recirculation (CPR) and for a degree of peripheral compartmentalisation in the short dialysis it was possible to achieve excellent fits between the observed urea data and the two-pool model assuming an intracellular to extracellular transfer coefficient (K_{ie}) of 750ml/min.

Phosphate Kinetics

Model A Two-pool kinetics

An attempt was made to fit the phosphate data to the same two-pool model. It was clear that phosphate kinetics were markedly different, figure 10.

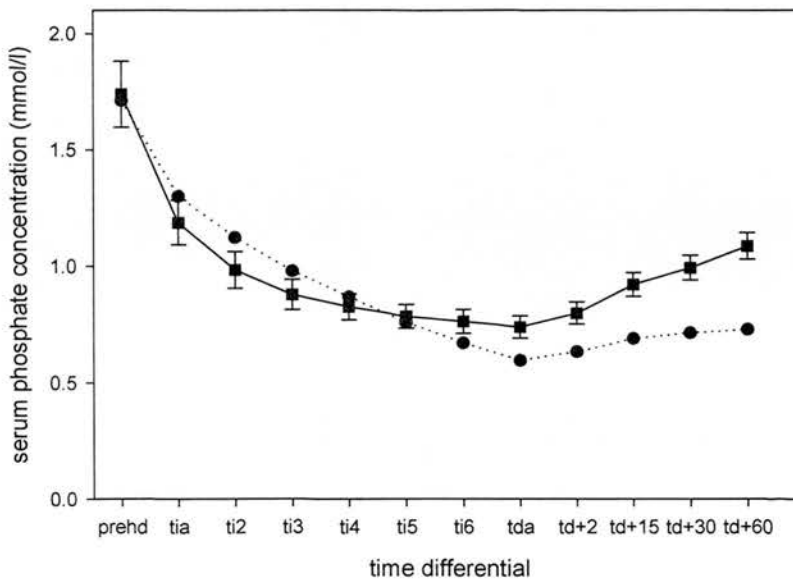


Figure 6.10. Mean observed phosphate levels during dialysis for all 58 treatments vs. phosphate levels predicted by two-pool model.

A clear discontinuity exists where the phosphate data plateaus and deviates from the two-pool model. The degree of rebound can also be seen to be greater in the observed data than the two-pool prediction. Symbols are: (■) observed phosphate data; (●) two-pool model. Observed phosphate data is mean \pm 95% confidence intervals.

During the initial part of the dialysis session, extracellular inorganic phosphate concentrations fell rapidly, but a point was reached beyond which the measured phosphate concentration plateaued or in some patients even began to rise. This phenomenon did not appear to be related to alterations in acid-base status as assessed by the serum bicarbonate concentration during dialysis, figure 11.

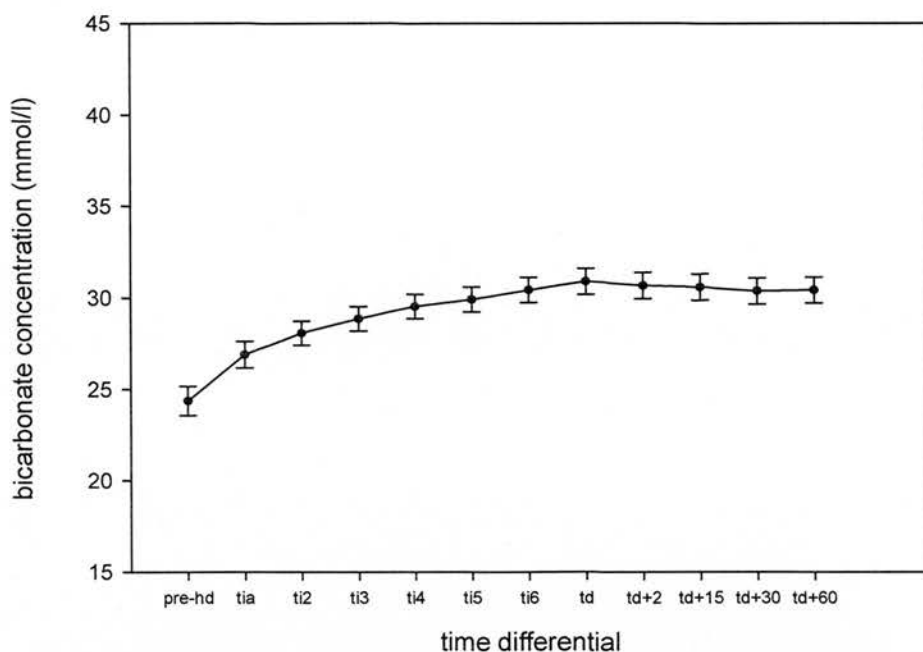


Figure 6.11. Serial mean plasma bicarbonate measurements throughout dialysis.

Bicarbonate levels increase throughout dialysis. No plateau is seen during dialysis in the bicarbonate data in contrast to the phosphate data, Figure 10. Data is mean \pm 95% confidence intervals

No correlation between the bicarbonate concentration and the phosphate concentration could be demonstrated at any point in the dialysis process. The average cumulative standard deviation between the observed phosphate data and the two-

pool model was 0.22 ± 0.04 mmol/l for long dialysis and 0.22 ± 0.03 mmol/l for short dialysis.

Model B Three-pool kinetics to maintain a target phosphate concentration

According to model predictions, the target concentration for phosphate averaged 1.22 ± 0.08 mmol/l for long dialysis and 1.15 ± 0.10 mmol/l for short dialysis (p=ns).

The target concentration almost always fell within the physiological range, Figure 12, but within this range showed significant correlation with the pre-dialysis serum phosphate concentration ($r=0.47$, $p<0.001$), Figure 13.

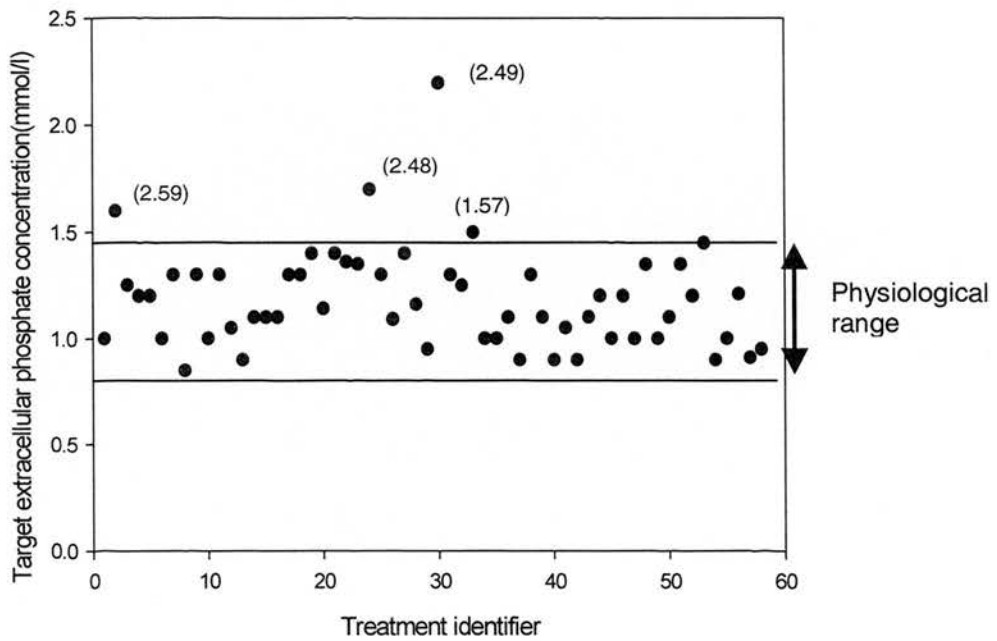


Figure 6.12 Target phosphate concentration. The majority of values fell within the physiological range (0.75-1.40mmol/l) and those that did not tended to have higher pre-dialysis phosphate concentrations (shown in parenthesis).

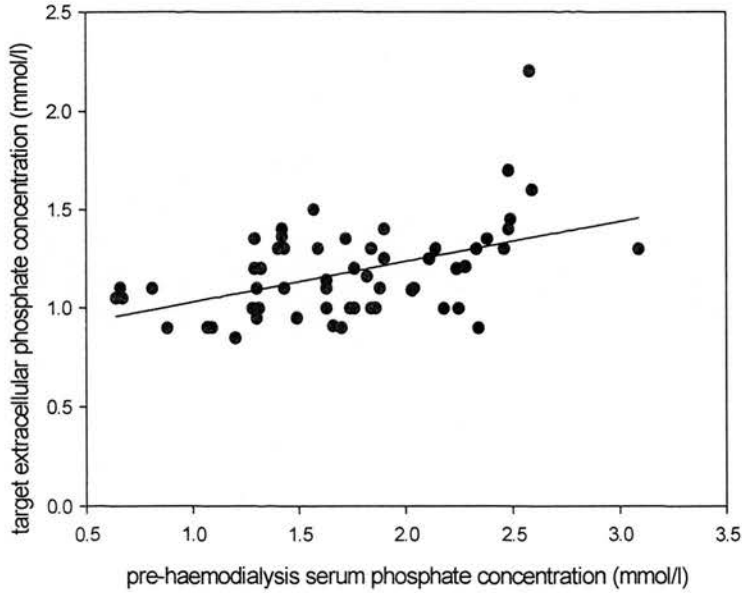


Figure 6.13 Correlation between pre-dialysis serum phosphate concentration and target extracellular phosphate concentration according to the model. ($r=0.47$ $p<0.001$).

During the period of active phosphate regulation the dialysis process continued to remove phosphate at an average rate of 0.16 ± 0.02 mmol/min in long dialysis and 0.18 ± 0.02 mmol/min in short dialysis. In order to maintain the phosphate plateau the generation rate from the third pool must be of a similar order.

In eight long dialysis treatments and six short dialysis treatments, this three-pool model could adequately explain the observed phosphate data. The addition of fourth pool kinetics in these treatments provided no further improvement in the degree of fit between the model and the observed data. The cumulative standard deviations were 0.03 ± 0.01 mmol/l and 0.04 ± 0.01 mmol/l for long and short treatments respectively.

One representative treatment is shown in figures 4 and 14 with the corresponding cumulative standard deviation curves shown in figure 15.

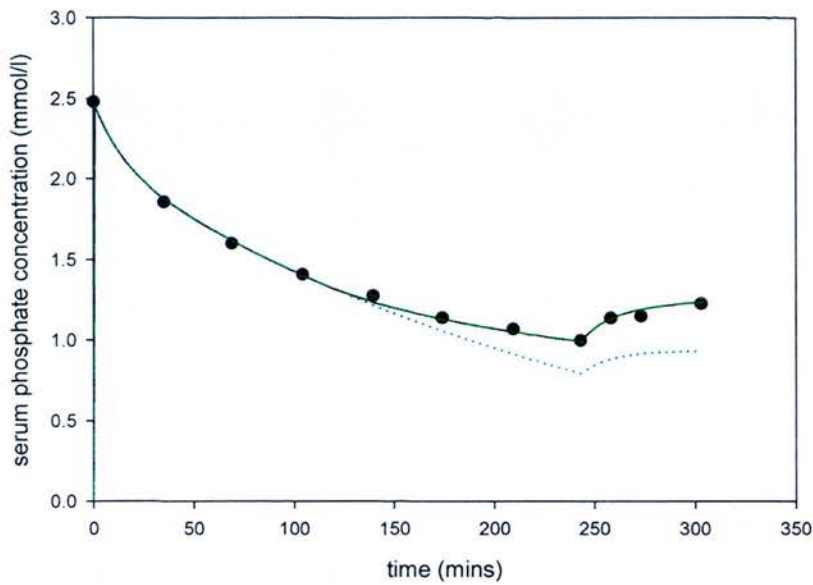


Figure 6.14. Representative treatment exhibiting three-pool kinetics. Symbols are: (●) extracellular phosphate data. Lines are: dotted, two-pool kinetics; dashed, three-pool kinetics.

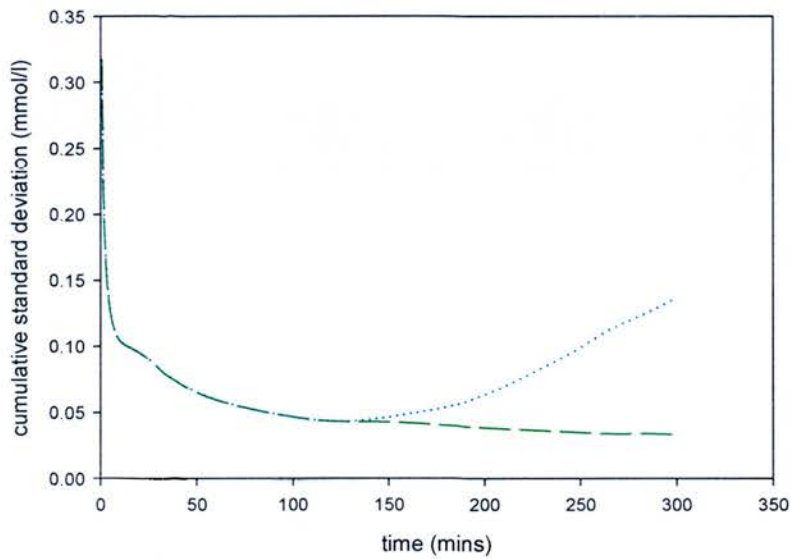


Figure 6.15 Cumulative standard deviation curves for figure 14. Symbols are as for figure 14

Model C Addition of a fourth pool and hysteresis element

The critically low concentration ranged from 0.41 mmol/l to 1.44 mmol/l with a mean of 0.86 mmol/l in long dialysis and 0.74 mmol/l in short dialysis ($p=ns$). Correlation was found between the critically low phosphate concentration and the pre-dialysis phosphate concentration ($r=0.67$, $p<0.001$), figure 16.

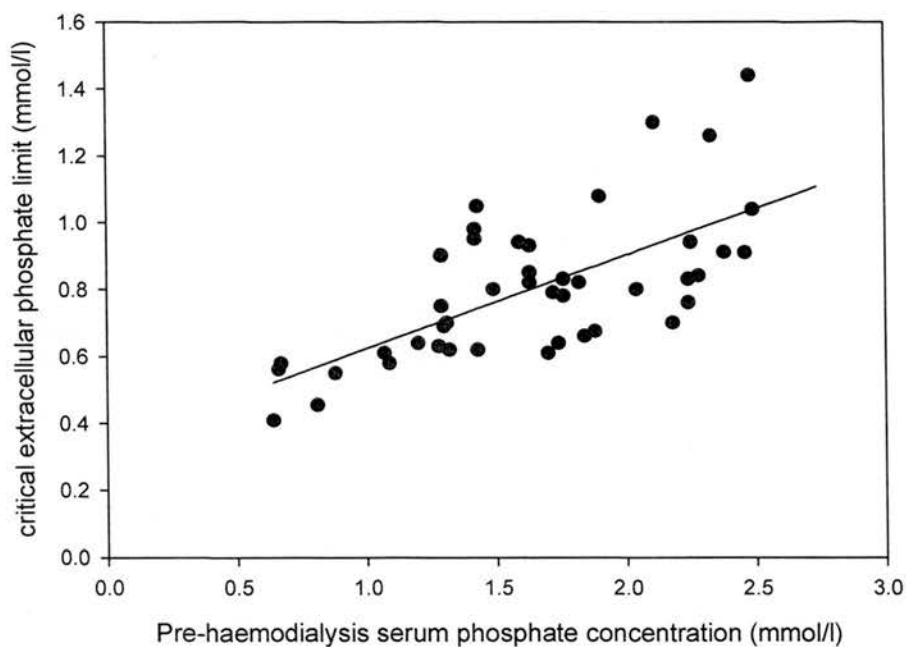


Figure 6.16 Correlation between pre-dialysis serum phosphate concentration and critically low extracellular phosphate concentration according to the model. ($r=0.67$ $p<0.0001$)

According to the model, the liberation of phosphate from this fourth pool ceased when the measured phosphate concentration had risen by a mean of 0.07 ± 0.02 mmol/l.

Model C shows an excellent fit with the observed data for the 21 long and 23 short treatments not explained by model B. The mean cumulative standard deviations were

$0.03 \pm 0.01\text{mmol/l}$ for both long and short dialysis treatments. Two representative treatments a and b are shown in figures 5, 6 and 17 with their corresponding cumulative standard deviation curves.

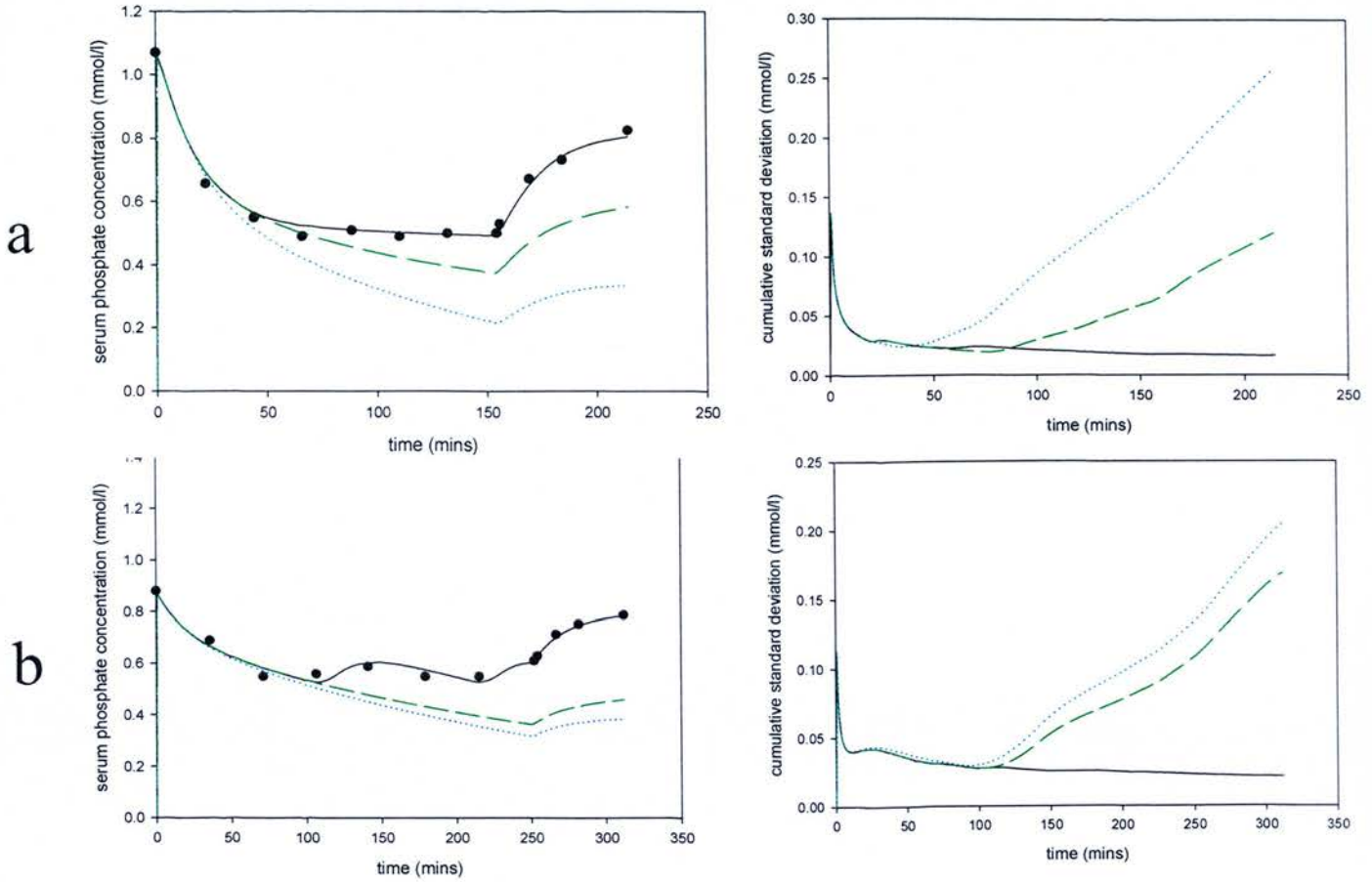


Figure 6.17. Two representative treatments (a and b) exhibiting four-pool kinetics with corresponding cumulative standard deviation curves. In figure 17a, the pre-haemodialysis serum phosphate concentration is 1.07mmol/l and there is a substantial contribution towards regulation from both the third and fourth pools. In figure 17b, the pre-haemodialysis serum phosphate concentration is 0.88mmol/l and the contribution towards regulation from the third pool is minimal. This is presumably because this individual has little in the way of third-pool phosphate stores.

Symbols are: (●) extracellular phosphate data. Lines are: dotted, two-pool kinetics; dashed, three-pool kinetics; solid, four-pool kinetics.

Summary Of All Models.

Models A, B and C were progressively implemented until the best approximation to the data was achieved. The application of model A demonstrated a reasonable fit for the initial part of dialysis corresponding to two-pool kinetics but a poor fit thereafter. The implementation of model B demonstrated a close correlation between the model and the data in a minority of treatments (14/58). The pre-dialysis serum phosphate concentration for these 14 treatments was significantly higher than for the remainder of treatments ($p < 0.01$). A close fit between the model and the data for the remaining 44 treatments was achieved when the hysteresis element of model C was applied. The cumulative standard deviation measurements for all 58 treatments in models A, B and C are shown in Tables 1 and 2.

Table 6.1. Comparison of cumulative standard deviation measurements for the fourteen treatments adequately explained by three-pool kinetics. Values are mean \pm 95% confidence intervals. Significance values are compared to model A (Student paired two sample for means); * $p < 0.05$, ** $p < 0.01$

	MODEL A (TWO POOL)	MODEL B (THREE POOL)
LONG DIALYSIS (8/29)	0.17 \pm 0.06	0.03 \pm 0.01**
SHORT DIALYSIS (6/29)	0.18 \pm 0.13	0.04 \pm 0.01*

Table 6.2. Comparison of cumulative standard deviation measurements for the forty-four treatments explained by four-pool kinetics. Values are mean \pm 95% confidence intervals. Significance values are compared to model A (Student paired two sample for means); ***p<0.001

	MODEL A (TWO POOL)	MODEL B (THREE POOL)	MODEL C (FOUR POOL)
LONG DIALYSIS (21/29)	0.24 \pm 0.05	0.15 \pm 0.05***	0.03 \pm 0.01***
SHORT DIALYSIS (23/29)	0.23 \pm 0.03	0.11 \pm 0.02***	0.03 \pm 0.01***

Comparisons Between Phosphate Data In Long And Short Dialysis

Pre-dialysis phosphate concentrations were 1.74 ± 0.23 mmol/l for long dialysis and 1.71 ± 0.19 mmol/l for short dialysis (p=ns). The minimum phosphate concentration achieved in short dialysis treatments was significantly lower p<0.001: figure 18, than that in long dialysis.

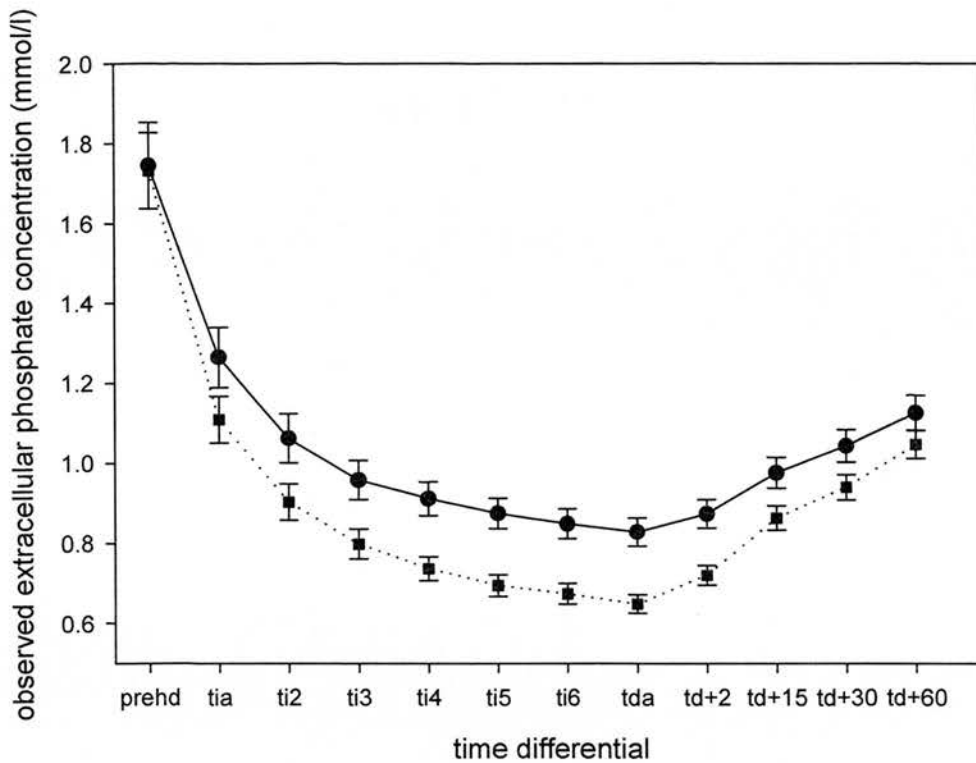


Figure 6.18. Comparison of observed serum phosphate concentrations in long and short dialysis for all 58 treatments. Symbols are: (■) short dialysis; (●) long dialysis. * $p < 0.001$; ** $p < 0.01$, paired t-test. Data are mean \pm SEM values

There was no statistical difference in the critically low limit between the long and short treatments suggesting that either there is a short time lag before the control mechanisms become active or that these mechanisms are overwhelmed by higher phosphate losses in short dialysis. The higher dialyser clearances and lower effective V in short dialyses result in a more rapid rate of decrease in the measured extracellular phosphate concentration in the initial stages of dialysis, Table 3.

Table 6.3. Intradialytic parameters. Values are mean \pm 95% confidence intervals.

Abbreviations are: T_d , length of dialysis; CPR, cardiopulmonary recirculation; $K_dPO_4(\text{model})$, dialyser clearance of phosphate used in model calculations; $K_dPO_4(\text{meas})$, dialyser clearance of phosphate calculated during dialysis; $K_{ie}PO_4$, intracellular to extracellular transfer coefficient for phosphate; $K_dUrea(\text{model})$, dialyser clearance of urea used in model calculations; $K_dUrea(\text{meas})$, dialyser clearance of urea calculated during dialysis; $K_{ie}Urea$, intracellular to extracellular transfer coefficient for urea.

	Long	Short	
T_d (min)	240.7 \pm 5.3	147.3 \pm 6.5	p<0.001
CPR (%)	4.3 \pm 0.4	5.4 \pm 0.8	p<0.01
%Watson volume (V)	96.6 \pm 3.2	68.0 \pm 4.5	p<0.001
$K_dPO_4(\text{model})$ (mmol/min)	158.4 \pm 12.1	226.4 \pm 13.1	p<0.001
$K_dPO_4(\text{meas})$ (mmol/min)	171.8 \pm 9.5	223.0 \pm 17.0	p<0.001
$K_{ie}PO_4$ (ml/min) effective	351.0 \pm 33.9	242.6 \pm 23.2	p<0.001
$K_{ie}PO_4$ (ml/min) corrected for V	363.8 \pm 32.7	363.8 \pm 32.7	p=ns
K_dUrea (model) (mmol/min)	151.1 \pm 9.3	214.8 \pm 13.5	p<0.001
K_dUrea (meas) (mmol/min)	185.6 \pm 10.2	285.2 \pm 15.2	p<0.001
$K_{ie}Urea$ (ml/min) effective	714.4 \pm 46.7	513.2 \pm 48.2	p<0.001
$K_{ie}Urea$ (ml/min) corrected for V	739.6 \pm 38.7	699.1 \pm 41.9	p=ns

$K_{ie}PO_4$ was significantly higher in long versus short dialysis (p<0.01) but this difference was not apparent when corrected for the effects of phosphate distribution volume (p=ns) (table 3). Post-dialysis phosphate levels in long dialysis (0.83 \pm 0.07mmol/l) were higher than in short dialysis (0.65 \pm 0.05mmol/l) (p<0.001).

Phosphate levels during the measured rebound up to 60 minutes were significantly higher in the long dialysis group although the rate of rebound with time in the short dialysis treatments was greater, Figure 18. Despite lower phosphate levels during dialysis the mass of phosphate removed during short dialysis was significantly less than during long dialysis (26.33 ± 4.38 vs. 30.89 ± 4.70 mmol: $p < 0.02$).

6.4 Discussion

Phosphate kinetics remains a poorly studied area of haemodialysis research despite the ubiquity of hyperphosphataemia. Existing strategies to achieve phosphate control rely heavily on dietary restriction and the use of phosphate binders. Utilising high-flux membranes for haemodialysis or haemodiafiltration and increasing the surface area of the dialyser membrane can remove phosphate more efficiently, although no improvement is seen in the pre-dialysis phosphate levels of patients dialysed this way^{102;103}. Bicarbonate replacement fluid has not been shown to be superior to acetate in terms of phosphate removal¹⁰⁴.

It is known that phosphate removal does not follow the same diffusion kinetics described for urea^{105;106}. Regardless of the duration of dialysis a characteristic plateau phase is rapidly reached below which phosphate levels do not fall. The reasons for this are complex and relate to the mobilisation of phosphate from unidentified stores during the dialysis procedure. Longer dialysis treatments remove a higher mass of phosphate but also result in significantly higher post-dialysis

phosphate concentrations according to our data. This is indicative of phosphate being mobilised to a greater extent in long dialysis. In the post-dialysis period, there is a rebound phenomenon persisting for in excess of one hour. The rate of the rebound with time is significantly higher in the short treatment group, which may be due to the additive effect of re-equilibration of phosphate from peripheral compartments that have been poorly perfused during the short dialysis treatment.

Recognition of the departure of the phosphate data from standard two pool kinetics has led to the hypothesis of a third pool of phosphate although its nature is not clear^{105;107-109}. The existence of a third pool is consistent with work demonstrating the absence of a plateau phase when bovine blood is dialysed *in vitro*¹¹⁰. Studies in erythrocytes demonstrate that intracellular to extracellular phosphate equilibrium was reached at a higher extracellular phosphate concentration in the erythrocytes of dialysis patients compared to controls suggesting adaptation to the hyperphosphataemic environment¹¹¹. This may explain the observation that the critically low point and target point are dependent on the pre-haemodialysis phosphate concentration, Figures 13, 16. ³²P and NMR studies demonstrate glycoposphates in uraemic erythrocytes not present in healthy controls that could conceivably act as a source of phosphate during dialysis^{111;112}.

In studies of haemodialysis patients, Sugasaki described a single-pool extracellular compartment model and proposed phosphate generation from reserves, triggered by the reduction of phosphate during dialysis to a nadir unique to each patient. These studies recognised the correlation of the phosphate nadir with the pre-dialysis serum

phosphate concentration^{112;113}. Maasrani studied four paediatric patients over eight dialysis sessions and suggested that the plateau phase of phosphate kinetics was maintained by a time-dependant influx from the intracellular space¹¹⁰. These studies all described the influx of phosphate in terms of a linear or exponential increase in the rate of phosphate generation (or gain) that did not fully explain the observed data. Poggitsch studied 28 haemodialysis patients and suggested that generation of phosphate was in response to falling extracellular phosphate levels. The point at which phosphate generation was initiated was again observed to be dependent on the pre-dialysis phosphate concentration. This study suggested that additional phosphate was released into the extracellular space in proportion to the difference between the momentary phosphate concentration and a critical level (equivalent to the target concentration in our model). This process continued into the post-dialysis period¹¹¹.

This model expands these ideas with the introduction of biphasic control involving sequential implementation of control mechanisms in response to changes in the prevailing phosphate concentration, Figures 2-9. In model A, dynamic equilibrium between the intracellular and extracellular compartments throughout the dialysis process is assumed, consistent with a two-pool model. Model B implements additional phosphate release from a third pool to maintain a pre-determined target phosphate concentration. Although the additional phosphate enters the extracellular space, which is consistent with the Poggitsch model, we propose that it is changes in the intracellular phosphate concentration that trigger the control mechanism. In theory, the generation process from the third pool would be switched off once intracellular phosphate concentrations reach the target level although this was not

evident in the current data since the rebound clearly extended beyond the final measurement at 60 minutes post-dialysis. In model C, the existence of a fourth pool is described that is operational in the majority of treatments studied. There is a hysteresis element deduced from the discontinuities in the data that reflects a “switching on” effect that exists to protect against critically low intracellular phosphate levels. Where the critically low intracellular phosphate concentration is not encountered during dialysis (in 24% of patients in this study) the hysteresis element is not invoked and the three-pool model can adequately explain phosphate kinetics.

Four pools of phosphate in this model best describe the behaviour of the data and the kinetics of phosphate clearly involves more complex mechanisms than simple diffusion. The gut is an unlikely candidate for the third or fourth pools as all the patients studied were fasting. Dialysis patients are likely to have pathological stores of phosphate, possibly in bone, as 86% of total body phosphate is in bone and approximately 250mg phosphate is mobilised daily in the process of remodelling. The third-pool phase of phosphate regulation can potentially be explained by efflux from such stores, possibly from a pool of phosphate not yet incorporated into bony matrix. If so, this phase would only be sustained in individuals with excess total body phosphate. The recognition that most haemodialysis patients have an excess of stored phosphate presents a challenge to achieve better phosphate control in the pre-dialysis period. Earlier referral of patients with chronic renal failure would allow the timely introduction of dietary phosphate restriction and phosphate binders to prevent

patients starting their dialysis careers in positive phosphate balance. Currently used phosphate binders may be inadequate for this task.

The observed time course of the hysteresis effect of the proposed model is difficult to explain if phosphate is released purely from a bone-related pool. The speed of the hysteresis response might imply phosphate release by a mechanism such as cell lysis, but the observation that the hysteresis can switch on and off repeatedly during a dialysis treatment is against this. The more probable explanation is the existence of a fourth pool local to the intracellular space that provides emergency protection against life threatening critically low intracellular phosphate concentrations. It is possible that glyco-phosphates as described in in-vitro studies^{111;112} could be involved in this short-term regulation mechanism. This mechanism is likely to represent physiological protection and may also operate in subjects with normal renal function.

This model sequentially implements the third and fourth pools allowing all treatments in both the long and short dialysis groups to be explained by passive two-pool, three-pool, and emergency fourth pool regulatory processes. This implies common mechanisms operating across all dialysis treatments and supports the physiological validity of the model. Phosphate generation from a third pool allows removal of more phosphate during dialysis than permitted by passive two-pool kinetics. This is theoretically beneficial in reducing the excessive phosphate stores that exist in chronic haemodialysis patients. However, the switch to fourth pool kinetics in the way described, may give cause for concern. It was observed that the majority of dialysis patients invoke fourth pool kinetics in response to critically low

phosphate concentrations. These low phosphate levels may contribute to dialysis-related symptoms and possibly to the excess morbidity and mortality seen in these patients in the longer term. This study did not appear to result in major intra-dialytic symptoms although this was not a primary end-point. This would be an interesting area for further study. In order to avoid the implementation of fourth pool kinetics it would be necessary to prevent phosphate concentrations reaching the critically low limit. This might be achieved by longer and slower treatments¹⁰⁰.

Daily nocturnal dialysis currently appears to be the only technique capable of safely depleting excess phosphate stores and maintaining patients in optimal phosphate balance, although whether in the current medico-political climate this will be practicable for the majority remains doubtful.

From existing data, it seems clear that a large part of the pathophysiology of hyperphosphataemia occurs in the pre-dialysis phase with patients building up excessive stores of phosphate long before they come on to dialysis. If patients can be identified earlier in the course of chronic renal failure, it would afford the chance to introduce appropriate dietary advice and phosphate binders at an earlier juncture thus preventing the build up of phosphate that undoubtedly contributes to the excess mortality demonstrated in these patients.

Chapter 7

Phosphate kinetics in acute haemodialysis

Phosphate kinetics in acute haemodialysis

7.1 Background

The study in chronic dialysis therapy (Chapter 6) showed clearly that the kinetic behaviour of phosphate deviates markedly from a two-pool model. It seems probable that mobilisation of phosphate from a third pool explains why phosphate levels appear to stabilise despite ongoing dialytic losses. It is assumed that patients build up a large store of excess phosphate starting in the pre-dialysis phase and that the ability to compensate for alterations in extracellular phosphate during dialysis may somehow relate to the size of this excess pool. Patients with acute renal failure are not expected to have large phosphate stores and as such may be less able to compensate for alterations in phosphate concentrations during dialysis.

In the four-pool study of patients undergoing chronic dialysis treatments, the fourth pool described is a mechanism to stop phosphate from falling below a critical intracellular level. It acts rapidly to correct critically low levels of phosphate and operates until a “safe” level is once again reached. In patients with acute renal failure, who are not expected to have high phosphate stores, this level may be reached earlier during dialysis.

7.2 Subjects and Methods

Five patients with acute renal failure of sudden onset were studied. The demographic data and causes of renal failure are described in Table 1.

Table 7.1. Demographic data and dialysis parameters of patients studied

Patient	Age	Assumed Duration	Cause of renal failure	Qb	Qd	Td	Ultrafiltration	Dialyser
t	(years)	of Renal Failure		(ml/min)	(ml/min)	(min)	volume (ml)	
1	74	5 days	Myeloma	200	500	180	100	KF201
2	67	7 days	Tubulo-interstitial nephritis	175	500	180	1000	KF201
3	60	3 days	HUS	200	500	130	0	KF201
4	63	14 days	Myeloma	200	500	180	1000	KF201
5	75	7 days	Sepsis	200	500	180	0	KF201

Dialysis parameters

Haemodialysis was performed using Fresenius 4008 haemodialysis machines and KF201 dialysis membranes. The duration of the treatment was set at 180 minutes although one patient stopped dialysis at 130 minutes due to onset of atrial fibrillation that caused a drop in blood pressure. The vascular access in all patients was via a double lumen internal jugular line. Blood flow (Qb) was set at 200ml/min in all subjects but was reduced to 175ml/min in one subject due to poor flows from the line. Ultra-pure dialysate was produced on-line and the flow rate set at 500ml/min. Samples taken from the venous limb of the haemodialysis circuit at 60 and 120 minutes were used to calculate the dialyser clearance of phosphate. The dialyser clearance was calculated taking haematocrit into account using method 1 as described in Chapter 4.

Sample preparation and analysis

Blood samples from the arterial limb of the haemodialysis circuit were taken every 15 minutes through the course of dialysis and for up to two hours thereafter. All samples for measurement of phosphate were prepared as described in the general methods chapter and assayed in-house in a single run on an Olympus AU600 autoanalyser. Samples were additionally tested for urea, and bicarbonate. Serial haematocrit was measured on heparinised capillary samples on an arterial blood gas analyser.

Mathematical Modelling

The blood flow (Q_b), dialysate flow (Q_d), dialysis time (T_d), ultrafiltration volume (UFV), sex, measured dialyser clearance (K), and the raw data series for phosphate and urea were introduced as inputs into the previously designed four pool model of phosphate kinetics. In the same manner as for the study in chronic dialysis patients, the components of the model were sequentially implemented until the best fit between the data and the model was achieved. Cumulative variance measurements were utilised to determine the accuracy of the fit.

7.3 Results

The urea data was fitted easily to a two-pool kinetic model for all patients (data not shown) but it was once again demonstrated that phosphate did not follow the two-pool pattern. The phosphate data from all five patients was fitted to the same four-pool model described for chronic renal failure, with the need for implementation of

all four pools of phosphate being apparent. The values for the adjustable parameters are given in Table 2 and the individual curve fits are shown in figures 1-5.

Table 7.2. Parameters used to fit data to four pool model.

Study	Pre-dialysis phosphate (mmol/l)	Post-dialysis phosphate (mmol/l) (Td+2)	Dialyser clearance (ml/min)	Phosphate target (mmol/l)	3 rd pool gain	Phosphate high limit (mmol)	Phosphate low limit (mmol/l)	4 th pool gain	Recirculation %	Cumulative Variance Td+2
1	1.49	1.00	120	1.1	2	1.05	1.00	3.1	4	0.024
2	2.00	1.08	170	1.6	1.6	1.25	1.24	2.5	12	0.053
3	1.16	0.89	100	1.15	2	1.15	1.00	0.8	4	0.012
4	1.8	1.22	95	1.35	2	1.29	1.26	6	4	0.054
5	2.75	1.67	85	1.7	3	1.88	1.88	2.5	4	0.039

Figure 7.1-7.5 Curve fits for individual patients

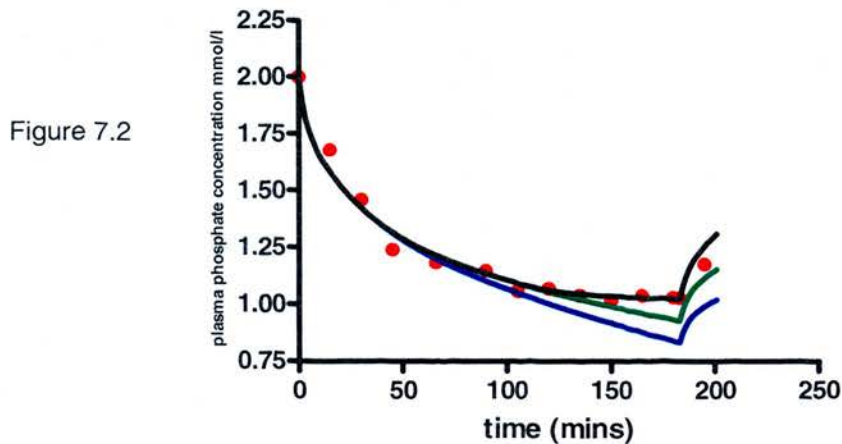
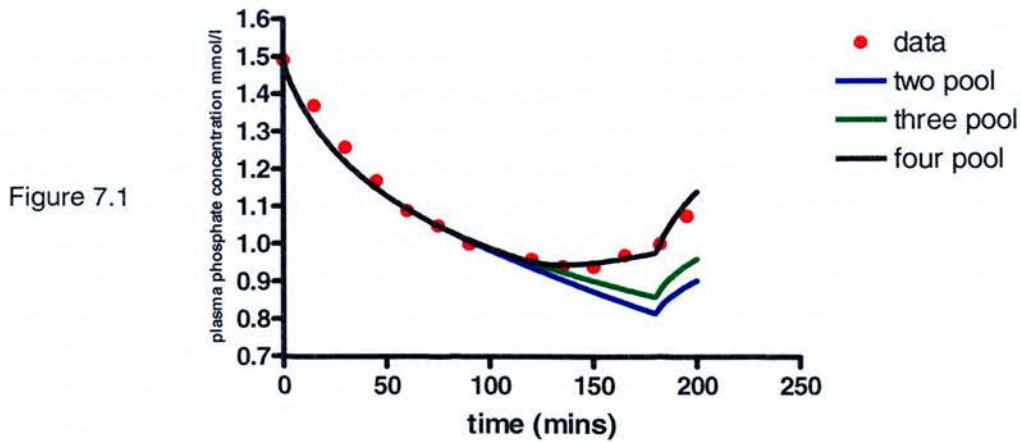


Figure 7.3

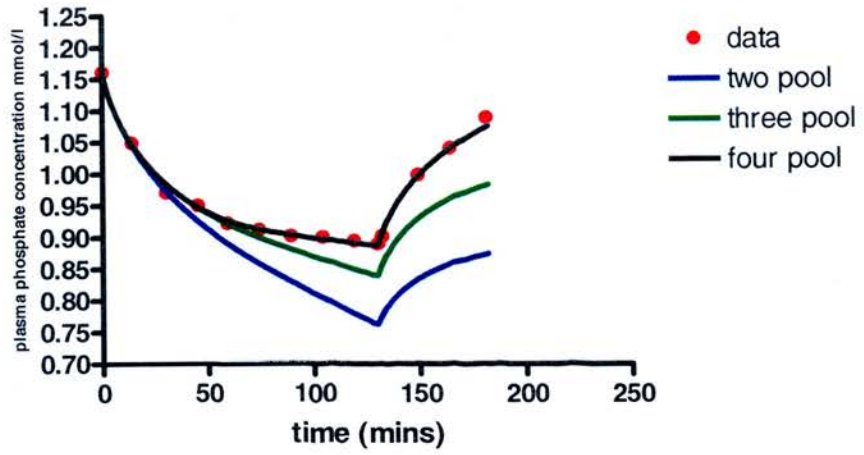


Figure 7.4

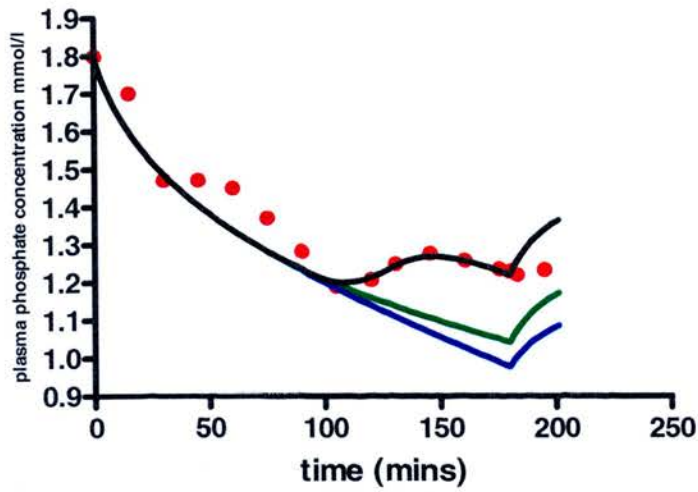
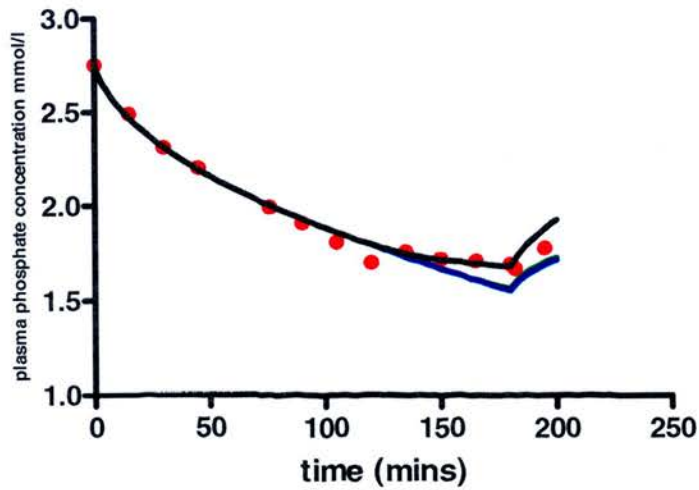


Figure 7.5



The pre-dialysis phosphate in the subjects with acute renal failure of 1.84 ± 0.27 mmol/l is slightly higher when compared to the patients with chronic renal failure previously studied (1.74 ± 0.05 mmol/l) but is not a significant difference. The target phosphate concentration (1.36 ± 0.13 mmol/l) again fell within the physiological range (0.75-1.40 mmol/l) unless the pre-dialysis phosphate was very high. The pre-dialysis phosphate correlated with the target phosphate concentration $r=0.72$, $p<0.001$, Figure 6.

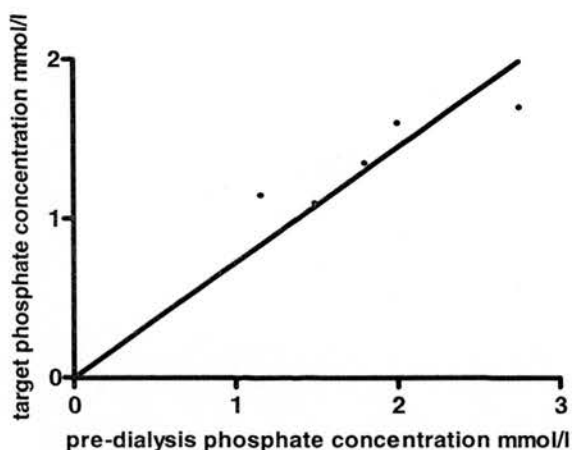


Figure 7.6. Correlation between pre-dialysis phosphate concentration and target phosphate concentration.

The same correlation with the critically low phosphate concentration seen in the chronic study was seen in these patients with acute renal failure. $r=0.68$, $p<0.001$, Figure 7, but the phosphate concentration at which this occurred was higher. (1.28 ± 0.16 mmol/l vs. 0.86 ± 0.30 mmol/l).

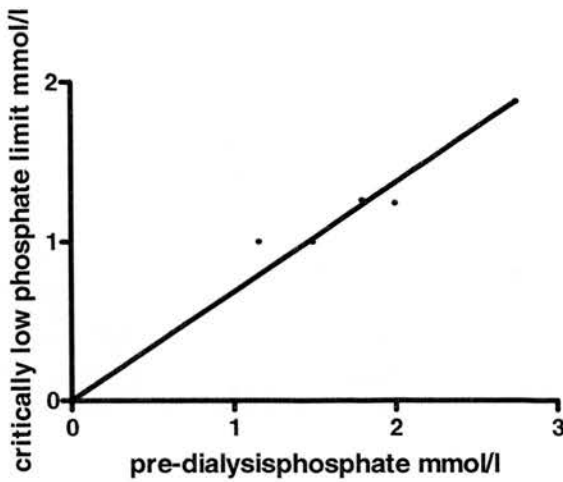


Figure 7.7. Correlation between pre-dialysis phosphate concentration and critically low phosphate concentration

In the patients with acute renal failure, the post dialysis phosphate was also higher than in the chronic renal failure study (1.17 ± 0.14 vs. 0.74 ± 0.02 mmol/l).

Additionally the pre-dialysis phosphate correlated with the post-dialysis phosphate $r=0.62$, $p<0.001$, Figure 8 which was not observed in the chronic renal failure study.

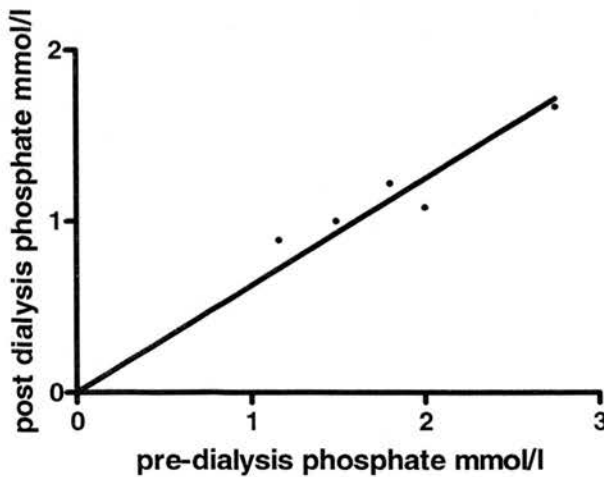


Figure 7.8. Correlation between pre-dialysis phosphate and post-dialysis phosphate concentration

The patients were more acidotic than in the chronic renal failure study but the same pattern of a rise in serum bicarbonate throughout dialysis was seen, Figure 9.

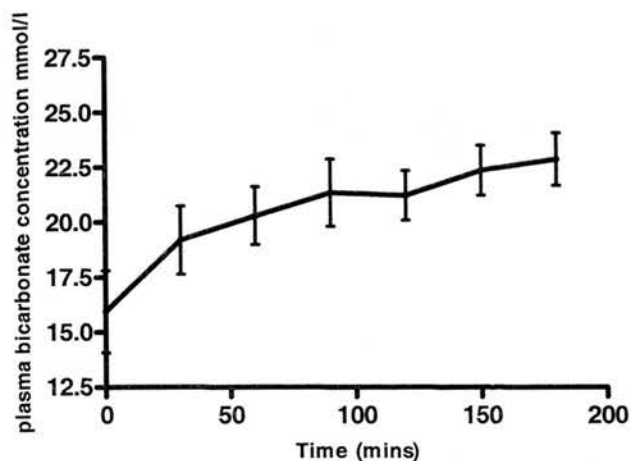


Figure 7.9. Pattern of change in bicarbonate concentrations through dialysis. Error bars are \pm SEM

7.4 Discussion

It was expected that patients with acute renal failure would be less able to maintain phosphate homeostasis due to a lack of phosphate stores, and would rapidly become hypophosphataemic during dialysis. The intra-dialytic phosphate kinetics were expected to reveal a limited source of additional phosphate for the “third pool” and an earlier activation of the “fourth pool” as a result.

Phosphate concentrations were well maintained in all the subjects studied with no individual becoming overtly hypophosphataemic during dialysis. The higher intra- and post-dialysis phosphate concentrations seen in the subjects with acute renal

failure could be a reflection of the lower clearance employed in acute dialysis due to the short duration of therapy and the lower pump speeds. If this were the only mechanism, the observation of a different kinetic profile, perhaps more in keeping with two-pool kinetics, would be expected. It appears, however, that the same kinetic profile apparent in subjects with chronic renal failure also exists in acute renal failure.

The nature of the “third pool” regulation in acute renal failure was similar when compared to patients undergoing chronic dialysis and the maintenance of phosphate concentrations during dialysis does appear to be dependant on continuing transfer of phosphate into the extracellular space from this “third pool”. The mechanism does appear to be exhausted more readily in acute renal failure leading to the involvement of the “fourth pool” at higher prevailing phosphate concentrations. In one subject there seemed to be a very limited contribution from this “third pool” mechanism, Figure 5.

The previous description of the “fourth pool” mechanism assumed that it is invoked by a fall in intracellular phosphate concentrations below a critical level. If some of the phosphate excess in chronic renal failure is sequestered within the cell, this could allow for more efficient buffering of intracellular phosphate concentrations, allowing lower extracellular phosphate concentrations during dialysis to be tolerated. In the absence of an efficient intra-cellular buffering mechanism in acute renal failure, the “fourth pool” mechanism would be activated at higher extracellular phosphate concentration levels.

An alternative explanation would be that the “fourth pool” simply becomes operational when influx of phosphate from the third pool is insufficient. This would again be in keeping with the finding of a higher “critical” concentration in acute renal failure.

7.5 Conclusions

In summary, this study demonstrates that, in keeping with the findings in chronic renal failure, a multi-pool kinetic model is required to explain phosphate removal in acute renal failure. It provides some evidence that the “third pool” mechanism is exhausted more readily in patients with acute renal failure supporting the premise that there are lower levels of accessible phosphate stores. The finding that the “fourth pool” mechanism becomes operational at higher prevailing phosphate concentrations in acute renal failure may simply be a reflection of lower availability of phosphate or could imply that buffering mechanisms are more easily exhausted in acute renal failure.

Chapter 8

Intracellular Phosphate Concentrations in Haemodialysis

Intracellular Phosphate Concentrations in Haemodialysis

8.1 Background

In chapter 6, the four-pool model describing phosphate kinetics in haemodialysis was described. The model identified four potential pools involved in phosphate regulation during dialysis and assumptions were made about the possible origin of these pools.

This study was designed to find out whether or not the intra-dialytic hypophosphataemia observed in haemodialysis patients had any effect on the intracellular concentration of inorganic phosphate.

It appeared from the four-pool model that additional phosphate moves into the extracellular space during dialysis and one possible source of this phosphate is the intracellular space. Only a very small proportion of intracellular phosphate exists as free inorganic phosphate and, if the cell is the source of additional phosphate, it is possible that there is a breakdown of organic phosphate molecules to liberate additional inorganic phosphate.

In order to investigate this further, serial plasma and intra-erythrocytic phosphate concentrations were measured during dialysis.

Previous studies that have attempted to assess the effect of dialysis on intracellular phosphate concentrations have mainly utilised the erythrocyte as a model^{111;114}.

While the erythrocyte is an atypical cell due to the absence of mitochondrial activity, it can still serve as a reasonable model due to the potential for liberation of phosphate from 2,3,DPG, ADP or ATP. Despite the recognised limitations it remains an attractive candidate for study due to the ease of obtaining samples and the existence of a well-validated assay technique⁶⁹.

8.2 Subjects and Methods

Subjects and Dialysis Technique

Ten patients were studied all of whom were stable on the chronic dialysis program at the Lister Hospital. None were suffering with intercurrent illness and their pre-dialysis phosphate concentration was 1.91 ± 0.32 mmol/l. All patients received haemodiafiltration using high-flux polysulphone membranes and replacement fluid volumes equivalent to 35% of the plasma flow. As different durations of dialysis were employed in the different subjects, for the purposes of clarity only the results at 0, 30, 60, 90, and 120 minutes are reported.

Laboratory Method

The method of Challa et al⁶⁹ as detailed below was used to measure plasma and whole blood phosphate concentration. Red cell phosphate concentration was assumed to be the difference between these two values.

Solutions

1. 1mol/l perchloric acid
2. 4.3mol/l potassium hydroxide containing 0.6mmol/l imidazole
3. 27mmol/l ammonium molybdate in 2.4mol/l HCl
4. Petroleum spirit (boiling point 80-100°C) / 2-methylpropan-1-ol 1:4 mix
5. Ethanol AR
6. 0.18mol/l stannous chloride in 1.65mol/l HCl

7. External standard (Na_2HPO_4) containing 4mmol/l PO_4
8. Internal standard (Na_2HPO_4) 0.96mmol/l in 1mol/l perchloric acid.

To measure the whole blood phosphate concentration, blood was collected from the arterial limb of the haemodialysis circuit into lithium-heparin sample tubes and chilled on ice. After 15 minutes, these were centrifuged at 4°C for 5 minutes at 1000g and plasma removed to yield a packed cell volume (PCV) of approximately 70%.

The plasma was saved and the red cells resuspended.

0.5ml of this suspension was then added to 1ml of ice-cold perchloric acid (1mol/l).

The tube was vortexed for 5-10 minutes then the precipitated protein removed by centrifugation at 2500g for 15 minutes at 4°C. The supernatant was then pipetted off into a polystyrene tube and stored in ice water for 15 minutes after which time the pH was adjusted to 6.0 ± 1.0 using potassium hydroxide/imidazole reagent.

Acid molybdate 0.2ml was added to 0.2ml of the neutralized extract in a polypropylene tube followed immediately by 0.4 ml petroleum

spirit/methylpropanolol. The tube was then vortexed for 1 minute at 1000g at 4°C.

0.25ml of the upper organic layer was transferred to a polystyrene tube containing 0.25ml ethanol. The rapid extraction of the phosphomolybdate complex into the organic solvent layer prevented hydrolysis of the organic phosphate.

Stannous chloride 0.025ml was then added and the absorbance read at 725nm after 10 minutes.

External standard

A standard curve was prepared from the stock Na_2HPO_4 containing 0.5, 1, 2, 3 & 4 mmol.

Internal standard

Solution 8 was used as the precipitating agent.

Plasma phosphate was analysed in a similar way with and without internal standard. Comparisons of the results for plasma phosphate were compared with results from a Beckman LX20 autoanalyser to check the validity of the method.

Calculations

External standards of 0.5 mmol, 1 mmol, 2 mmol, 3 mmol and 4 mmol were used in each experiment to calculate the external standard curve. Internal standards were utilised where 0.96 mmol phosphate was added to each sample. The phosphate concentration of samples was calculated from the external standard curve.

Differences between values obtained with and without the addition of internal standard were used to calculate the percentage recovery (r). To calculate the erythrocyte phosphate concentration, C , assay values were multiplied by $100/r$ then substituted into the equation,

$$C = \frac{100 B - (100 - PCV)P}{PCV}$$

Where B is the phosphate concentration in the enriched suspension and P is the phosphate concentration in plasma.

The samples were analysed in triplicate with the average value obtained with the internal standard quoted.

Data analysis

GraphPad Prism (version 4.00 for Windows, GraphPad Software, San Diego California USA) was utilised for all analyses. Repeated measures ANOVA with Bonferroni post-test analysis was used to determine the significance of changes in phosphate concentration with time. A p value of <0.05 was deemed significant.

Unfortunately, it proved to be extremely difficult to carry out this study due to the intensity of the sampling intervals and the sample preparation involved. Ten studies were initiated but results from only five were included in this analysis.

8.3 Results

The precision of the method for calculating phosphate concentrations was established from the standard curves ($y=0.28x$, $r^2=1$), Figure 1. The comparison of plasma phosphate concentrations measured using this method with values obtained on the same plasma samples analysed using a Beckman LX20 autoanalyzer ($y=0.99x$, $r^2=1$), is shown in Figure 2.

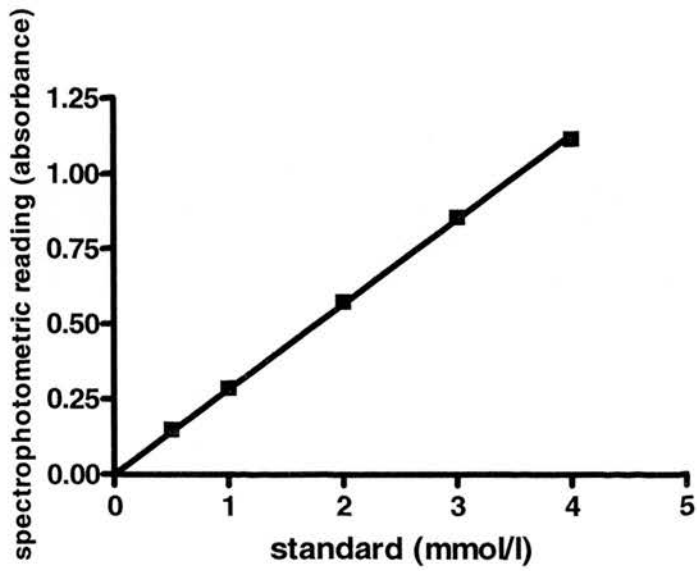


Figure 8.1 Standard curve.

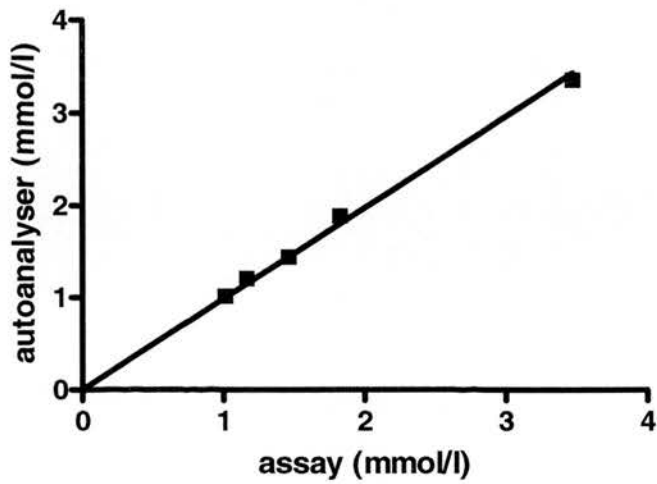


Figure 8.2 Plasma phosphate concentration. Comparison of assay results vs. autoanalyser

Previous observations have shown that urea is cleared by two-pool kinetics whereas phosphate reaches a plateau after the first 60-90 minutes of dialysis. This finding is replicated in this data set, Figure 3.

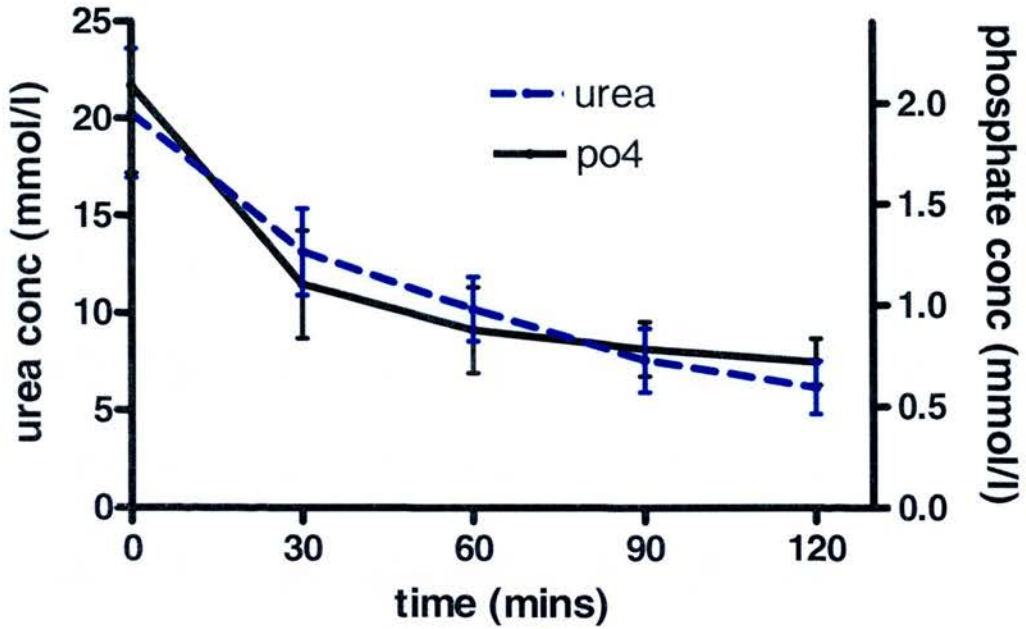


Figure 8.3 Comparison of urea and phosphate kinetics during dialysis. Error bars are \pm SEM.

Plasma phosphate levels fell from 2.08 ± 0.43 mmol/l at the beginning to 0.72 ± 0.11 mmol/l at 120 minutes of dialysis. Erythrocyte phosphate fell from 0.61 ± 0.12 mmol/l to 0.29 ± 0.10 mmol/l, Figure 4.

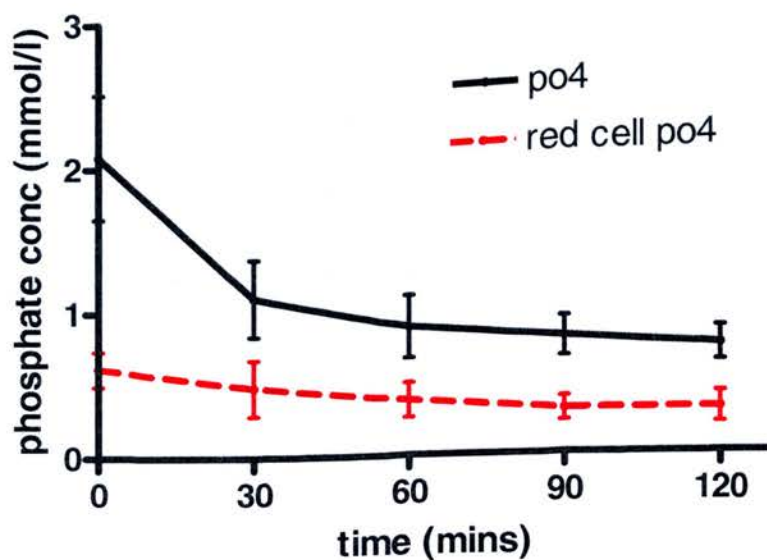


Figure 8.4 Plasma and erythrocyte phosphate concentrations during dialysis.
Error bars are \pm SEM.

The fall in plasma phosphate was significant from 0 to 30 mins and insignificant thereafter when assessed by repeated measures ANOVA with Bonferroni post-test analysis. The change in erythrocyte phosphate was not significant at any time.

The ratio between intracellular to extracellular phosphate at the beginning of dialysis was 0.29 ± 0.01 , Figure 5 and during the remainder of dialysis was 0.43 ± 0.03 , Figure 6. When the individual time points were compared, it could be seen that the ratio of the intracellular to extracellular phosphate concentration remained fairly constant after the first 30 minutes of dialysis.

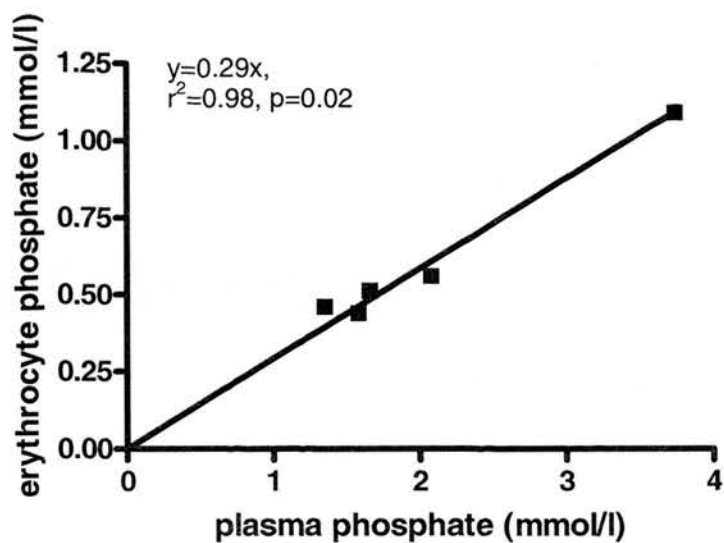


Figure 8.5. Correlation between plasma phosphate and erythrocyte phosphate at beginning of dialysis for the five treatments studied.

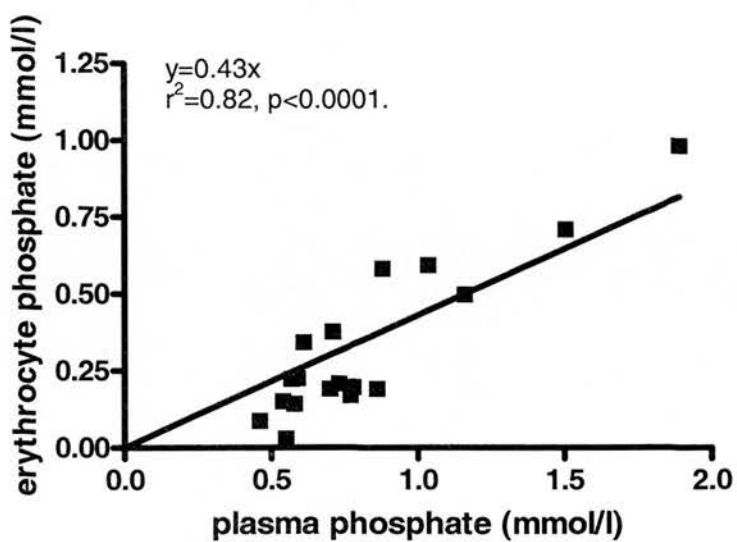
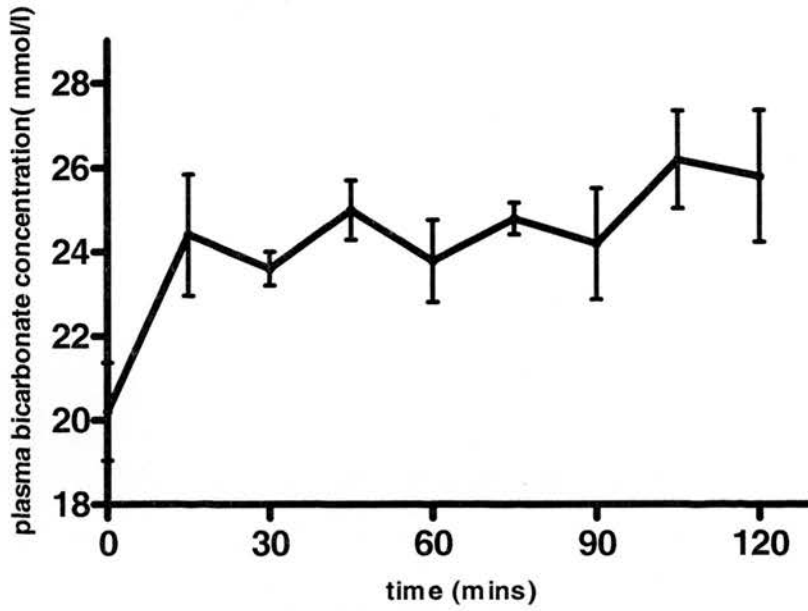


Figure 8.6. Correlation between plasma phosphate and erythrocyte phosphate from 30 minutes to end of dialysis in the five treatments studied.

All patients showed a marked change in plasma bicarbonate during dialysis with pre-dialysis concentrations of 20.2 ± 1.2 mmol/l and concentrations at 120 minutes of 25.8 ± 1.6 mmol/l, Figure 7.



8.7. Change in bicarbonate concentration with dialysis. Error bars are \pm SEM

Figure

8.4 Discussion

It was presumed that phosphate would move out of intracellular stores when a critically low level of phosphate concentration was reached. A rise in intracellular phosphate might reasonably be expected at this point. This phenomenon was not observed despite very low extracellular phosphate concentrations being reached. It has previously been shown that even when there are marked perturbations in extracellular concentration of phosphate there is a smaller percentage difference in intracellular phosphate concentration which is said to be consistent with the presence of buffers within the cell¹¹⁵. The observed maintenance of the intracellular to extracellular phosphate ratio supports the notion of buffering within the cell to maintain the trans-cellular gradient.

If phosphate moved across the cell membrane by passive diffusion alone, at steady state, it would be expected to show an intracellular to extracellular concentration dictated by the Gibbs Donnan ratio of 0.53 as previously described¹¹⁶. Taking the water content of erythrocytes (72%) and plasma (93%) into account this would give an intracellular to extracellular ratio of 0.41, which is very close to the observed intracellular to extracellular phosphate gradient of 0.43 ± 0.03 observed during dialysis in this study.

If the pre-dialysis sample is excluded the observations would be consistent with passive diffusion being the main trans-membrane transport mechanism, but other factors must be contributing at the start of dialysis. The finding of a lower intracellular to extracellular gradient at the beginning of dialysis suggests that there is an excess of phosphate in the extracellular fluid at the beginning of dialysis. The

rapid fall in extracellular phosphate in the first 30 minutes of dialysis could represent a period of time where single pool kinetics dominates, depleting this extracellular excess.

The acidic environment may also be important in altering the pre-dialysis transcellular gradient as, in the presence of acidosis, the transfer of phosphate from the intra to extracellular space is enhanced.

It can be seen from Figure 4 that the absolute difference between intra and extracellular concentrations becomes relatively small as dialysis progresses.

It is clear from previous work that, despite prolonged dialysis treatment, there comes a point beyond which phosphate does not fall further, despite ongoing removal. This data gives no evidence that the additional phosphate is liberated from the intracellular space, at least in the erythrocyte, as there is no abrupt discontinuity observed within the time course of the intracellular phosphate measurements.

It has previously been shown that even in the presence of markedly abnormal serum phosphate levels (down to 0.35 mmol/l) there is remarkably little effect on concentrations of ATP. When phosphate concentrations fall even lower, buffering mechanisms are no longer sufficient and a reduction in the levels of ATP can be seen¹¹⁴. Theoretically, prolonged phosphate depletion in haemodialysis could have similar effects. The absence of mitochondria in the red cell, while making it an attractive candidate cell for study might also explain why no intracellular perturbation in phosphate concentration is observed.

8.5 Conclusion

In conclusion, these observations support the theory that, in the erythrocyte, intracellular to extracellular transport of phosphate occurs by passive diffusion.

There appears to be a buffering mechanism within the cell to maintain the intracellular concentration and thus the trans-membrane phosphate gradient.

However, there is no evidence of a sharp discontinuity in the data that could represent breakdown of organic phosphate compounds.

Section 5

Beta2-microglobulin and Dialysis Related Amyloid

Chapter 9

Beta2-Microglobulin Kinetics in Haemodialysis

Beta2-Microglobulin Kinetics in Haemodialysis

9.1 Introduction

The “adequacy debate” is a topical issue in dialysis research. It is recognised that the definition of dialysis adequacy with reference to indices of urea clearance, does not accurately reflect the clearance of “middle molecules” such as beta2-microglobulin (11.8kD)¹¹⁷. Recent studies have suggested that there is a strong association between retention of middle molecules and patient mortality regardless of otherwise apparently “adequate” dialysis³⁷.

Beta2-microglobulin is an 11.8kD protein that is an integral part of the major histocompatibility complex expressed on the surface of all cells. It synthesised at 2-4mg/kg/day (or 980-1960mg/week in a 70kg individual) and, in the presence of normal renal function, is freely filtered at the glomerulus before being catabolised in the proximal tubule. There is no good evidence that β 2m production is increased in dialysis patients unless there is a co-existing pathology such as a clonal B cell neoplasm. In situations of reduced renal function, there is retention of beta2-microglobulin and removal by conventional dialysis techniques is poor. The clearance of beta2-microglobulin may be enhanced by use of high-flux dialysis therapies when the maximum removal is around 130mg/week¹¹⁸, which clearly falls far short of the weekly synthesis. The effect of residual renal function should not be underestimated as even a small amount of residual renal function can have a dramatic impact on β 2m levels¹¹⁹. It has been demonstrated that conversion from low-flux to high-flux dialysis¹²⁰ or by switching to nocturnal dialysis¹¹⁸ can reduce beta2-microglobulin levels although the levels can never be fully normalised. The removal

during haemofiltration is thought to be higher than on haemodialysis due to the improved clearance of higher molecular weight substances using convective therapies¹²¹.

In 1985 Geiyo identified beta2-microglobulin as a constituent part of dialysis related amyloid¹²², and it is widely acknowledged that accumulation of beta2-microglobulin is associated with development of carpal tunnel syndrome, symptomatic destructive arthropathies, and subchondral bone erosions and cysts. At present, there is no way of predicting the presence or extent of dialysis related amyloid from the serum beta2-microglobulin level.

Traditional low-flux therapies remove so little β 2m that kinetic modelling studies have been difficult. With the increasing prevalence of high-flux therapies that incorporate an element of convection, it has been possible to study the kinetic behaviour of beta2-microglobulin in more detail.

Previous modelling studies fall into two groups.

i) The first group utilised radio-labelled beta2-microglobulin to study the kinetic profile of beta2-microglobulin uptake and removal during haemodialysis^{123;124}. Odell et al studied a total of 5 anuric haemodialysis patients in two different studies and injected them with ¹²⁵I labelled β 2m. In one study, plasma activity of the tracer was followed for 72 hours and, in a second study, low-flux haemodialysis was carried out after 24 hours and high-flux dialysis after 48 hours. It was demonstrated that clearance characteristics during both types of dialysis fitted a three-pool variable volume model and that there was a significant non-renal clearance of beta2-

microglobulin from the plasma. In the study by Vincent et al¹²⁴ the excretion of ¹²⁵I labelled β 2m in 5 subjects with normal renal function and 17 functionally anephric subjects on dialysis was studied. Excretion was complete within 96 hours in subjects with normal renal function but the tracer was seen to accumulate in subjects with renal failure. There was a tendency for more residual tracer to be present in patients on haemodialysis when compared to those on haemofiltration or CAPD. A two-pool kinetic model was used to explain beta2-microglobulin removal in subjects with normal renal function but a multi-pool model was necessary in subjects with renal failure.

In summary, these studies show that, in patients with little native renal function, there is a tendency for β 2m to accumulate and that the presence of residual renal function is important in removal. The studies suggest that the kinetic behaviour of beta2-microglobulin in dialysis is more complex than can be explained by a two-pool model. These studies have given insight into some of the issues with beta2-microglobulin accumulation and removal but are difficult to apply in the clinical context due to problems with the radioactive tracer and the fact that kinetic behaviour of an injected tracer may be different to the endogenous processes.

ii) The second group used inference from modelling theory to define equations based on blood sampling during dialysis¹²⁵⁻¹²⁸. Gotch et al published the first β 2m modelling study in 1989. This group used data from serial measurement of β 2m concentration during and after high-flux dialysis to derive a multi-pool variable volume model. They identified the theoretical existence of a third pool representing polymerised β 2m. This multi-model was further developed by Da Lian et al who

emphasised the contribution of fluid shifts when considering changes in beta2-microglobulin concentrations. More recent discussion has reverted back to consideration of a two pool model taking into account the effect of changes in compartmental volumes¹²⁷. Ward et al have recently published a study of beta2-microglobulin kinetics in 10 patients receiving post-dilutional HDF. They model β 2m clearance according to a two-pool model with variable volume and conclude that haemodiafiltration fails to deliver higher β 2m removal as a result of resistance to transfer of β 2m between body compartments¹²⁸.

From the existing information, it is clear that a single pool model is insufficient to explain beta2-microglobulin kinetics. The recognition of a rebound phenomenon in the post-dialysis period^{129;130} has led the majority of investigators to conclude that there have to be at least two pools involved in the kinetic model. The importance of residual renal function and the contribution of internal convection to beta2-microglobulin removal are acknowledged. It is additionally recognised that there may be a further pool of intact beta2-microglobulin that could influence the kinetics of beta2-microglobulin removal during dialysis and that, theoretically, this pool could represent the load of dialysis related amyloid.

There seem to be several distinct stages of beta2-microglobulin deposition¹³¹. The first stage is beta2-microglobulin deposition on the surface of cartilage around joints. The layer gradually thickens, but at this stage, patients are generally asymptomatic. The characteristic sites of deposition in the musculoskeletal system may result from an apparent affinity of β 2m for collagen¹³² and glycosaminoglycans¹³³ that are found

preferentially in osteoarticular structures. With time, the deposits undergo chemical modification with polymerisation of the β 2m fibrils and interaction with advanced glycosylation end-products^{134;135}. Whether AGEs render β 2m more amyloidogenic or whether they in some way stabilise existing deposits is not known, but the absence of AGEs in early deposits is supportive of the latter theory¹³⁶. The final phase of beta2-microglobulin deposition involves a chemotactic phase where monocytes and macrophages become involved in the deposits, releasing pro-inflammatory cytokines and leading to potential destruction of tissue¹³⁷⁻¹³⁹. It is likely that patients begin to experience symptoms at this stage.

The amount of β 2m that can be removed during dialysis may depend on the equilibrium between free circulating β 2m and these different stages of β 2m deposition. When considering the development of a potential model of beta2-microglobulin kinetics it seemed logical to consider these chronic deposits of beta2-microglobulin as further theoretical pools of solute that, under the right conditions, could be mobilised by dialysis therapy.

This study of beta2-microglobulin kinetics used this theory as a basic premise for the construction of a multi-pool variable volume model to explain the observed behaviour of beta2-microglobulin during dialysis.

9.2 Methods

Study Design and Patient Selection

The dialysis program at the Lister Hospital has uniquely utilised “high-flux” techniques since the unit’s inception in 1989. The Royal Free Hospital dialysis unit is in close geographical proximity to the Lister Hospital and has a comparable population treated predominately with a “low-flux” technique. Both centres use an ultrapure water source and biocompatible membranes. This study became possible as a sufficiently large cohort of patients now exists who have never been exposed to “low-flux” membranes in over 5 years of dialysis. It has been demonstrated that membrane flux not biocompatibility is the main determinant of beta2-microglobulin levels¹⁴⁰, and it was assumed that any differences between the two groups would be due to membrane flux alone.

Beta2-microglobulin kinetics were studied in 36 patients. All patients who had received haemodialysis for in excess of five years were eligible for the study unless they had current sepsis, a chronic inflammatory disorder or malignancy. 24 patients from the Lister Hospital and 12 patients from the Royal Free Hospital were recruited. The patients at the Lister Hospital had received exclusively high-flux therapy for at least five years while those at the Royal Free Hospital, until six months prior to the study, had dialysed on low-flux membranes. At the time of the study, there were 14 patients on haemodiafiltration (HDF) and 10 on high-flux dialysis (HFHD) at the Lister Hospital. Of the 12 patients at the Royal Free, 8 patients were receiving low-efficiency haemodialysis (IFHD) and 4 were receiving high-efficiency dialysis using high-flux membranes (HFHD). For the purposes of baseline data, the patients were

grouped according to their hospital, while for the modelling studies they were grouped according to their current modality of dialysis.

At the time of the study, the Lister Hospital had a policy of dialyser re-use. It has been shown that the peracetic acid technique when used to reprocess high-flux synthetic dialysers has no effect on the clearance of β_2m ¹⁴¹.

Sampling Technique

The elimination of beta2-microglobulin by dialysis was assessed from blood samples taken every fifteen minutes during dialysis, and after a two-minute washback.

Dialyser clearance was calculated from paired arterial and venous samples taken every 30 minutes during dialysis according to method 2 described in Chapter 4. The entire volume of spent dialysate was collected for the measurement of beta2-microglobulin. Beta2-microglobulin was measured in all samples using an immunoturbidimetric technique (Olympus systems). This method was capable of measuring the very low beta2-microglobulin levels present in some dialysate samples. Samples obtained at the Royal Free dialysis unit were centrifuged and kept refrigerated at 4°C until transported to the Lister Hospital. These samples were analysed in a single run within 8 hours which has been demonstrated to give consistent results. There is limited interference with this technique in the presence of icterus (<3%), haemolysis (<5%) and lipeamia (<10%) and no documented interference in the presence of uraemia or in the range of physiological pH¹⁴².

Kinetic Modelling

The measured dialyser clearance ($K_{d \text{ meas}}$), Q_b , Q_d , dialysis time (T_d), sex, pre- and post-dialysis weights, and HDF rate were provided as inputs to the model.

Mathematical expressions describing a multi-pool variable volume model were written from first principles and introduced into commercially available software (VisSim, Visual Solutions Inc) to provide dynamic simulation of the haemodialysis environment. The model was then further adjusted and refined to give an accurate fit with the data as assessed by cumulative variance between the data and model.

Hypotheses regarding potential mechanisms were then developed. This is a similar approach previously used in the modelling of phosphate kinetics in Chapter 6.

The kinetic model for patients on low-efficiency dialysis was expected to reveal little clearance of beta2-microglobulin. The model in high-flux dialysis and haemodiafiltration was anticipated to be similar, but with different clearance characteristics relative to the different contributions of diffusion and convection.

In a previous study¹⁴⁰ in similar patient groups, a considerable difference in beta2-microglobulin levels between high-flux and the low-flux dialysis was seen (22.3 ± 5.4 mg/l vs. 43.4 ± 13.7 mg/l). Based on these observations the power of this study was deemed sufficient to detect differences in beta2-microglobulin kinetics between the groups.

9.3 Results

The demographics for the patients recruited from the two centres are shown in Table 1.

Table 9.1 Demographics of patients recruited to study on basis of hospital

	Lister	Royal Free	
Male:Female	17:7	6:6	
Age (years)	65.4±12.7	57.0±16.5	p=ns
Time on dialysis (years)	7.8±2.2	13.0±10.5	p=0.03
Pre-dialysis β 2m level (mg/l)	28.7±8.0	36.5±12.4	p=0.03

The demographics, dialysis parameters and pre-dialysis bloods for the groups of patients based on dialysis modality are shown in Table 2.

Patients receiving low-efficiency intermediate-flux dialysis had been on dialysis for significantly longer, had higher pre-dialysis β 2m concentrations, lower percentage reduction in β 2m level, lower β 2m clearance and lower mass removal of β 2m when compared to those on high-flux dialysis or HDF. The only significant difference between the patients on high-flux dialysis and HDF was in the plasma clearance of β 2m achieved by the membrane, but this did not translate into a lower pre-dialysis β 2m level, a higher mass removal of β 2m or higher percentage reduction in β 2m levels.

Table 9.2 Demographics and dialysis parameters of patients recruited to study on basis dialysis modality

	Intermediate flux (IFHD)	High flux (HFHD)	HDF
Male:Female	3:5	11:3	10:4
Age (years)	54.7±18.1	68.2±12.5	61.4±12.3
Time on dialysis (years)	15.8±12.0	7.3±1.5*	8.4±2.6^
KRU (ml/min)	0.01±0	0.56±0.83	0.35±0.94
Qb (ml/min)	331.3±72.8	376.4±79.0	417.9±72.3^
Qd (ml/min)	706.1±129.4	796.4±13.36*	800±0*
Td (min)	225.3±17.6	204.4±41.7	186.4±32.7*
UF (ml)	2085±723.3	2163±1106	2087±831.0
Pre-dialysis β2m (mg/ml)	41.0±13.0	27.1±6.8*	30.1±8.2^
Urea clearance (ml/min)	219.6±24.2	260.2±35.1*	240.6±31.3
% β2m reduction	26.9±10.0	54.8±14.6*	61.4±16.0*
Mass urea removed (mmol)	365.5±214.3	460.4±188.5	417.3±129.4
β2m clearance (plasma) (mg/min)	6.4±6.6	50.1±18.1*	81.8±19.9 [#]
β2m clearance (whole blood) (mg/min)	3.9±13.1	81.9±38.2*	95.3±38.2*
Mass β2m removed (mg)	69.6±40.6	147.0±87.3*	148.2±57.1*
Mass β2m removed /min (mg/min)	0.3±0.2	0.7±0.3*	0.8±0.3*

- *p<0.01, ^p<0.05 when compared to IFHD
- [#]p<0.05 when HFHD compared to HDF.

Kinetic modelling

From simple inspection of the data, it was immediately clear that β_{2m} is not removed by first order kinetics. The clearance achieved is always significantly less than would be predicted by the instantaneous clearance measurements across the dialyser. This suggests that there is refilling of the vascular compartment during dialysis that acts against the expected fall of β_{2m} . When dealing with urea kinetics this refilling occurs by intracellular to extracellular transfer governed by the transfer coefficient K_{ie} . β_{2m} is confined mainly to the intravascular and interstitial spaces so any transfer would be either from the interstitial space or from generation or liberation of β_{2m} at the cell surface. This could occur either from degeneration of the solid phase or from de novo generation.

In the first instance, a two-pool variable volume model akin to that proposed by Ward et al¹²⁸ was constructed to assess the degree of fit with the data. It was not possible to achieve a good fit between the data and the model on this basis. It was decided therefore to begin the modelling again from first principles.

Several different models were constructed in the search for one that would be physiologically valid.

Model 1

In all patients it was assumed that at steady state there was a constant generation rate, G , of beta2-microglobulin of 0.16mg/kg/hr that fed into the interstitial space. In addition it was assumed that all patients had a degree of endogenous extra-renal clearance, K_{nr} , assumed to deplete the concentration in the interstitium, and a degree of residual renal function, K_r , assumed to lower the intravascular compartment concentration, Figure 1.

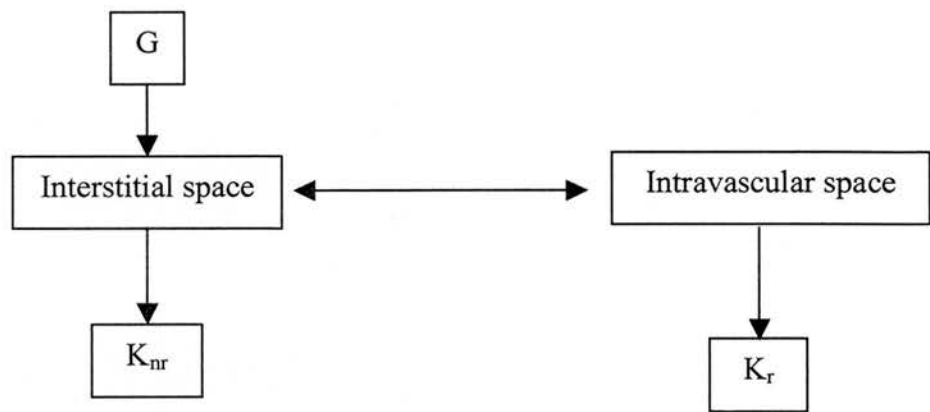


Figure 9.1 Situation at steady state with beta2-microglobulin influx from steady state generation and removal via extra-renal routes and residual renal function. Where G is the endogenous generation rate, K_{nr} is the native extra-renal clearance, and K_r is the clearance due to residual renal function.

It was assumed that when dialysis is commenced, removal of beta2-microglobulin from the intravascular space occurs according to the laws of inter-related diffusion and convection, Figure 2.

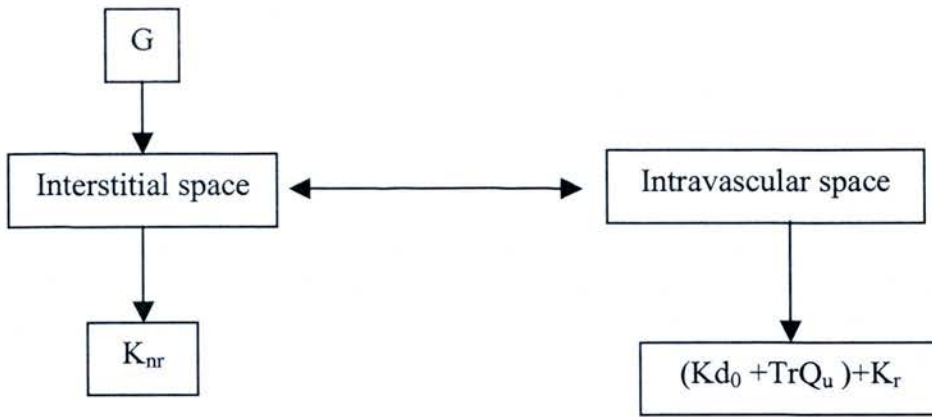


Figure 9.2. Single pool model of beta2-microglobulin removal. Abbreviations as for figure 1 plus Kd_0 is the diffusive clearance in absence of ultrafiltration, Tr is the transmittance coefficient and Q_u is the ultrafiltration rate.

An attempt was made to fit the data to this one-pool model but after the first 15-20 minutes the data deviated markedly from this, Figure 3, and thus this model was abandoned.

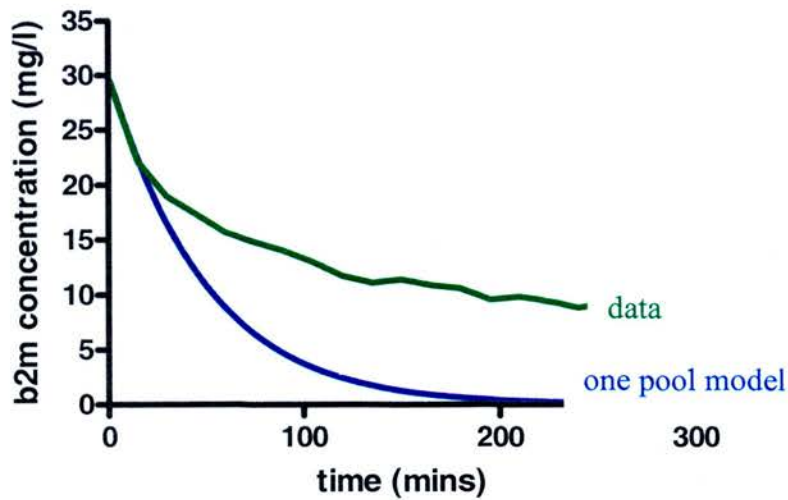


Figure 9.3. Representative treatment comparing data to Model 1 (single pool model).

Model 2

It was therefore assumed that there is movement of beta2-microglobulin from the interstitial compartment to the intravascular space by a process of diffusion in response to the fall in intravascular concentration. This would occur according to the concentration difference between the interstitial and intravascular compartments and the mass transfer coefficient across the capillary wall. Additionally, it was assumed that β_2m would transfer from the interstitial space to the intravascular space by convection as a result of fluid movement in response to ultrafiltration and vascular refilling. For the purposes of the model, it was assumed that 20% of the final ultrafiltered volume would originate from the intravascular space and 80% from the interstitial space. Obviously additional fluid fluxes occur between the intracellular and interstitial spaces but it was felt this added too much complexity to the model at this stage, Figure 4.

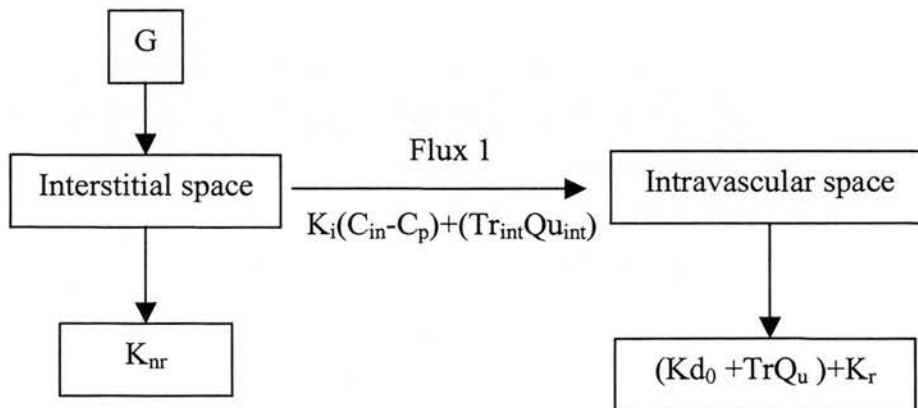


Figure 9.4. Model 2. Situation during dialysis when fall in intravascular beta2-microglobulin occurs. Abbreviations as for figures 1+2 plus K_i is interstitial to intravascular mass transfer coefficient, C_{in} is the interstitial concentration, C_p is the intravascular concentration, Tr_{int} is the transmittance coefficient for internal transfer and $Q_{u_{int}}$ is the internal ultrafiltration rate.

This two-compartment model was implemented but it was found that, although the fit improved up to around 40 minutes of dialysis, there were at least two further points where the data deviated from predicted, Figure 5.

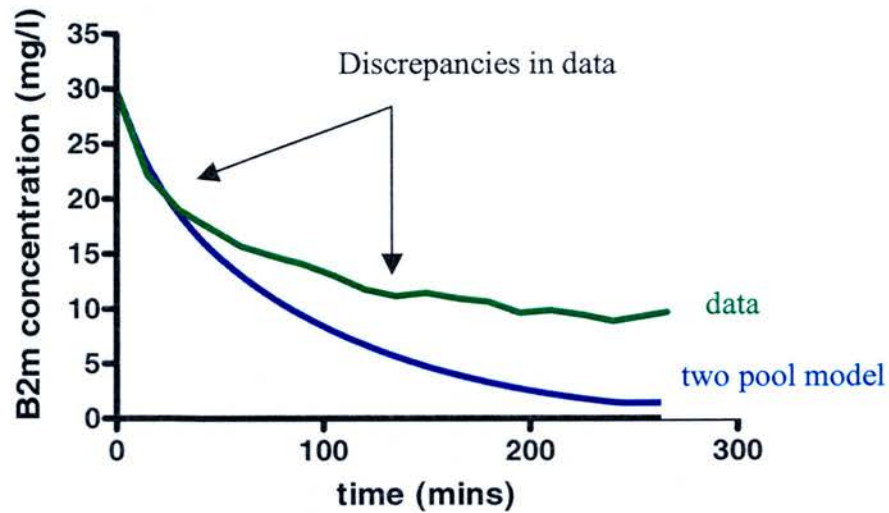


Figure 9.5. Representative treatment comparing data to Model 2 (two pool model).

It appeared therefore from the time course of the data that single pool kinetics probably operated for the first 15-20 minutes of dialysis and, during this time, beta2-microglobulin was cleared solely from the intravascular compartment. After this period, transfer from the interstitial compartment began to take place by diffusion, in accordance with two-pool kinetics. There was also assumed to be additional transfer of beta2-microglobulin by convection due to refilling of the vascular compartment from the interstitial space. The features of this two-pool model were implemented in all cases. It was assumed that the mass transfer coefficient between the interstitial space and the intravascular compartment was constant throughout dialysis for a given patient.

Model 3

After the initial 40-45 minutes of dialysis the rate of decrease of beta2-microglobulin fell. This could have been due to a reduction in the dialyser clearance of beta2-microglobulin, but there was no evidence of this from repeated estimations of clearance calculated during dialysis from simultaneous arterial and venous samples. An increased concentration could also reflect contraction of the intravascular compartment due to ultrafiltration but, based on serial haematocrit measurements, this did not appear to be a sufficient explanation. Another possibility is a rise in concentration due to an increased rate of beta2-microglobulin influx into the intravascular compartment. This either could occur because of an increased rate of generation of beta2-microglobulin de novo, or could be due to breakdown of beta2-microglobulin stores. Based on previous research which does not suggest large increases in beta2-microglobulin generation during dialysis¹²³, it was felt that the latter mechanism was more probable. It was assumed that the rate of transfer of beta2-microglobulin from this "store" would be in proportion to the ease with which the store could be mobilised and the magnitude of the store itself. A total clearance value K_2 was attributed to this flux. It was assumed that once this flux was initiated it continued at a constant rate for the remainder of dialysis. The mass transfer coefficient for transfer from the interstitial space to the intravascular space was kept unchanged, Figure 6.

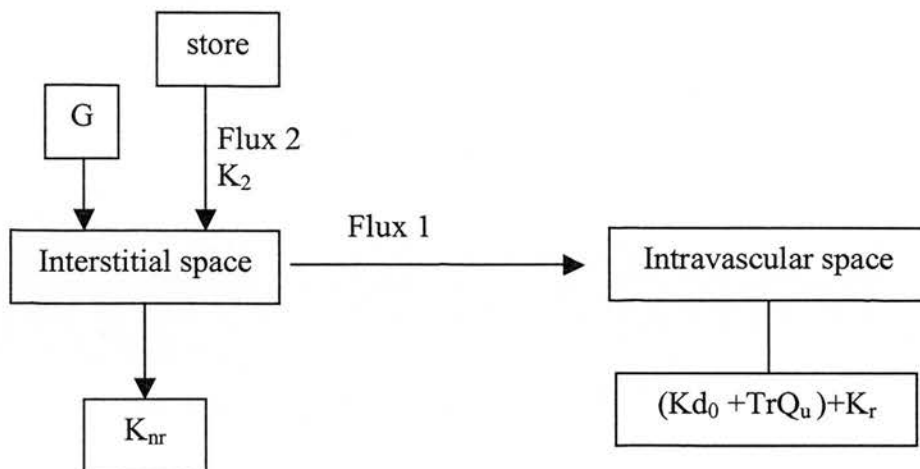


Figure 9.6 Model 3a Situation when beta2-microglobulin released from “store” into interstitial space

In the first attempt at the model, flux 2 was added to the interstitial space, Figure 6, but it was not possible to achieve a good fit with the observed data. This model was therefore abandoned. In a second attempt, flux 2 was added directly to the intravascular compartment and improved fits were obtained, Figures 7, 8.

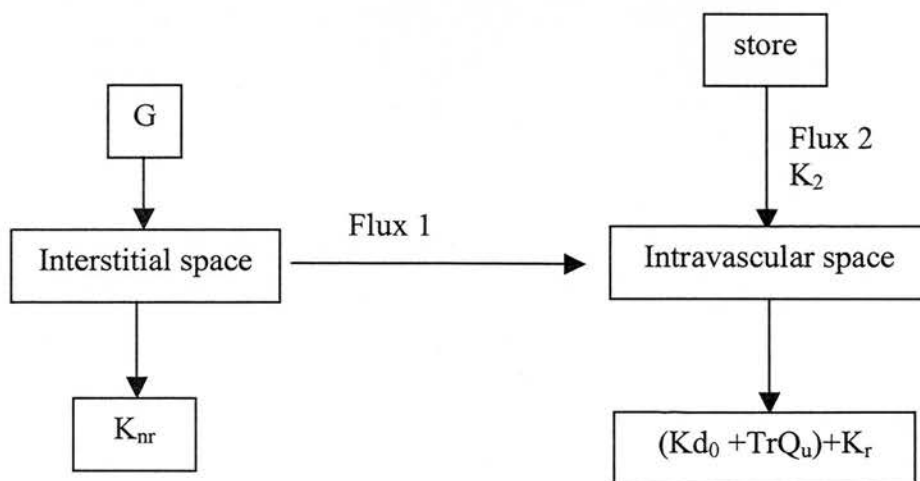


Figure 9.7 Model 3b. Situation when beta2-microglobulin released from “store” into intravascular compartment

The addition of this mechanism improved the fit once more, figure 8, but there was still one further discrepancy in the data that required to be explained.

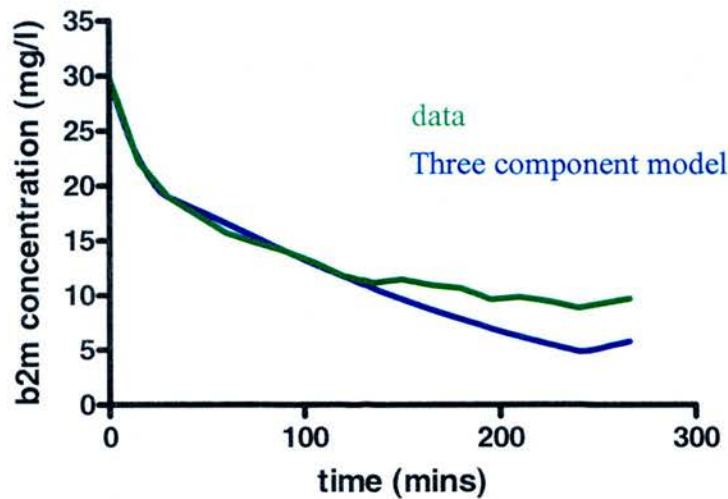


Figure 9.8. Representative treatment showing improvement in fit with implementation of flux from storage pool.

Model 4

In most of the patients, there was one final discrepancy in the data that occurred towards the end of dialysis. Removal by dialysis was ongoing as evidenced by the measured dialyser clearances but the influx of beta2-microglobulin into the intravascular space was occurring at an even higher level than before. It was postulated that this represents a phase of treatment where stores of beta2-microglobulin that have possibly been more resistant to breakdown finally become available for dialysis and occurred at around 90 minutes into dialysis.

The magnitude of this response could correlate in some way with the extent of beta2-microglobulin deposition and therefore dialysis related amyloid. No inference was

made about the origin of this additional influx (represented by K_3) and it was not assumed to depend on the prevailing intravascular concentration, Figure 9.

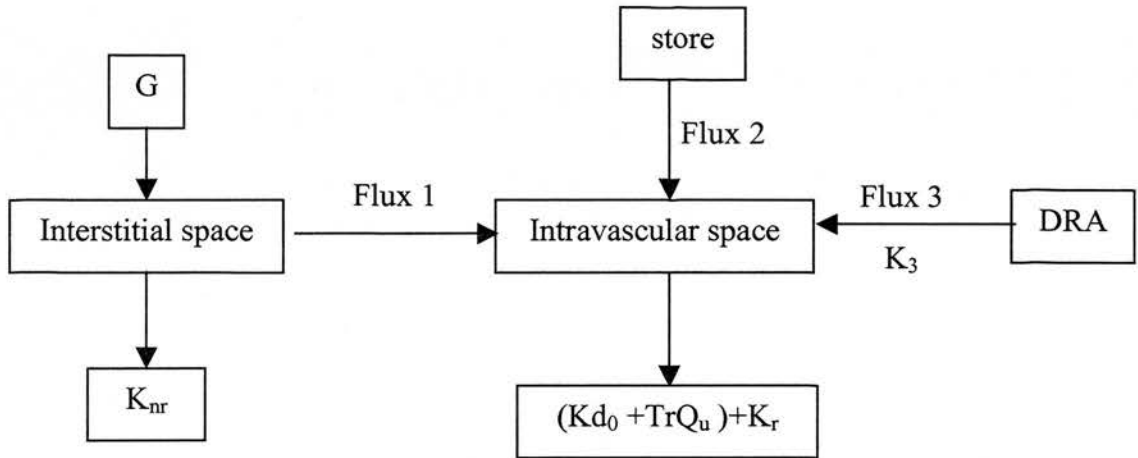


Figure 9.9. Model 4. Implementation of flux from potential dialysis related amyloid stores.

When this final flux was added to the model, it was possible to achieve excellent fits between the data and the model, Figure 10. The cumulative variance curve that gives a quantitative assessment of the goodness of fit is shown in figure 11. The cumulative variance at $Td+2$ for this treatment was 6.9.

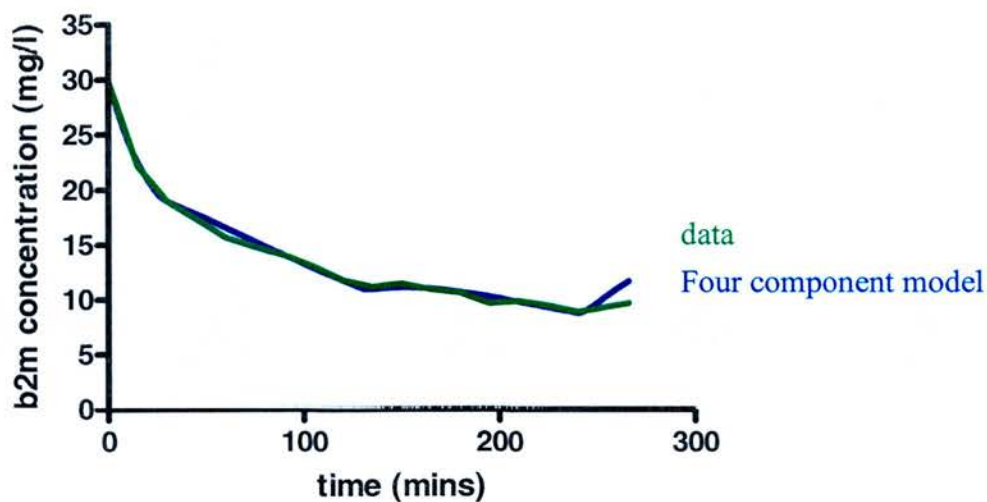


Figure 9.10. Representative treatment with Model 4 (four component model) implemented.

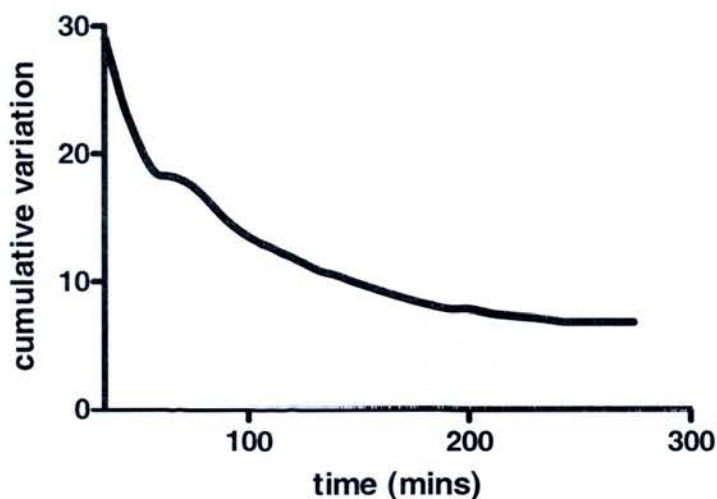


Figure 9.11. Cumulative variance curve for representative treatment shown in figure 10.

There were 25 patients who fitted the four-component model and 11 who did not.

Whether or not a fit was achieved was determined by visual inspection of the curves and the calculation of a cumulative variance of $< 20 \text{mmol}^2/\text{l}^2$ at $\text{td}+2$.

There were several distinct differences between the two groups of patients shown in table 3.

Table 9.3. Summary of patient characteristics

	Fit	Non-fit	
HFHD/HDF:IFHD	25:0	3:8	
Measured plasma β 2m clearance (ml/min)	69.2 \pm 21.9	13.0 \pm 14.6	p<0.001
% β 2m reduction	61.5 \pm 11.9	27.7 \pm 10.2	p<0.001
Initial β 2m (mg/l)	28.8 \pm 7.6	38.0 \pm 12.1	p<0.01
Qb (ml/min)	396.8 \pm 78.0	334.0 \pm 66.0	p<0.05
Qd (ml/min)	798.0 \pm 10.0	755.0 \pm 92.6	p<0.01
Td (min)	194.4 \pm 38.7	217.5 \pm 24.1	p=ns

The clear difference between the groups was the membrane utilised during dialysis and thus the beta2-microglobulin clearance. All the patients in the fit group dialysed using a high-flux membrane Arylane H9, HF80, 18GWS, Polyflux 21S or PAN11. The majority of the patients in the non-fit group used HF60LS or HF80LS although there were also one each of HF80, KF201 and 18GWS.

Using the clearance measurements for inulin as an approximation for β 2m clearance, the mass transfer coefficient-area (KoA) for each of these membranes can be estimated. It is clear that the membranes in the fit group have a far higher KoA for β 2m compared to the membranes in the non-fit group with the exception of the 18GWS. Issues with regards to the ability of this membrane to remove middle molecules have previously been noted¹⁴³. The one patient using an HF80 membrane

in the non-fit group had reused the membrane 18 times which perhaps explains this discrepancy as no other patient had reused a membrane this many times. The urea clearance obtained by this membrane was also far lower than would have been predicted.

Where the measured instantaneous $\beta_2\text{m}$ clearance across the dialyser was $<28\text{ml/min}$ it was not possible to fit the data to the model. For all patients with a measured $\beta_2\text{m}$ clearance $>28\text{ ml/min}$ the fit was achieved with a mean cumulative variance of 13.7 ± 10.0 at T_d+2 .

The magnitude of the different fluxes and dialysis characteristics for the 25 treatments that fitted the model are shown in table 4. There was a significant correlation between the pre-dialysis $\beta_2\text{m}$ level and the magnitude of flux 1 ($p=0.03$). This suggests that when there is a high prevailing $\beta_2\text{m}$ level there is a large pool of accessible $\beta_2\text{m}$ that is readily available for dialysis. The magnitude of flux 3 correlated with the percentage $\beta_2\text{m}$ reduction indicating that the mobilisation of $\beta_2\text{m}$ is necessary to achieve maximal $\beta_2\text{m}$ removal. There was, however no correlation with the pre-dialysis $\beta_2\text{m}$ level. Using multiple regression analysis it was not possible to find any other significant relationships between any of the clearance parameters, mode of dialysis, type of dialyser and magnitude of fluxes 2 or 3. Based on this model is only possible therefore to comment with any certainty on the size of the circulating $\beta_2\text{m}$ pool at the onset of dialysis.

Table 10.4. Dialysis parameters and magnitude of flux in treatments fitted to model 4

Dialyser membrane	Flux 1	Flux 2	Flux 3	Q_{HDF} (ml/min)	Measured plasma clearance (ml/min)	Pre-dialysis β_2m (mg/l)	Percentage reduction β_2m
Arh9	8.1	37	13	0	45.21	23.88	75
Arh9	18	62	43	90	109.33	24.98	67.3
Arh9	14	40	44	100	62	26.69	73.3
Arh9	6	18	35	0	61.27	35.22	69.2
Arh9	16	40	40	110	70	27.97	32.7
Arh9	13	55	55	70	87.63	35.76	64.9
Arh9	20	70	10	0	69.52	29.2	43.3
Arh9	8	34	12	0	55.98	26.19	70
Arh9	12	40	50	0	66.03	33	79.8
Arh9	14	50	45	0	68.17	29.8	41.8
Arh9	12	55	35	60	69.53	28.65	56.8
Hf80	15	30	8	0	58.85	19.88	75.5
Hf80	16	17	30	90	101.47	25.92	75.5
Hf80	13	85	32	110	105.31	30.25	55.7
Hf80	2.8	29	25	90	66.08	32.16	70.6
Hf80	25	41	13	0	60.09	15.23	77.4
Hf80	9	30	29	0	38.01	39.49	85.3
Hf80	1	3	11	0	37.15	25.4	70
Hf80	12	36	6	70	70.32	21.79	60.7
KF201	5	30	10	50	57.58	24.53	77.3
PAN11	4.3	31	0	90	92.21	54.97	43.1
POLYFLUX 21S	6	11	2	0	28.25	31.58	60.3
18gws	8	40	22	0	70.48	24.15	69.5
18gws	30	100	65	110	98.25	23.86	59.8
18gws	4	38	10	100	75.96	30.04	70.8

10.4 Discussion

The model described here gave a good fit with virtually all patients receiving high-flux dialysis therapies. It assumed that there is a large amount of beta2-microglobulin sequestered in the body, some of which is directly accessible at the beginning of dialysis, and some that requires time to be released. A pictorial representation of this is given in figure 12.

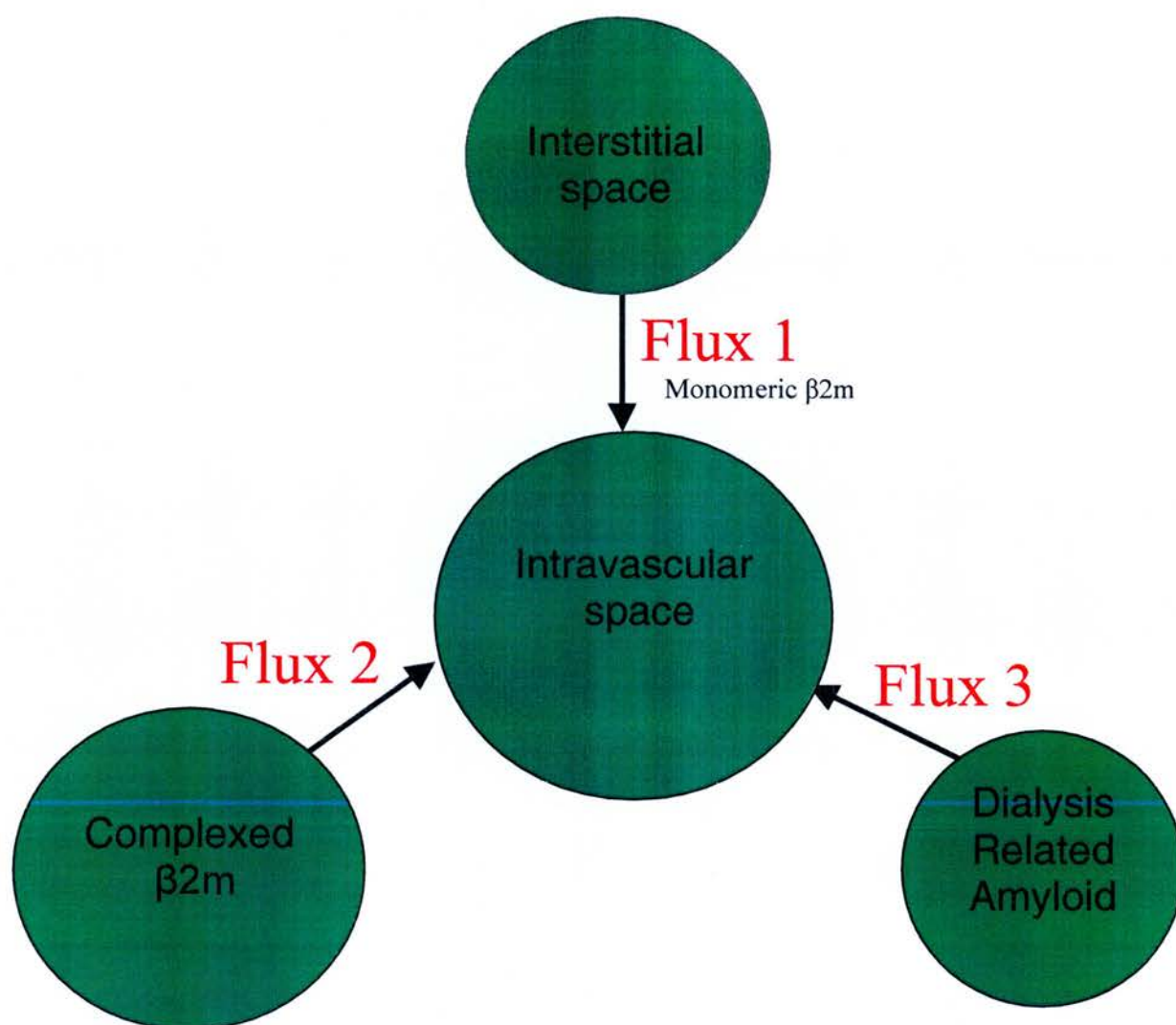


Figure 9.12 Pictorial representation of proposed model

As dialysis progresses it appears, from blood-side measurements, that the clearance of β_2m falls. This can be explained in one of three ways. The first is that the clearance truly does fall due to changes in the membrane but this is disproven by the serial estimations of clearance across the dialysis membrane, which is not seen to fall significantly. The second possibility is that blood concentrations rise artefactually due to contraction of the intravascular space, but the magnitude of the changes in haematocrit seem to argue against this. The third possible explanation, and the one that we propose here, is that there are a number of different pools of β_2m in the body that become available at different times depending on the duration and intensity of the dialysis session. As each of these pools becomes available, they have the effect of increasing the prevailing β_2m concentration, which results in the measured extracellular β_2m concentration falling less slowly.

As stated in the introduction, several different studies have postulated that there are different stages of β_2m amyloid deposition. Assuming that some of the β_2m is circulating in monomeric form this would become available for dialysis relatively quickly, and would be represented by flux 1. β_2m that is bound reversibly could be represented by flux 2, which operates from around 40 minutes of dialysis. The β_2m that is complexed and deposited with other substances ($A\beta_2m$) would be relatively more stable and much less likely to be available for dialysis unless the duration and intensity were increased. This would be consistent with the presence of flux 3, which does not become operational until at least 90 minutes of dialysis have elapsed. If there was any way of predicting the load of dialysis related amyloid from the model it would be by inspection of the magnitude of these fluxes. Unfortunately, at present,

there does not appear to be any way of utilising this information in a clinically relevant way.

The finding that some of the high-flux membranes did not operate in a high-flux manner is concerning. In one of the situations, it can be explained on the basis of reuse where clearly the membrane was less efficient in terms of both small and middle molecule clearance. In the other situation however, the membrane was being used for the first time and clearly did not deliver the projected performance. Within a dialysis unit it is therefore of utmost importance that the efficiency of high-flux membranes in terms of middle molecule clearance as well as small solute clearance is assessed.

The inter-compartmental resistance to beta2-microglobulin removal has previously been suggested as a potential problem in the clearance of beta2-microglobulin during dialysis¹²⁸. The failure of haemodiafiltration to reduce the pre-dialysis levels of beta2-microglobulin is a direct result of this phenomenon. Even in the presence of a more efficient membrane, it is not possible to remove more β 2m unless the treatment time is increased to permit mobilisation of β 2m from stores. In addition to increasing the duration of therapy, the provision of a more frequent dialysis schedule is likely to enhance β 2m removal, as more of the β 2m load will be mobilised and available for removal. In the short term, this might be reflected in higher circulating β 2m levels. I am not aware at this time of any studies that have addressed this question specifically. One further method of achieving enhanced β 2m removal is through the use of specific absorption columns. Studies of these have shown improved removal

of $\beta 2m$ ¹⁴⁴ and possibly an improvement in joint-related symptoms but it is not clear if the benefit relates to removal of $\beta 2m$ itself or to related uraemic toxins¹⁴⁵.

9.5 Conclusions

The model proposed here is a multi-compartmental model that is based on the premise that beta2-microglobulin exists in many different phases of accumulation and deposition. The model sequentially implements fluxes from different pools of beta2-microglobulin depending on their relative resistance to mobilisation. It seems probable that current convective therapies will remain insufficient to achieve neutral $\beta 2m$ balance unless the duration and/or frequency of therapy are increased.

Chapter 10

The Use of SAP Scans in Chronic Dialysis Patients

The Use of SAP Scans in Chronic Dialysis Patients

10.1 Background

It has been recognised for over three decades that patients on long-term dialysis are at risk of developing dialysis related amyloidosis (A β 2m) with the first report of carpal tunnel syndrome in a dialysis patient in 1975¹⁴⁶. This type of amyloid has a propensity for musculoskeletal tissues manifesting as bone cysts and destructive arthropathies, especially carpal tunnel syndrome. The largest studies of amyloid prevalence in haemodialysis have looked at post-mortem findings, with the histological prevalence estimated to be 48% in patients dialysed for a median of 4 years⁵⁷. There are also reports of visceral involvement although this occurs almost exclusively in patients dialysing for in excess of 10 years and is less often symptomatic¹⁴⁷. The studies that have attempted pre-mortem assessment have largely used either tissue biopsy or x-ray documentation as diagnostic tools¹⁴⁸. The use of biopsy techniques is limited by the potential for sampling error and the invasive nature of the technique. Imaging of dialysis related amyloid by plain x-rays, CT scanning or ultrasound is sub-optimal, particularly for early disease. MRI is probably the most useful radiological investigation, but even this does not yield infallible results^{149;150}.

Since the 1980s the technique of Serum Amyloid P component (SAP) scanning has been developed at The National Amyloidosis Centre allowing a non-invasive, highly specific, semi-quantitative assessment of total amyloid load¹⁵¹⁻¹⁵³. SAP is present as a component of all types of amyloid deposit and is derived from circulating serum

amyloid P (which represents 0.04% of serum protein but 15% of amyloid deposits)¹⁵⁴. SAP combines non-covalently to amyloid fibrils in a calcium-dependant manner and is in dynamic equilibrium with the SAP in the circulation. The function of SAP is not known but there are suggestions that it prevents proteolytic breakdown of amyloid deposits¹⁵⁵.

SAP Scan Technique

Following intravenous injection of ¹²³I-labelled SAP, distribution of the tracer is seen in the circulating pool of native SAP. In normal subjects the half-life of the tracer in the plasma averages 31 hours but in patients with massive amyloidosis the half-life is very much shorter. Where there are amyloid deposits, the tracer enters these due to the dynamic equilibrium between the circulating and deposited SAP and, once localised there, is cleared much more slowly than it is from plasma. The presence of this sequestered SAP in amyloid deposits is then identified on scintigraphic imaging. The SAP scan is most effective in picking up amyloid deposits in joints distant from the central body-pool such as hands and knees.



Figure 10.1. SAP scan in a normal individual

Clinical Utility of SAP Scans

The value of the scans has been demonstrated in patients with all types of systemic amyloidosis where studies show regression of amyloid deposits following treatments aimed at reducing the level of circulating amyloid P component precursors^{156,157},

Figure 2.

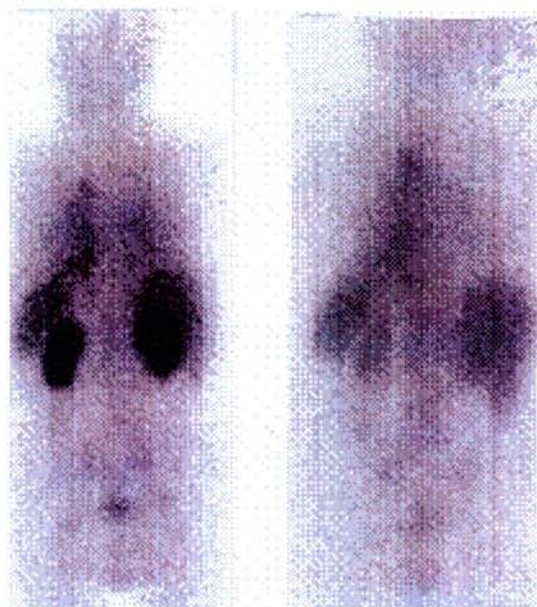


Figure 10.2 Serial SAP scans after treatment for systemic AL amyloid showing regression of amyloid deposits, left, before treatment, right, 6 years later after chemotherapy. <http://www.ucl.ac.uk/medicine/amyloidosis/research/index.html>

In the dialysis population, there has been little rigorous research on the use of SAP scans to determine the presence and extent of beta2-microglobulin associated amyloid (A β 2m) deposition. The original studies carried out by Nelson et al in 38 dialysis patients suggested that the sensitivity of the technique was good, but did note that there was poor definition of amyloid deposits in the hips even when symptoms were apparent. The specificity of the scan appeared good as there was no uptake of tracer in six patients who had dialysed for less than 1.5 years, but could also be

questioned due to the uptake of tracer by the spleen in 36% of patients¹⁵¹. This was interpreted at the time as being evidence of splenic amyloid but, in a subsequent post-mortem study, splenic amyloid has only been detected in 5% of patients⁵⁷. Some concern has also been raised about the inability of the SAP scan to differentiate between A β 2m and non-beta2-microglobulin amyloid deposits due to the ubiquity of SAP in amyloid deposits of all types.

The majority of evidence for the use of SAP scans in patients with renal failure comes from studies looking at the regression of amyloid deposits following successful renal transplantation, but even these studies are limited. In one study, there was a reduction in the extent of A β 2m seen on serial SAP scans in eight out of nine transplanted patients, studied over a five year period¹⁵⁸. Other studies examining the effect of transplantation on A β 2m have concluded that there is no doubt that the symptoms of A β 2m resolve in the post-transplant period, but that there is no definite evidence of regression in amyloid deposition¹⁵⁹⁻¹⁶¹.

β 2m scintigraphy

Due to the potential problems with SAP scintigraphy, alternative tracers that are more specific for β 2m amyloid have been proposed. Floege et al report improved specificity for A β 2m with the use of ¹³¹I- β 2m, ¹¹¹In- β 2m and ¹¹¹In-rh β 2m scintigraphy¹⁶². These tracers are not currently available for use in the UK but do offer the potential for increased diagnostic accuracy in dialysis patients. These tracers are not suitable for use in the post-transplant period as they suffer from rapid excretion of the tracer in the presence of residual renal function.

The pathophysiology of A β 2m is incompletely understood and the best strategies for preventing and treating A β 2m have not yet been fully established. As more patients spend long periods of time on haemodialysis the clinical need for assessment of amyloid load is likely to increase.

The study presented in this thesis aimed to determine whether high-flux therapies have beneficial effects on the formation of dialysis related amyloid as detected by SAP scanning. An additional aim was to correlate the results of the SAP scans to the study of beta2-microglobulin kinetics. There has been little rigorous research studying beta2-microglobulin kinetics and dialysis related amyloid in tandem and, to my knowledge, none in such well-defined populations. It is unlikely to be practical to perform SAP scans on more than a small number of haemodialysis patients due to geographical and financial considerations, so the development of a method to predict amyloid load from beta2-microglobulin kinetics without the need for a SAP scan would be an important advance.

10.2 Methods

Fifteen patients were studied, all of whom had participated in the study of beta2-microglobulin kinetics described in the preceding chapter. Not all subjects included in the previous study could be recruited to this study due to the extent of their co-morbidities and the difficulty of travelling to London on two consecutive days. Seven

of the fifteen patients were from the Lister Hospital and dialysed using haemodiafiltration (6) or high-flux dialysis (1). The remainder were from the Royal Free Hospital with four receiving high-flux dialysis and four intermediate-flux dialysis. SAP scans were carried out at the National Amyloidosis Centre. The patients received an injection of ^{123}I -labelled purified serum amyloid P component (SAP) following a routine dialysis session then re-attended the following day for whole body SAP scintigraphy. The results were scored based on activity at six joints (knees, wrists, shoulders) and were assessed by a single observer who had no knowledge of the patient's dialysis history. The following scoring system was used to give a maximum score for an individual patient of 18.

No activity =	0
mild signal =	1
moderate signal =	2
strong signal =	3

10.3 Results

The scores obtained from the SAP scans and the dialysis parameters for the 15 patients are given in table 1. All except one of the patients had evidence of dialysis related amyloid on the SAP scan, but only four were symptomatic. An example of the scan output in a patient with amyloid deposition at the wrists is shown in Figure 3.

Table 10.1 SAP scan scores and dialysis parameters

Patient	SAP scan score	Hospital	Dialysis membrane	Dialysis modality	Measured		Age (years)	KRU (ml/min)	Length of time on dialysis (years)	Fits with 4 pool model	Symptomatic Arthropathy or carpal tunnel surgery
					dialyser clearance (ml/min)	Pre-dialysis β_2m (mg/l)					
1	7	Lister	Hf80	HDF	66.1	32.2	71	0.01	19.8	Yes	No
2	5	Lister	Arh9	HDF	109.3	25.0	67	0.01	11.5	Yes	No
3	2	Lister	Hf80	HDF	70.3	21.8	61	3.52	6.9	Yes	No
4	4	Lister	Arh9	HDF	87.6	35.8	65	0.01	10.7	Yes	No
5	2	Lister	Arh9	HDF	69.5	28.6	57	0.01	13	Yes	No
6	3	Lister	Kf201	HDF	37.1	24.5	77	0.01	8.2	Yes	No
7	3	Lister	Hf80	HFHD	57.6	25.4	70	0.66	6.5	Yes	No
8	2	Royal Free	Arh9	HFHD	56.0	26.2	70	0.01	8	Yes	Yes
9	4	Royal Free	Arh9	HFHD	45.2	23.9	75	0.01	8.7	Yes	No
10	0	Royal Free	Arh9	HFHD	69.5	29.2	43	0.01	5	Yes	No
11	3	Royal Free	Polyflux21s	HFHD	28.2	31.6	60	0.01	7.9	Yes	No
12	1	Royal Free	Hf60ls	IFHD	0	51.7	30	0.01	16.5	No	Yes
13	11	Royal Free	Hf60ls	IFHD	7.9	21.0	63	0.01	35.4	No	Yes
14	4	Royal Free	Hf60ls	IFHD	0	59.5	51	0.01	20	No	Yes
15	2	Royal Free	Hf80ls	IFHD	11.5	44.9	30	0.01	12.8	No	No

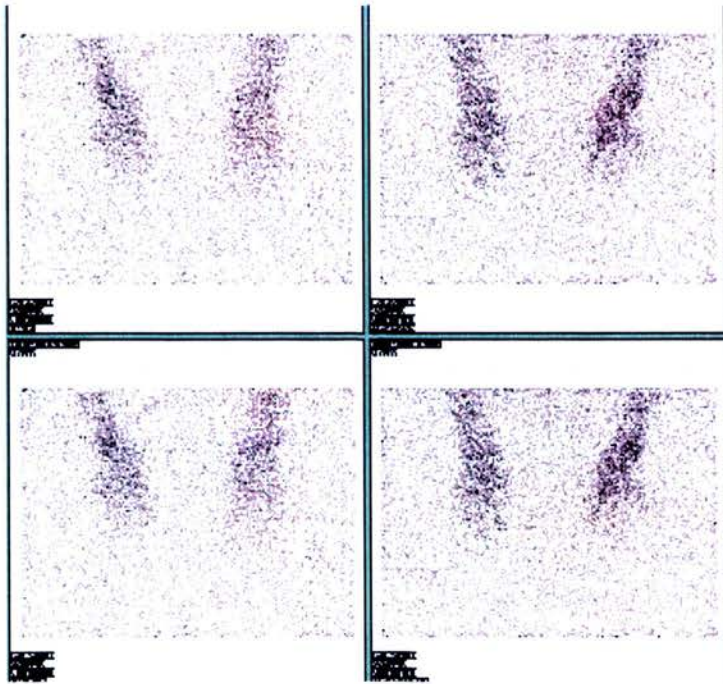


Figure 10.3 SAP scan showing uptake at wrists

Factors that may be relevant to the presence or absence of $A\beta_{2m}$ are the prevailing level of beta2-microglobulin, the age at which a patient commenced haemodialysis¹⁶³, the duration of haemodialysis treatment, the level of residual renal function, the flux of the dialyser and the microbiological purity of the haemodialysis water.

Pre-dialysis beta2-microglobulin level

The pre-dialysis beta2-microglobulin level was significantly higher in the patients receiving intermediate-flux dialysis but there was no difference between the patients at the Lister Hospital and those on high-flux dialysis at the Royal Free. The prevailing pre-dialysis level of beta2-microglobulin did not predict the score achieved on SAP scan ($p=0.47$) and, additionally, did not predict the presence or

absence of symptoms of A β 2m. The pre-dialysis β 2m level did not correlate significantly with the level of residual renal function, the measured dialyser clearance of β 2m or the length of time on dialysis but there was a significant negative correlation between the pre-dialysis β 2m level and advancing age, Figure 4.

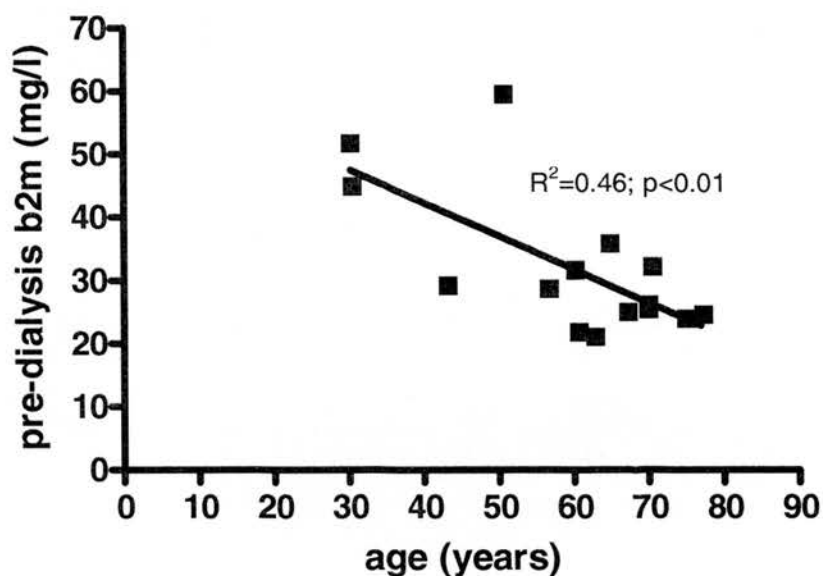


Figure 10.4. Correlation between pre-dialysis beta2-microglobulin concentration and age

Age

The age of the patients was 59.3 ± 14.9 years. The patients at the Lister Hospital were significantly older than those receiving intermediate-flux dialysis at the Royal Free Hospital, but there was no difference in the ages of the two groups at the Royal Free or between the patients at the Lister Hospital and those on high-flux dialysis at the Royal Free.

As detailed above there was a significant negative correlation between age and the pre-dialysis beta2-microglobulin level, Figure 4. There was no apparent relationship between age and the scan score or the presence or absence of symptoms.

Duration of Dialysis Therapy

The patients had been on dialysis for 12.7 ± 7.8 years. Those on intermediate-flux dialysis had been receiving dialysis therapy for significantly longer than those on high-flux dialysis or HDF. The duration of dialysis therapy correlated with the SAP scan score $p < 0.001$. Additionally, the patients who had symptomatic dialysis related amyloid had been dialysing for significantly longer than the patients who did not (20.0 ± 11.5 vs. 10.1 ± 4.1 years; $p = 0.02$).

Residual Renal Function

Very few of these patients had any residual function remaining so this was disregarded for the purposes of this analysis, and KRU for all subjects was assumed to be zero. It is clear that there is a wide variation in SAP scan score for the same level of residual function.

Membrane Flux

The pre-dialysis β_2m level was higher in patients receiving intermediate-flux dialysis when compared to those receiving high-flux dialysis or HDF, but the membrane flux did not predict the scan score or the presence or absence of symptoms of $A\beta_2m$.

All seven patients (6 receiving haemodiafiltration, 1 receiving high-flux dialysis) at the Lister Hospital had evidence of amyloid deposition but none had symptomatic arthropathy or had required carpal tunnel surgery. The duration of dialysis and the age of the patients in this group were not different when compared to all patients dialysing at the Royal Free Hospital or to the subset of patients with symptomatic arthropathies. However, when compared to the patients receiving intermediate-flux

dialysis, the patients on HDF were significantly older and had been on dialysis for a shorter period of time, Table 2.

Table 10.2 Clinical Parameters according to place of dialysis and membrane flux

	Lister HDF/HFHD	Royal Free High-Flux	Royal Free Intermediate-Flux
Pre-dialysis β 2m (mg/l)	27.6 \pm 4.9*	27.7 \pm 3.4*	44.3 \pm 16.6
Symptoms	0/7	1/4	3/4
Scan Score	3.7 \pm 1.8	2.2 \pm 1.7	4.5 \pm 4.5
Time on dialysis (years)	10.9 \pm 4.6*	7.4 \pm 1.6*	21.2 \pm 9.9
Age (years)	66.9 \pm 6.6*	62.0 \pm 14.1	43.5 \pm 13.3

*= $p < 0.05$ when compared to Royal Free Intermediate-Flux

Predictors of SAP scan score

Multiple regression analysis with backward elimination of variables revealed duration of dialysis therapy, and age to be the most significant factors in the prediction of the SAP scan score, with duration of dialysis being the strongest predictor, Table 3.

Table 10.3 Regression model to predict scan score

Variable	Coefficient	Standard error of coefficient	t	p
Constant	-5.723	1.170	-4.89	0.000
Length of time on dialysis	0.38798	0.03883	9.99	0.000
Age at start of dialysis	0.09253	0.01707	5.42	0.000

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F	p
Regression	3	90.117	30.039	34.36	0.000
Residual error	11	9.616	0.874		

Using age and length of time as predictors the regression equation is

$$\text{Scan score} = -5.72 + 0.388 * \text{length of time on dialysis} + 0.0925 * \text{age}$$

And $R^2=90.3\%$

Correlation between Kinetic modelling and SAP scans

In the eleven of these fifteen patients in whom it had been possible to model beta2-microglobulin kinetics, an attempt was made to correlate the findings of the mathematical model with the results of the SAP scans, Table 4. Using multiple regression analysis there was no clear relationship between any modelled parameter and either the scan score or the presence or absence of A β 2m. Particularly there was no relationship between the magnitude of flux 3 and the scan score.

Table 10.4 SAP scan scores and modelling parameters.

Patient	SAP scan score	Symptomatic Arthropathy or carpal tunnel surgery	Flux 1	Flux 2	Flux 3
1	7	No	2.8	29	25
2	5	No	18	62	43
3	2	No	12	36	6
4	4	No	13	55	55
5	2	No	12	55	35
6	3	No	5	30	10
7	3	No	1	3	11
8	2	Yes	8	34	12
9	4	No	8.1	37	13
10	0	No	20	70	10
11	3	No	6	11	2

10.4 Discussion

It is generally accepted that in the era of low-flux dialysis therapies the longer a patient spent on dialysis, the higher the beta2-microglobulin level became, and the higher was the likelihood of developing A β 2m. There is a widely held view that the introduction of high-flux therapies may have altered this natural progression of disease⁶⁰. The results presented here are entirely consistent with the first observation, but give no evidence that high-flux therapies prevent the deposition of A β 2m as

detected on a SAP scan. The results indicate that the only factors that influence the amount of A β 2m detectable on a SAP scan are the duration of dialysis therapy and the age of the patient.

Duration of dialysis therapy

The average duration of dialysis in these fifteen patients was 12.7 years [range 5.0-35.4 years] so the prevalence of symptomatic A β 2m would be expected to be high. The finding that 93% of patients had evidence of A β 2m on a SAP scan is consistent with previous observations but the 27% prevalence of symptoms is lower than estimates in the literature. The patients with symptoms had been on dialysis significantly longer than the rest of the group as a whole, but not longer than the subset of patients on haemodiafiltration.

Age

The observation of a relationship between chronological age and propensity to develop A β 2m is consistent with all previous work in this field. In vitro studies suggest that the propensity for dialysis related amyloid to deposit in musculoskeletal tissues may be due to its obligate co-deposition with collagen¹³². It is possible that degenerative change in the osteoarticular structures acts as a nidus for deposition of β 2m. The presence of osteoarticular damage is certainly one plausible explanation for the increase in peri-articular amyloid deposition in the elderly.

Against this explanation is the finding that the prevailing levels of beta2-microglobulin are lower in the older patients. Kabanda et al have previously noted lower beta2-microglobulin levels in the elderly and showed in multiple regression analysis that age was negatively correlated to β 2m concentrations when corrected for residual renal function and time on dialysis¹⁶⁴.

Whilst, at first, these two observations seem contradictory, they perhaps suggest that, in the elderly, the dynamic equilibrium in beta2-microglobulin is shifted away from circulating β 2m and towards deposition of A β 2m deposits. This could be due to the presence of a favourable environment for formation of A β 2m.

A second potential factor is the higher level of advanced glycosylation end products in the elderly. Advanced glycation end products (AGEs) are formed by the Maillard Reaction where nonenzymatic glycation and oxidation (glycoxidation) reactions occur between carbohydrates and proteins. This reaction renders proteins more resistant to degradation. Levels of these modified proteins are elevated in situations of impaired renal function in diabetics and non-diabetics alike. The concentration of AGEs additionally rises in an age-dependant manner even in the presence of normal renal function¹⁶⁵. Beta2-microglobulin seems to bind preferentially to AGE-modified collagen which can then be further modified by non-enzymatic glycosylation¹³⁴. This tendency could provide a further explanation of the observed association between peri-articular amyloid deposition and increasing age.

Biocompatibility

Haemodiafiltration is considered to be a more biocompatible process and less likely to activate the immune system. This would be one possible explanation for the absence of symptoms in patients on haemodiafiltration. However, there is no evidence that the patients with symptomatic amyloid deposition in this study had been exposed to a less biocompatible environment. Modified cellulose or synthetic membranes had been used in both centres for at least five years and the water quality was comparable. It seems therefore that, consistent with previous observations¹⁴⁰, the membrane flux rather than the biocompatibility of the dialysis process predicts the likelihood of developing symptomatic dialysis related amyloid.

Membrane Flux and the Importance of Co-precipitants

One of the quoted advantages of haemodiafiltration is its ability to clear increased quantities of beta2-microglobulin, but it is by no means certain that this will lead to a reduced incidence of A β 2m. This study suggests that the use of HDF or other high-flux therapies is still associated with the deposition of A β 2m but that the symptoms experienced by patients are different.

If the symptoms of A β 2m are related more to the substances that are co-deposited with beta2-microglobulin it may be that high-flux therapies prevent the accumulation of another, as yet unidentified, uraemic retention product that is acting in concert with the deposition of beta2-microglobulin to cause symptomatic dialysis related amyloid. In dialysis patients it is known that the presence of apolipoprotein E, glycosaminoglycans and proteoglycans exacerbates the formation of beta2-microglobulin deposits¹⁶⁶. Further work suggests that, in the early stages of beta2-

microglobulin deposition there is a limited inflammatory reaction, but, as time goes on, the interaction with AGEs leads to chemotactic attraction of macrophages and release of pro-inflammatory cytokines^{137;138}. It may be at this stage that destruction of the articular tissues occurs and symptoms develop. High-flux dialysis and haemodiafiltration may be acting to prevent or delay this final phase.

10.5 Conclusion

One of the most striking findings in this study is that there is no obvious correlation between the amount of dialysis related amyloid detected on a SAP scan, the prevailing level of beta2-microglobulin and the presence of symptomatic arthropathies. This leads to the assumption that the deposition of beta2-microglobulin as amyloid is not the only factor responsible for the development of symptomatic amyloid-related disease in long-term dialysis patients. If it is the final inflammatory phase that causes the symptoms of A β 2m this would be consistent with the observations in the post-transplant period of improved symptomatology without definite evidence of regression of amyloid deposition^{160;161;167}.

This study has not identified a way in which to predict the extent of A β 2m from blood analysis, and has highlighted potential problems with the use of SAP scanning as a diagnostic tool in the context of haemodialysis. The technique seems good at detecting amyloid deposits but correlates poorly with symptomatic disease. As high-flux therapy and HDF become more prevalent, it may be possible to study patients after the conversion from low-flux to high-flux therapy to determine if there is any change in symptomatology and to monitor the response of existing amyloid deposits.

Section 6

Discussion and Conclusions

Chapter 11

General Discussion and Conclusions

General Discussion and Conclusions

The work presented in this thesis arose from a recognition of the failure of modern dialysis therapy to deliver all that it offered in the early days of the technique. While the longevity of patients receiving haemodialysis has improved, the technique is not providing a good quality of life. Patients are at risk of profound morbidity and early death, principally from premature cardiovascular disease. The solutes that I chose as my principle areas of study are well recognised to be retained in the uraemic state and have been widely implicated in the pathophysiology of dialysis complications. Knowing how best to monitor dialysis adequacy is another challenge as it is clear that traditional urea kinetic modelling is an imperfect tool.

11.1 Mathematical Modelling

The technique of mathematical modelling has been shown in these studies to be a very useful and clinically applicable tool in the context of haemodialysis therapies. In order for clinicians to understand the reasons for sub-optimal delivery of dialysis, it is first necessary to understand the mechanism of solute removal. In my experience, this is an aspect of renal medicine that is poorly taught and poorly understood. Many papers on modelling of the dialysis process suffer from the complexity of the equations presented, and become very inaccessible to anyone outside of the field of knowledge. By using the modelling technique presented in this thesis I have been able to express the ideas in a manner that can be understood in principle, without needing an advanced knowledge of the underlying kinetic mechanisms. In this way, I believe the process of modelling dialysis therapy has been demystified and is made

more accessible to all. The technique of building the model from first principles has allowed the nature of solute removal to be understood in more detail and deviations from a predicted pattern identified. It is only when the barriers to solute removal are identified that strategies can be devised to improved on standard practice.

11.2 Phosphate

The modelling study in chronic dialysis patients has shown clearly that the retention of phosphate in chronic haemodialysis patients is a major obstacle to the achievement of controlled serum phosphate levels. It seems probable that the mechanism behind the multi-pool nature of phosphate kinetics has its origins in the pre-dialysis period. Chronic phosphate overload, which is probably occurring from stage 3 or 4 CKD onwards, presumably deposits in various tissues throughout the body. Once a patient comes onto dialysis, there is a potential pathway for removal of solute and some of the phosphate moves out of these stores. Unfortunately due to the relatively low amounts of phosphate that can be removed on conventional dialysis, and the large size of the store, it is almost impossible to achieve neutral phosphate balance on a thrice-weekly dialysis schedule. It seems clear that either more frequent dialysis scheduling or longer, slower treatments would improve phosphate removal. The work on diurnal variation makes this discussion more interesting. It is clear that patients dialysed during the night will be dialysing at the peak of their phosphate concentration and may therefore have an advantage in terms of diffusive clearance. The study presented in this thesis examined patients with stage 5 CKD to prevent any confounding issues from fluctuations in phosphate concentration brought about by dialysis. It is not entirely certain that these findings can be applied to the dialysis

population but it would seem reasonable to assume that similar findings will exist as long as patients maintain a degree of residual renal function. Care should therefore be taken when interpreting phosphate concentrations to consider the timing of the sample. The current pre-dialysis guidelines of a phosphate ≤ 1.8 mmol/l should also stipulate the timing of this sample. The maximum target phosphate concentration should perhaps be lower than this level to take into account the peak in phosphate concentration that occurs at night.

11.3 Beta2-microglobulin

The modelling study presented has again demonstrated a multi-pool kinetic model that explains β_2 m removal during haemodialysis. The model presented is attractive when considered in terms of potential stages of β_2 m deposition. The internal transfer of solute that occurs during the haemodialysis process again limits removal of beta2-microglobulin. In the presence of high membrane clearance there appears to be more potential for the mobilisation of beta2-microglobulin from stores but additionally the duration of dialysis therapy seems to be of importance as it takes some time for the different fluxes to become operational.

The use of high-flux therapies does seem to make a difference to the symptoms experienced by patients but does not alter the likelihood of amyloid deposition as might have been predicted. It seems most probable that high-flux therapies reduce symptoms by altering the potential for co-deposition of substances that promote an inflammatory response.

From the point of view of the SAP scans, it was disappointing that they did not add much to the clinical diagnosis of A β 2m. It is probable that the utility of this imaging technique will become more apparent when monitoring progression of disease using different dialysis techniques and schedules. Further studies using serial scanning of patients changing from low-flux dialysis to high-flux dialysis or haemodiafiltration, and studies of patients changing from thrice weekly to daily dialysis are likely to be give valuable information about the potential to modify the disease process.

11.4 New Dialysis Techniques

The majority of clinicians now accept the potential benefits that high-flux therapy can offer and, where there is an adequate water supply, this technique is often utilised. There are still a large number of dialysis units that are unable to deliver high-flux therapy due to concerns about the quality of the water supply and the potential for backfiltration. This is clearly an issue that needs to be addressed, particularly where there is an opportunity to develop new dialysis facilities.

There is increasing evidence that haemodiafiltration will produce clinical benefit although the absolute proof of this is lacking at present. It is the most physiologically valid of all the available dialysis therapies and with increasing experience of the technique, it is likely to become more prevalent. Longer-term outcome studies such as the Membrane Permeability Outcome (MPO) study that is underway in Europe may still prove it to be a superior technique.

We are probably now at the limit of what thrice-weekly dialysis can achieve and it is now time to think about the structure of service delivery. It is not surprising that an intermittent therapy that permits wide fluctuations in physiological parameters does

not deliver the optimum outcomes. In an effort to make dialysis treatments more physiological, there are two main issues to consider – the duration of therapy and the frequency of therapy.

11.5 The Time vs Frequency Debate

Time

It is clear that time is an important factor in achieving adequate dialysis. This was largely discounted in the original NCD study as the difference between short and long dialysis failed to reach significance. This led to a continual reduction in treatment times in the USA which did not really occur in Europe or Japan¹⁶⁸. It became apparent that outcomes varied in different countries and much of the difference has been attributed to the discrepancies in treatment times. In Japan where treatment times were longest, there was a significant reduction in mortality when compared to Europe and the USA. The results from the DOPPS study showed that when treatment time was >240 mins vs. <240 mins the relative risk of death was 0.81 $p=0.0005$. There was also a clear interaction between length of dialysis and delivered dose with the best outcomes in patients with highest Kt/V and longest treatment times¹⁶⁹, Figure 1.

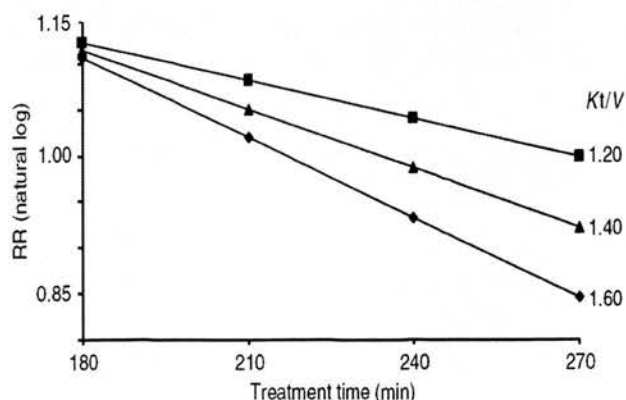


Figure 11.1

The relationship between treatment time, Kt/V and outcome.

[Reproduced with permission from ¹⁶⁹]

The experience from Tassin is in line with this with improved outcomes when a schedule involving long hours (8 hrs 3x weekly) is utilised³².

The modelling of phosphate and beta2-microglobulin kinetics helps to explain why the duration of dialysis is so important. For solutes such as urea, the duration of therapy is probably not vital providing the membrane characteristics and blood flow are optimised. The improved survival with increased $Kt/V(\text{urea})$ probably does not relate to removal of urea per se but is rather a surrogate marker for improved removal of other uraemic toxins. For solutes with resistance to internal transfer, as demonstrated for phosphate and beta2-microglobulin, allowing enough time for internal movement of solute is essential. Increasing the blood flow or membrane clearance characteristics will not necessarily lead to reductions in the pre-dialysis concentrations of these solutes as total mass removal is dictated as much by internal transfer of solute as it is by clearance across the dialyser membrane. There is currently a lot of interest in reducing the duration of dialysis therapies, which is justified on the basis of the maintenance of the urea removal characteristics. A note of caution should be sounded however, as the modelling studies presented here indicate that a reduction in duration is likely to be accompanied by a disproportionate reduction in middle molecule clearance. This appears to be demonstrated in a comparison of phosphate clearance in nocturnal dialysis (6-8 hours 6 or 7x per week) versus short daily dialysis (1.5-2.5 hrs 6 or 7x per week)⁹⁶. Short daily dialysis is seen to be little better than conventional haemodialysis in terms of control of hyperphosphataemia but nocturnal long hours haemodialysis delivers marked improvements in phosphate clearance. I am unaware of any similar studies that have been conducted comparing beta2-microglobulin clearance in short daily with

nocturnal haemodialysis but it would be expected that a similar pattern would be found.

Frequency

Increasing the frequency of dialysis brings it more in line with the physiological situation of continuous clearance. There is increasing evidence that both short daily dialysis and nocturnal daily dialysis improve control of solute concentrations and additionally improve blood pressure and general well being. It also appears that patients experience less in the way of disequilibrium symptoms with far less of a “dialysis hangover” when compared to a thrice weekly program. The propensity for development of dialysis related amyloid may be reduced with daily dialysis regimes as there is some evidence that more frequent scheduling has a beneficial effect on the levels of AGEs^{170;171}.

The future of the Time vs Frequency Debate

At present, there are no randomised controlled trials that address the issues of time vs. frequency and many of the existing observational studies can be criticised on the basis of patient selection or duration of follow-up. However, the results of the studies reported to date makes this a very interesting area for further study.

The National Institute of Health are currently recruiting for several studies to assess the impact of more frequent scheduling of dialysis on outcome. The results of these studies are likely to be of considerable interest. There are four trials that are relevant

in this field. The first two are randomised controlled trials while the other two are observational studies.

1. The impact of short daily dialysis (1.5-2.75 hrs 6x per week) vs. standard thrice weekly (3-4 hrs) dialysis
2. Comparison of nocturnal dialysis (6 hrs 6x per week) with conventional dialysis delivery (3-4 hrs 3x per week)
3. Comparison of Daily Nocturnal Haemodialysis With Daily Haemodialysis
4. Comparison of Four and Eight Hours Dialysis Sessions in Thrice Weekly Haemodialysis

In the current political climate, delivering these types of dialysis schedules within the NHS would present a huge challenge as the dialysis units are not funded or equipped for such operation. The only practical way to deliver a more frequent program of dialysis therapy is to develop the home dialysis services. With this development will come increased pressure on vascular access services to ensure that patients have stable vascular access and innovative techniques such as the use of a buttonhole needling technique may need to be developed in order to preserve vascular access. In the trials that have reported to date, failure of vascular access was not a major problem¹⁷².

11.6 New Measures of Dialysis Adequacy

By increasing the frequency of dialysis, it is possible to increase the weekly Kt/V by a far greater extent than by increasing the adequacy of the individual dialysis session

Figure 2.

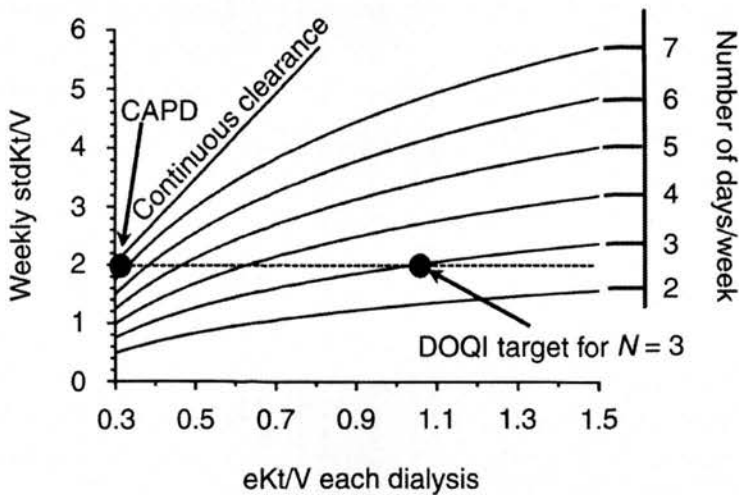


Figure 11.2 The effect of increasing adequacy and increasing frequency on weekly Kt/V.

[Reproduced with permission from¹⁷³]

New equations are needed to describe these new frequent treatments as, if clearance is continuous, the URR will be zero and the Kt/V cannot be calculated as the system is constantly in steady state with no fluctuations. The currently favoured option is the standardised Kt/V.

$$\text{standardised Kt/V} = \frac{\text{continuous removal rate}}{\text{average peak concentration}} = \frac{G}{\text{average peak concentration}}$$

To calculate the standardised Kt/V from a measured single pool Kt/V the following equation can be used. This was developed by Gotch¹⁷³ and refined by Leypoldt¹⁷⁴.

$$\text{stdKt/V} = \frac{10080 \frac{1 - e^{-eKt/V}}{t}}{\frac{1 - e^{-eKt/V}}{\text{spKt/V}} - \frac{10080}{Nt} - 1}$$

where N is the number of treatments per week, spKt/V is the measured single pool Kt/V and eKt/V is the equilibrated Kt/V.

This equation does not take into account residual renal function or changes in volume status and assumes equally spaced dialysis treatments. It is therefore not entirely accurate.

This still only gives information about small solute clearance and from the modelling studies presented here, it is clear that this falls far short of providing a description of a truly adequate treatment. Alternative or perhaps complementary adequacy measures such as beta2-microglobulin clearance have been proposed, but while there are still a large number of centres where high-flux dialysis and haemodiafiltration are not utilised, it is unlikely that estimates of middle molecule clearance will be accepted as standard measures of dialysis adequacy for some time yet.

The studies presented here give weight to the notion of duration of dialysis being of utmost importance in the achievement of adequate solute clearance when there are large deposits of solute to remove. Perhaps when the body is in equilibrium and solute generation matches solute removal the more important factor will be

frequency of therapy, as this will prevent the unphysiological peaks and troughs in solute concentration.

Only by monitoring the control of variables with different dialysis schedules will the question about the “best” therapy be answered. For patients who have been receiving dialysis for long periods of time the best delivery at present would appear to be daily long hours dialysis, but perhaps in the future when patients begin their dialysis careers on a more frequent schedule, a shorter daily regime will be adequate as they will not have the same excess stores of solute.

11.7 The Future of Modelling in Haemodialysis.

I believe that we are entering an exciting period of dialysis delivery in terms of addressing the real problems experienced by our patients. It is apparent that the traditional “one size fits all” approach to dialysis therapy is no longer appropriate and dialysis parameters need to be tailored to individual patients. As physicians, we are faced with the challenge of maximising the potential benefits of an imperfect treatment and need to facilitate alternative ways of delivering the service. It is imperative that the barriers to solute removal are understood to prevent the mistakes of the past being repeated as occurred with the reduction in dialysis duration. By understanding the kinetics underlying the dialysis process it is much more possible to recognise the barriers to solute removal and to design therapies that are specific to controlling these variables. Modelling studies can give insight into the mechanisms underlying solute clearance and can be used to predict the likely outcome from a particular dialysis schedule. As new dialysis schedules become more widespread it

will be possible to model solute clearance in the longer and more frequent therapies to determine if they truly deliver the benefits that they theoretically appear to offer. If discrepancies are then identified between the predicted behaviour and actual dialysis delivered, further investigation can be targeted to elucidate the problem. It will also be important to ensure that in striving for optimal solute clearance, we do not expose patients to depletion syndromes and this can easily be investigated using intradialytic modelling.

By improving the understanding of solute kinetics, the long-term goal is to improve the outcome for patients with end-stage renal failure on dialysis and to reduce the unacceptably high morbidity and mortality that these patients currently experience.

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Papers and Presentations Arising From This Work

Published Papers

Spalding EM, Chamney PW, Farrington K. Phosphate Kinetics in Haemodialysis: Evidence For Biphasic Regulation. *Kidney Int* 2002. 61(2). 655-667.

Spalding EM, Farrington K. Dialysis quality and quantity: How much and how often? *Hemodialysis International* 2007 11(s2). S5-S9.

Spalding EM, Pandya P, Farrington K. High Haematocrit Does not Adversely Affect Solute Clearance in High-Flux Dialysis Therapies. *Nephron Clinical Practice*. 2008. In press.

Submitted Papers

Spalding EM, Farrington K. Diurnal Variation is Maintained in End Stage Renal Disease

Oral Presentations

Spalding EM, Chamney PW, Farrington K. Phosphate Kinetics in Haemodialysis: Evidence For Biphasic Regulation. East Anglia Renal Meeting.

Spalding EM. Phosphate Control in Dialysis Patients. British Dietetic Society.

Spalding EM, Farrington K High Haematocrit does not adversely affect solute clearance in high-flux dialysis therapies. Scottish Renal Association.

Spalding EM, Farrington K Diurnal Variation is Maintained in End-stage renal disease Scottish Renal Association.

Poster Presentations

Spalding EM, Chamney P, Farrington K. Phosphate Kinetics in Haemodialysis
Renal Association

Spalding EM, Chamney P, Farrington K. Phosphate Kinetics in Haemodialysis
American Society of Nephrology.

Spalding EM, Farrington K Increasing Haematocrit and Effect on Solute Clearance
ERA-EDTA

Spalding EM, Farrington K Diurnal Variation is Maintained in End-stage renal
disease ERA-EDTA

Phosphate kinetics during hemodialysis: Evidence for biphasic regulation

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Phosphate kinetics during hemodialysis: Evidence for biphasic regulation.

Background. Hyperphosphatemia in the hemodialysis population is ubiquitous, but phosphate kinetics during hemodialysis is poorly understood.

Methods. Twenty-nine hemodialysis patients each received one long and one short dialysis, equivalent in terms of urea clearance. Phosphate concentrations were measured during each treatment and for one hour thereafter. A new model of phosphate kinetics was developed and implemented in Vis-Sim™. This model characterized additional processes involved in phosphate kinetics explaining the departure of the measured data from a standard two-pool model.

Results. Pre-dialysis phosphate concentrations were similar in long and short dialysis groups. Post-dialysis phosphate concentrations in long dialysis were higher than in short dialysis ($P < 0.02$) despite removal of a greater mass of phosphate ($P < 0.001$). In both long and short dialysis serum phosphate concentrations initially fell in accordance with two-pool kinetics, but thereafter plateaued or increased despite continuing phosphate removal. Implementation of an additional regulatory mechanism such that a third pool liberates phosphate to maintain an intrinsic target concentration (1.18 ± 0.06 mmol/L; 95% confidence intervals, CI) explained the data in 24% of treatments. The further addition of a fourth pool hysteresis element triggered by critically low phosphate levels (0.80 ± 0.07 mmol/L, CI) yielded an excellent correlation with the observed data in the remaining 76% of treatments (cumulative standard deviation 0.027 ± 0.004 mmol/L, CI). The critically low concentration correlated with pre-dialysis phosphate levels ($r = 0.67$, $P < 0.0001$).

Conclusion. Modeling of phosphate kinetics during hemodialysis implies regulation involving up to four phosphate pools. The accuracy of this model suggests that the proposed mechanisms have physiological validity.

Hyperphosphatemia is a well-recognized complication of chronic renal failure (CRF), and is important in the pathogenesis of secondary hyperparathyroidism. Chronic

hyperphosphatemia and its treatment with calcium-containing phosphate binders also may be a factor in the progressive calcific vascular disease, which occurs in dialyzed patients and is a potential contributor to the increased incidence of premature cardiovascular death in this population [1]. It has been shown that a pre-dialysis serum phosphate concentration of >2.1 mmol/L is associated with increased mortality [2, 3]. In recognition of such factors, the British Renal Association has instituted a target pre-hemodialysis serum phosphate value of 1.2 to 1.7 mmol/L [4]. There is no firm evidence that reducing pre-hemodialysis phosphate levels to this degree is beneficial, however, and only about 30% of British hemodialysis patients achieve this target [5]. Non-compliance with diet and phosphate binders is often cited as the major cause of poor phosphate control, but shortened dialysis times undoubtedly contribute. We have studied intradialytic phosphate kinetics in long and short dialysis. We have used a mathematical modeling approach to investigate the degree to which serum phosphate levels during hemodialysis may be controlled, and to infer the main elements of the regulatory process.

METHODS

Patients

Twenty-nine stable hemodialysis patients (26 male, 3 female) were studied. Their median age was 54 years (19 to 81 years) and they had been stable on hemodialysis for at least three months. All patients dialyzed via an arteriovenous fistula and the absence of access recirculation was confirmed by a saline dilution method with a sensitivity of 5% [6]. Residual renal solute clearance (KRU) varied from 0 to 4.0 mL/min (mean 1.2 ± 0.3) but was disregarded for the purposes of the study. Patients who were severely malnourished (normalized protein catabolic rate (NPCR) <0.7 and albumin <35 g/L) or hospitalized with infection or heart failure were excluded.

Dialysis techniques

Hemodialysis (HD) treatments were performed using a Fresenius 2008D hemodialysis machine, bicarbonate di-

Key words: hyperphosphatemia, kinetic modeling, intracellular fluid, extracellular fluid, two-pool model, three-pool model, four-pool model.

Received for publication May 16, 2001

and in revised form September 4, 2001

Accepted for publication September 5, 2001

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ysis fluid and polysulphone dialyzers (Fresenius HF80 or 60). Blood flow rates (Q_b) were 253 to 545 mL/min and dialysis fluid flow rates (Q_d) were 500 to 800 mL/min. The same equipment was used for hemodiafiltration (HDF), but 100 to 120 mL/min filtration was performed simultaneously. Replacement fluid was generated by filtration of the dialysate using the Fresenius on-line HDF system.

Study protocol

Patients were studied during a conventional hemodialysis (mean 240.7 ± 5.3 min) and a short hemodiafiltration (mean 147.3 ± 6.5 min) on the same day of consecutive weeks [7]. Dialysis prescriptions were adjusted to achieve a desired two-pool Kt/V (urea) of 1.0, where K is the dialyzer clearance rate, t is the duration of the dialysis session, and V is the urea distribution volume or total body water volume (TBW). The dose of dialysis required to achieve the desired Kt/V (dKt/V) was calculated from the equation:

$$t = \text{desired } Kt/V \times \left(\frac{V}{K} + t_p \right) \quad (\text{Eq. 1})$$

where t_p compensates for urea rebound in the post-dialytic period in accordance with two-pool kinetics. In all treatments t_p was set to 35 minutes as described previously [7].

Sampling and assay techniques

Blood samples were taken from the arterial needle before the start of HD/HDF (t_{i1}), from the arterial line at five equally spaced time intervals during HD/HDF (t_{i2} – t_{i6}) and at the end of the treatment without slowing the blood pump (t_d). Further samples were taken at 2, 15, 30 and 60 minutes post-dialysis from the fistula needle. The washback was performed after the two-minute sample. The ultrafiltration rate (Q_f) was calculated from pre- and post-dialysis weight. The blood flow rate (Q_b) was measured after the dialysis by timed volumetric measurement under the same conditions as obtained during dialysis. Dialyzer clearance was calculated approximately twenty minutes after the start of HD/HDF from the dialyzer inlet and outlet concentration measurements, taking hematocrit into account. Concentrations of urea, creatinine, phosphate and bicarbonate were measured using a Hitachi-717 autoanalyzer.

Data analysis

Statistical analysis. Statistical analysis was carried out using the Student t test for paired samples and linear correlation as appropriate. A P value <0.05 denoted the presence of a statistically significant difference. Mean values are quoted with 95% confidence intervals unless otherwise stated.

Mathematical modeling. Models were implemented

using VisSim™ (Visual Solutions Inc.) modeling software. This environment offered the possibility to import the observed data, facilitating direct comparison against the models. The measured dialyzer clearance ($K_{d, \text{meas}}$), Q_b , Q_d , dialysis time (T_d), sex, pre- and post-dialysis weights were provided as inputs to the model.

For each of the models the standard deviation between the data and the model was calculated via the expression:

$$\sqrt{\frac{1}{N} \sum_{i=0}^N (C_{e, \text{Data}}(i) - C_{e, \text{Model}}(i))^2} \quad (\text{Eq. 2})$$

This provided an objective measure of the degree of fit over the whole treatment and measured rebound period.

Urea model

The two-pool model of urea kinetics during hemodialysis is well validated and assumes a dynamic equilibrium between intracellular and extracellular compartments. The model can be represented schematically (Fig. 1), graphically (Fig. 2), and mathematically (**Appendix**). The dialyzer clearance of solute (K_d) induces diffusive flux of solute (F_d) from the extracellular space and introduces disequilibrium between the intracellular and extracellular compartments. Solute then diffuses freely down the concentration gradient (F_1) at a rate determined by the intracellular to extracellular transfer coefficient (K_{ie}). In addition the ultrafiltration process adds convective flux of solute (F_c). Total body water volume (V) was calculated according to the Watson formula [8] with an assumed intracellular to extracellular volume ratio of 2:1. The mass transfer coefficient (K_{ie}) between the intracellular and extracellular spaces was manipulated to achieve the best fit with the two-pool model. Where adjustments to the total body water were required the intracellular and extracellular volumes were reduced in equal proportions as this was considered to be more physiologically representative of the effects of peripheral compartmentalization and thus effective solute distribution volume. An element of cardiopulmonary recirculation (CPR) was included in the design of the model.

Phosphate models

Since the kinetics of urea is more straightforward, this marker was used as the reference for establishing the compartment volumes, intracellular to extracellular ratio, and cardiopulmonary recirculation effects. Phosphate was assumed to be distributed in the same spaces and subject to the same basic factors influencing urea kinetics. The initial conditions were set to the prevailing phosphate concentration at the start of simulation.

Model A. This model was characterized by a simple two-pool kinetic system as described for urea kinetics. A schematic representation of the model is shown in Figure 1 with the derivation of the model provided in the **Appendix**.

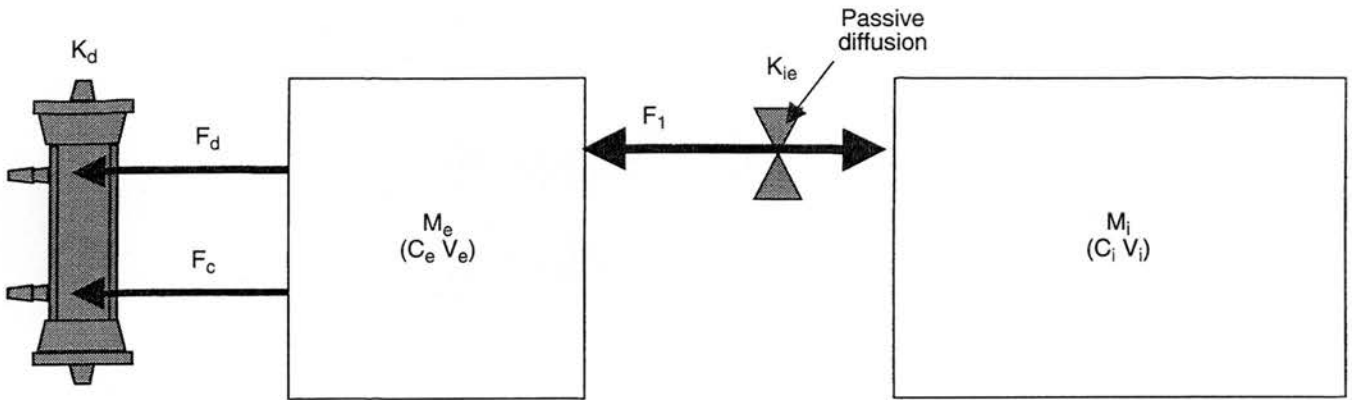


Fig. 1. Model A: Schematic representation of two-pool kinetics. Abbreviations are: M_e , extracellular mass of solute; C_e , extracellular concentration of solute; V_e , extracellular volume; M_i , intracellular mass of solute; C_i , intracellular concentration of solute; V_i , intracellular volume; F_1 , intercompartmental flux; F_d , diffusive flux across dialyzer membrane; F_c , convective flux across dialyzer membrane; K_d , dialyzer clearance; K_{ie} , cell membrane mass transfer coefficient.

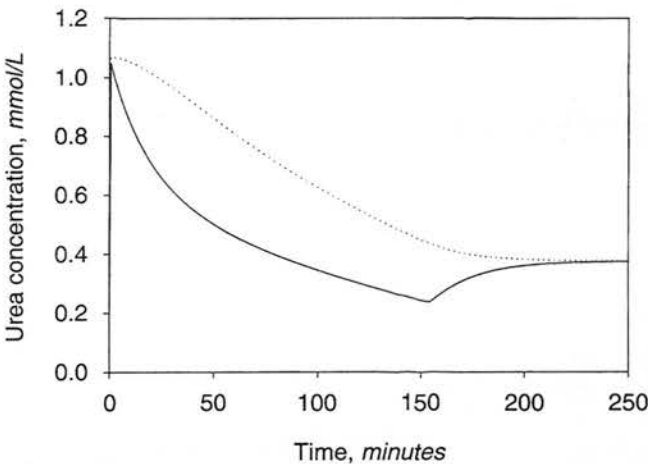


Fig. 2. Graphic representation of two-pool kinetics. Symbols are: (solid line) extracellular concentration according to the model; (dotted line) intracellular concentration according to the model.

Model B. The established elements from model A were implemented, and a regulatory mechanism involving a third pool was added. This regulatory mechanism endeavored to maintain a predetermined target phosphate concentration that was initially inferred by inspection of the nature of the rebound period and subsequently optimized. Initially it was assumed that phosphate would enter the intracellular environment from an internal third pool and that the intracellular to extracellular transfer coefficient for phosphate ($K_{ie}PO_4$) would remain constant throughout the treatment. Using this variant of the model, the fit with the data was particularly poor and this approach was therefore abandoned.

In a second approach additional phosphate flux into the extracellular space was explored. In this model, phosphate was dialyzed initially according to two-pool kinetics. A regulatory system was invoked releasing phosphate

from a third pool into the extracellular space limiting the fall of phosphate levels below an intrinsic target concentration. No assumptions were made regarding the source of the additional phosphate. The phosphate generation process was assumed to be proportional to the difference between the momentary extracellular phosphate concentration and the intrinsic target concentration. The constant of proportionality or "gain" determined the magnitude of the rate of phosphate generation. Values for the intrinsic target concentration and the gain were optimized manually by direct inspection of the data and comparison of cumulative standard deviation measurements. The rate of removal by the dialysis process was matched by concomitant liberation of phosphate via the regulatory process, thus maintaining phosphate concentrations in accordance with the data. It is probable that intracellular phosphate is protected, since phosphate plays a key role in all energy dependant processes within the cell. Intracellular phosphate concentrations are not readily measured so the extracellular phosphate concentration was used as a surrogate of the intracellular concentration. The mass transfer of phosphate across the cell membrane was assumed to be a bi-directional process, thus enabling the intracellular phosphate concentration to re-establish equilibrium with the extracellular space in the post-dialysis period. A schematic representation of the model is shown in Figure 3 with the derivation of the model provided in the **Appendix**. One representative treatment is shown in Figure 4.

Model C. This model implemented all the elements of model B with the further introduction of a fourth pool and a hysteresis element. We postulated that in addition to the phosphate that is released to maintain a target intracellular concentration, there must be a critically low intracellular phosphate concentration, below which energy dependant processes are compromised and a life-threatening situation exists. The existence of such a protective

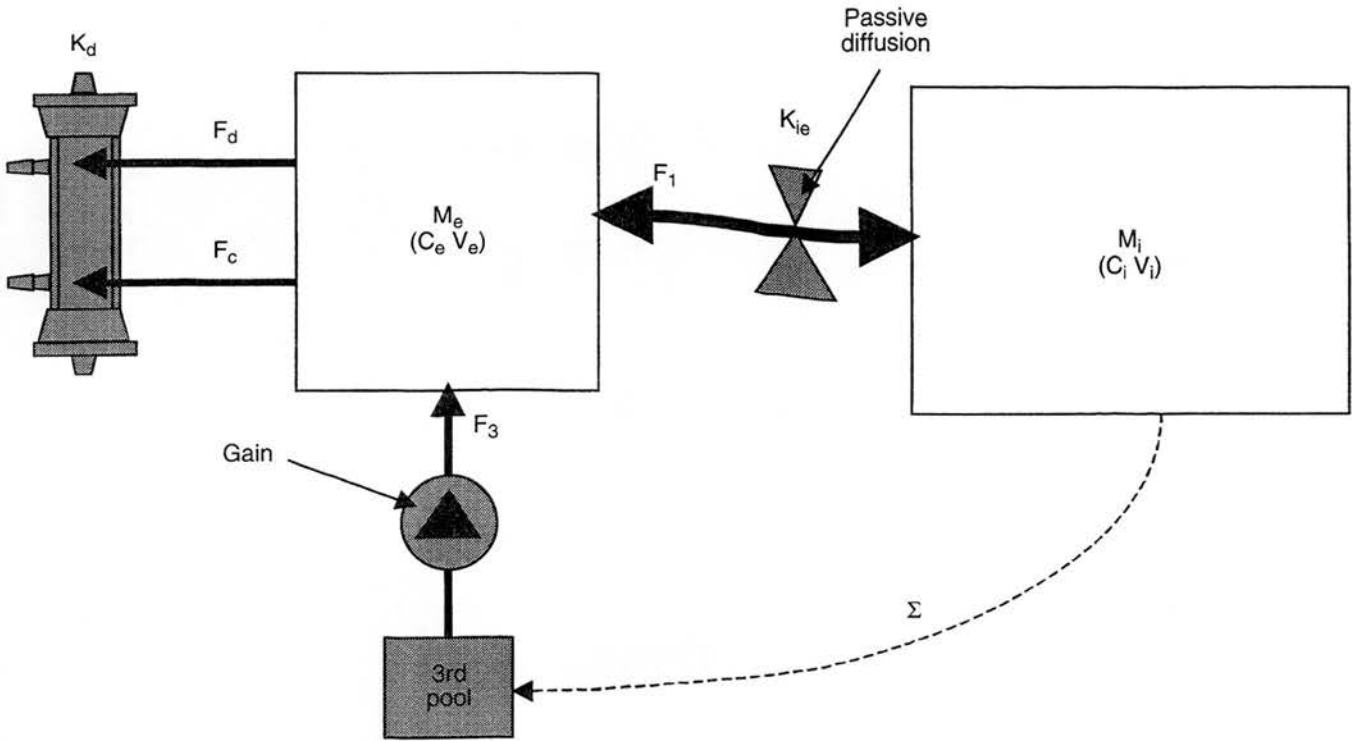


Fig. 3. Model B: Schematic representation of three-pool kinetics. The two-pool kinetic model of model A (Fig. 1) forms the basis of this model with additional phosphate flux (F_3) into the extracellular space from a third pool. This occurs in proportion to the phosphate error (Σ) or the difference between the momentary intracellular phosphate concentration and an intrinsic intracellular phosphate target concentration. The magnitude of phosphate generation is dependent on a constant factor termed the gain. Abbreviations are in the legend to Figure 1.

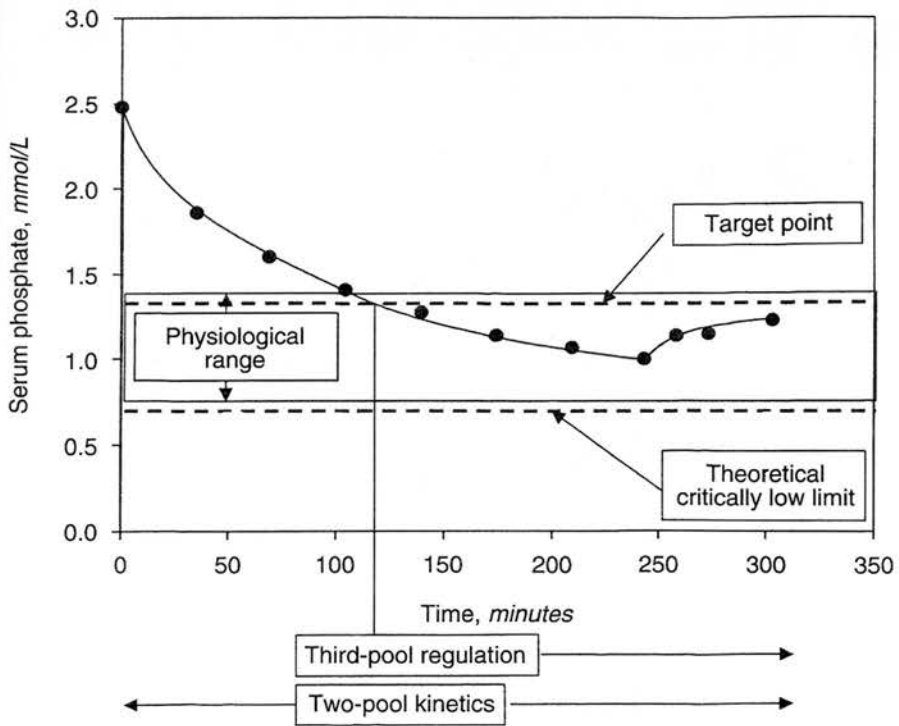


Fig. 4. Representative treatment exhibiting features of model B. Two-pool kinetics are operational beyond the duration of dialysis. When the prevailing phosphate concentration falls below the target point phosphate is released from the third pool. The critically low limit is not reached in this treatment.

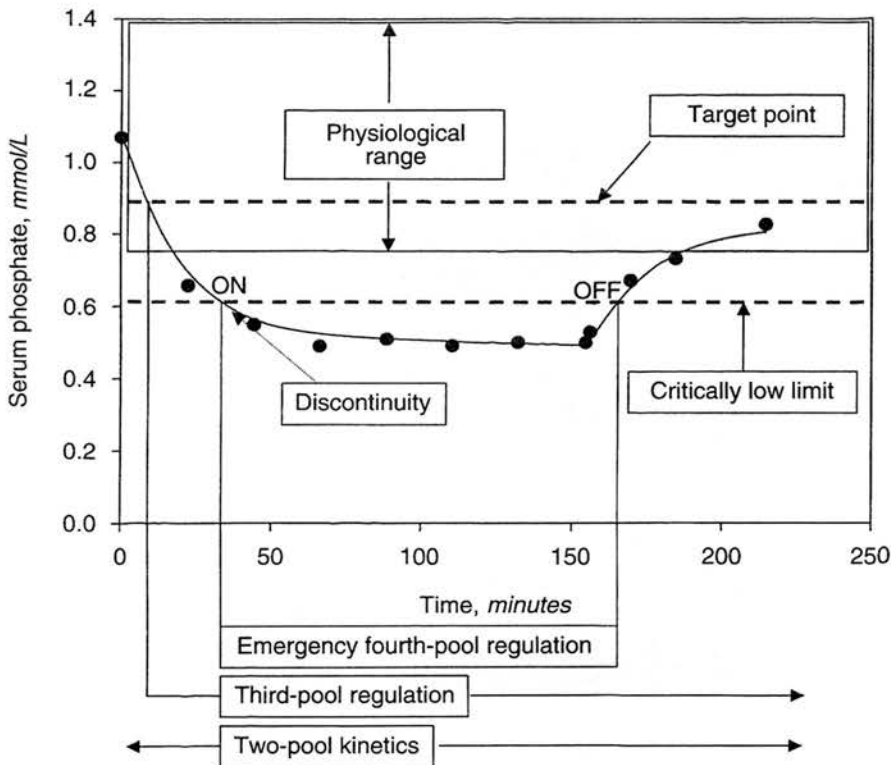


Fig. 5. Representative treatment exhibiting features of model C. Kinetics are as for model B (Fig. 4) but, in addition, when the critically low limit is exceeded, fourth pool kinetics become operational. The influx from this pool switches off when a safe phosphate level has been re-established.

mechanism was evident from the clear discontinuity that could be observed in the time course of phosphate variation, as shown in Figure 5. The switching point of the hysteresis element can be inferred from the observed discontinuity. When the intracellular phosphate concentration falls to the critically low level, the hysteresis element results in a rapid switching on of the generation process with phosphate generated from a fourth pool local to the intracellular space. The hysteresis element ensures liberation of phosphate over a limited concentration band, which prevents oscillations about the critically low limit. Once intracellular phosphate concentrations are restored, normal proportional control applies as described for model B. The critically low limit may be encountered several times during the course of a treatment, as shown in Figure 6. This is particularly likely to occur when the pre-dialysis serum phosphate concentration is low. The model can be represented schematically (Fig. 7) and mathematically (**Appendix**).

RESULTS

Urea kinetics

It was possible to achieve excellent fits with a two-pool model for the urea data in long dialysis (Fig. 8A) with a median intracellular to extracellular transfer coefficient for urea (K_{ie} urea) of 750 mL/min and total body

water $96.6 \pm 3.2\%$ of the predicted Watson volume (V). In contrast, during short dialyses it was necessary to assume a lower effective total body water $68.0 \pm 4.5\%$ of predicted volume, and a corresponding reduction in K_{ie} urea in order to achieve the same degree of accuracy. (Fig. 8B) The values used to compensate for cardiopulmonary recirculation (CPR) averaged $4.3 \pm 0.4\%$ in long dialysis and $5.4 \pm 0.8\%$ in short dialysis ($P < 0.01$).

Phosphate kinetics

Model A: Two-pool kinetics. An attempt was made to fit the phosphate data to the same two-pool model. It was clear that phosphate kinetics were markedly different (Fig. 9). During the initial part of the dialysis session extracellular inorganic phosphate concentrations fell rapidly, but a point was reached beyond which the measured phosphate concentration plateaued or in some patients even began to rise. This phenomenon did not appear to be related to alterations in acid-base status as assessed by the serum bicarbonate concentration during dialysis (Fig. 10). No correlation between the bicarbonate concentration and the phosphate concentration could be demonstrated at any point in the dialysis process. The average cumulative standard deviation between the observed phosphate data and the two-pool model was 0.22 ± 0.04 mmol/L for long dialysis and 0.22 ± 0.03 mmol/L for short dialysis.

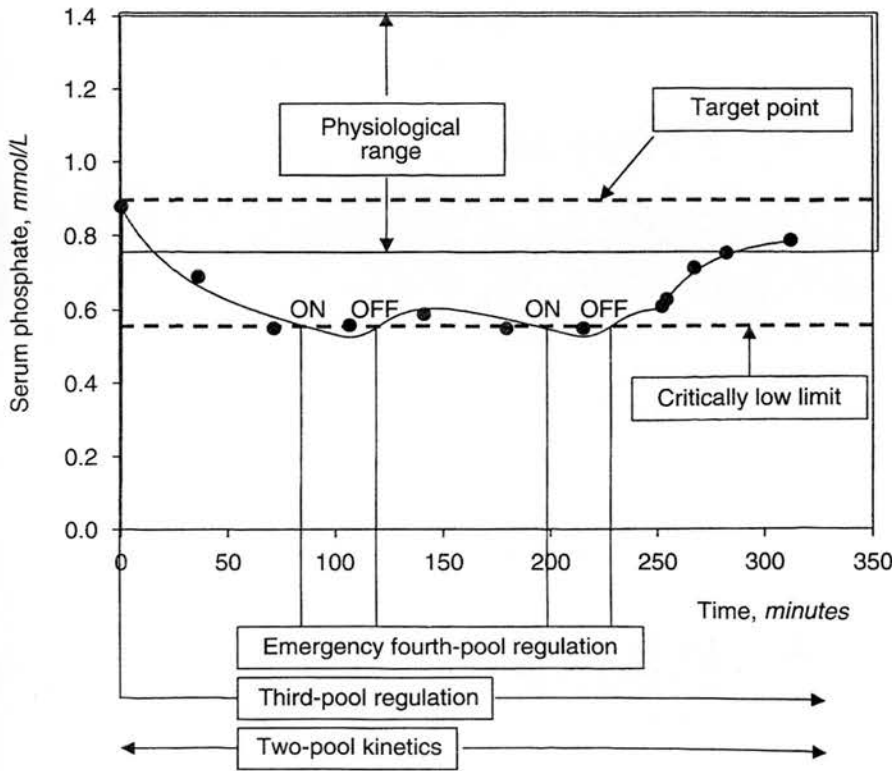


Fig. 6. Representative treatment exhibiting features of model C with repeated activation of fourth pool kinetics.

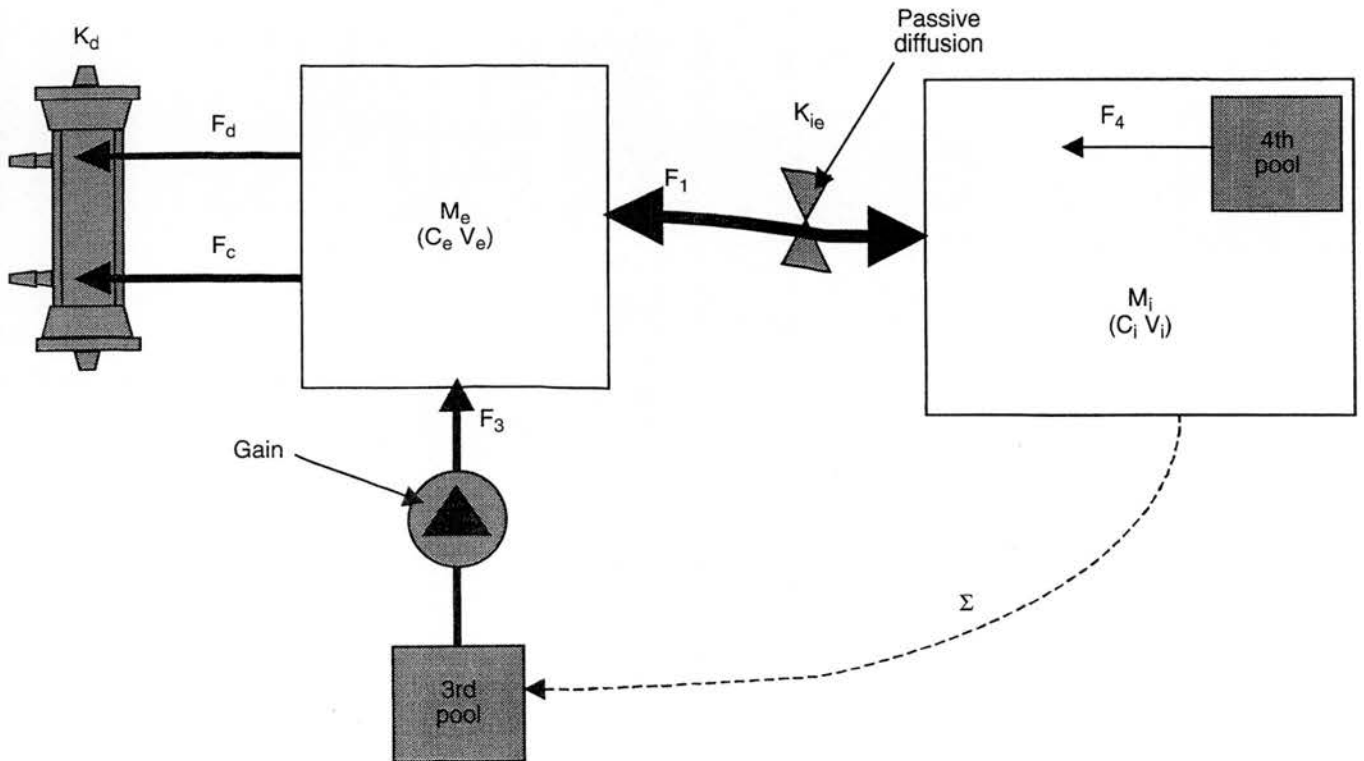


Fig. 7. Model C: Addition of hysteresis element to model B. In addition to the proportional control mechanism shown in the three-pool model (Fig. 3), critically low intracellular phosphate levels trigger immediate release of phosphate from a fourth pool local to the intracellular space (F_4). This independent mechanism serves to protect the intracellular environment from dangerously low phosphate concentrations. Abbreviations are in the legend to Figures 1 and 3.

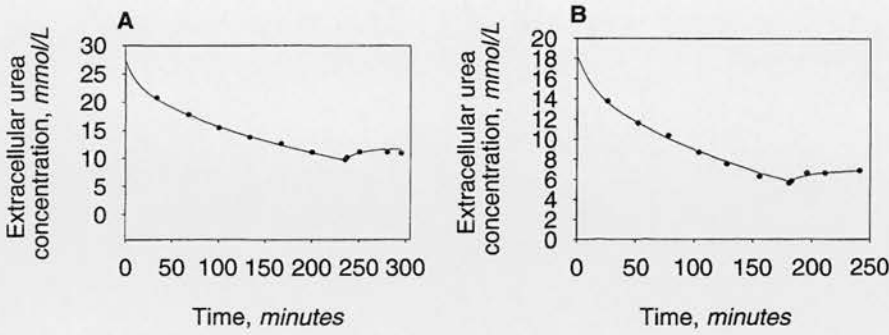


Fig. 8. Representative data from one patient showing observed urea levels during dialysis (●) versus urea levels predicted by two-pool model (represented by the line) in (A) long dialysis and (B) short dialysis. Allowing for cardiopulmonary recirculation (CPR) and for a degree of peripheral compartmentalization in the short dialysis, it was possible to achieve excellent fits between the observed urea data and the two-pool model, assuming an intracellular to extracellular transfer coefficient (K_{ie}). In A, K_{ie} = 750 mL/min; CPR = 4%; volume = 100%. In B, K_{ie} = 413 mL/min; CPR = 4%; volume = 57.5%.

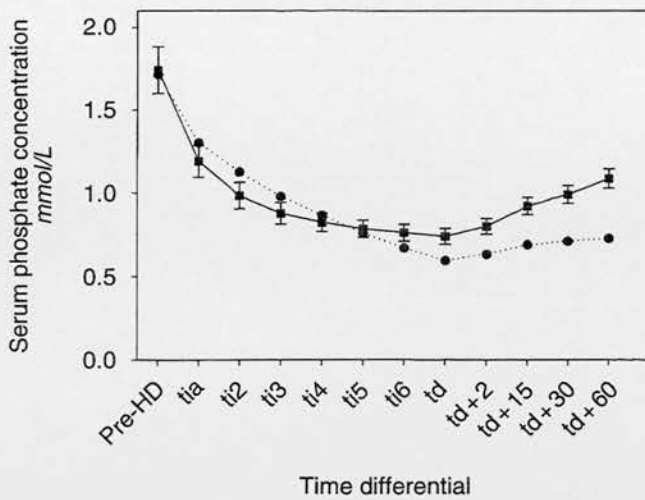


Fig. 9. Mean observed phosphate levels during dialysis for all 58 treatments versus phosphate levels predicted by two-pool model. A clear discontinuity exists where the phosphate data plateaus and deviates from the two-pool model. The degree of rebound can also be seen to be greater in the observed data than the two-pool prediction. Symbols are: (■) observed phosphate data; (●) two-pool model. Observed phosphate data are mean \pm 95% confidence intervals. For the key to the time differential axis, see "Sampling and assay techniques."

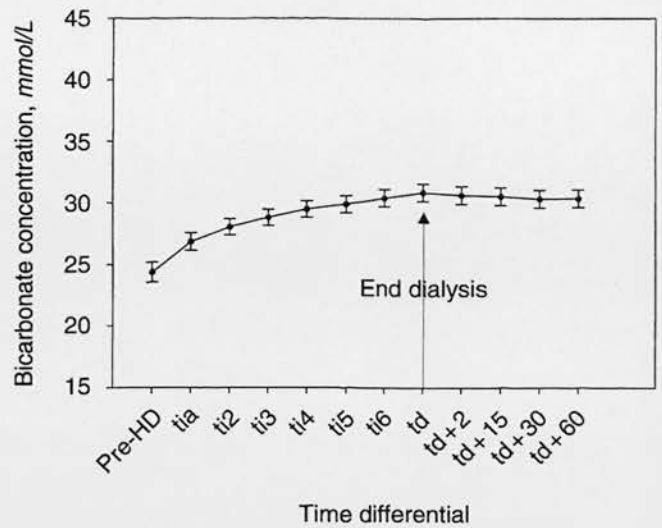


Fig. 10. Serial mean plasma bicarbonate measurements throughout dialysis. Bicarbonate levels increase throughout dialysis. No plateau is seen during dialysis in the bicarbonate data in contrast to the phosphate data (Fig. 9). Data are mean \pm 95% confidence intervals.

Model B: Three-pool kinetics to maintain a target phosphate concentration. According to model predictions the target concentration for phosphate averaged 1.22 ± 0.08 mmol/L for long dialysis and 1.15 ± 0.10 mmol/L for short dialysis ($P = NS$). The target concentration almost always fell within the physiological range (Fig. 11A), but within this range showed significant correlation with the pre-dialysis serum phosphate concentration ($r = 0.47$, $P < 0.001$; Fig. 11B).

During the period of active phosphate regulation the dialysis process continued to remove phosphate at an average rate of 0.16 ± 0.02 mmol/min in long dialysis and 0.18 ± 0.02 mmol/min in short dialysis. To maintain the phosphate plateau the generation rate from the third pool must be of a similar order.

In eight long dialysis treatments and six short dialysis treatments this three-pool model could adequately ex-

plain the observed phosphate data. The addition of fourth pool kinetics in these treatments provided no further improvement in the degree of fit between the model and the observed data. The cumulative standard deviations were 0.03 ± 0.01 mmol/L and 0.04 ± 0.01 mmol/L for long and short treatments, respectively. One representative treatment is shown in Figures 4 and 12B with the corresponding cumulative standard deviation curves shown in Figure 12B.

Model C: Addition of a fourth pool and hysteresis element. The critically low concentration ranged from 0.41 mmol/L to 1.44 mmol/L with a mean of 0.86 mmol/L in long dialysis and 0.74 mmol/L in short dialysis ($P = NS$). Correlation was found between the critically low phosphate concentration and the pre-dialysis phosphate concentration ($r = 0.67$, $P < 0.001$) (Fig. 13). According to the model the liberation of phosphate from this fourth pool ceased when the measured phosphate concentration had risen by a mean of 0.07 ± 0.02 mmol/L.

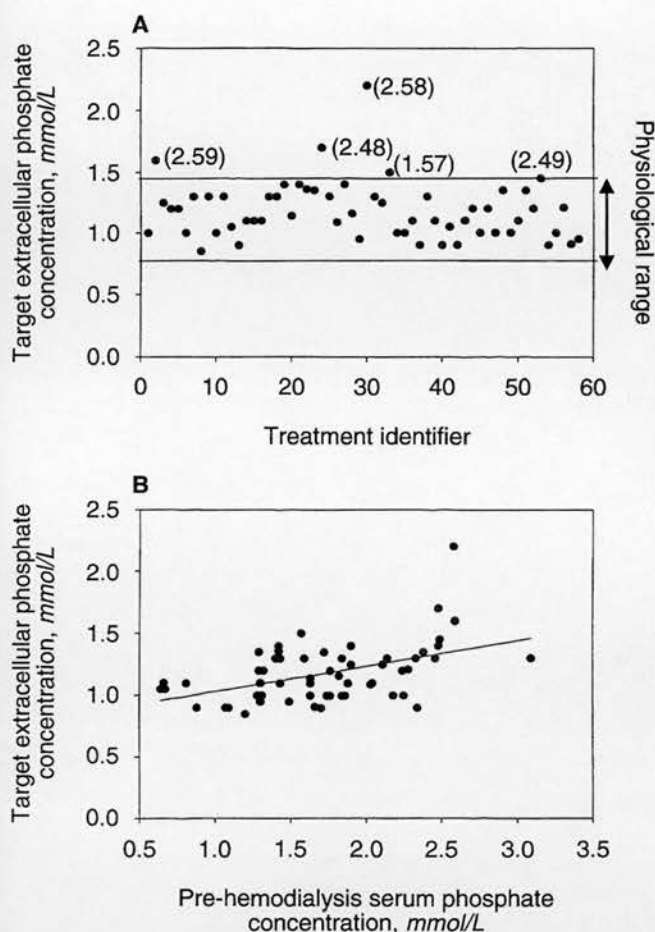


Fig. 11. (A) Target phosphate concentration. The majority of values fell within the physiological range (0.75 to 1.40 mmol/L) and those that did not tended to have higher pre-dialysis phosphate concentrations (shown in parenthesis). (B) Correlation between pre-dialysis serum phosphate concentration and target extracellular phosphate concentration according to the model ($r = 0.47$, $P < 0.001$).

Model C shows an excellent fit with the observed data for the 21 long and 23 short treatments not explained by model B. The mean cumulative standard deviations were 0.03 ± 0.01 mmol/L for both long and short dialysis treatments. Two representative treatments are shown in Figures 5, 6 and 14 with their corresponding cumulative standard deviation curves shown in Figure 14.

Summary of all models

Models A, B and C were progressively implemented until the best approximation to the data was achieved. The application of model A demonstrated a reasonable fit for the initial part of dialysis corresponding to two-pool kinetics but a poor fit thereafter. The implementation of model B demonstrated a close correlation between the model and the data in a minority of treatments (14 of 58). The pre-dialysis serum phosphate concentration for these 14 treatments was significantly higher than

for the remainder of treatments ($P < 0.01$). A close fit between the model and the data for the remaining 44 treatments was achieved when the hysteresis element of model C was applied. The cumulative standard deviation measurements for all 58 treatments in models A, B and C are shown in Tables 1 and 2.

Comparisons between phosphate data in long and short dialysis

Pre-dialysis phosphate concentrations were 1.74 ± 0.23 mmol/L for long dialysis and 1.71 ± 0.19 mmol/L for short dialysis ($P = \text{NS}$). The minimum phosphate concentration achieved in short dialysis treatments was significantly lower ($P < 0.001$; Fig. 15) than that in long dialysis. There was no statistical difference in the critically low limit between the long and short treatments, suggesting that either there is a short time lag before the control mechanisms become active or that these mechanisms are overwhelmed by higher phosphate losses in short dialysis. The higher dialyzer clearances and lower effective V in short dialyses result in a more rapid rate of decrease in the measured extracellular phosphate concentration in the initial stages of dialysis (Table 3). $K_{ie}PO_4$ was significantly higher in long versus short dialysis ($P < 0.01$), but this difference was not apparent when corrected for the effects of phosphate distribution volume ($P = \text{NS}$; Table 3). Post-dialysis phosphate levels in long dialysis (0.83 ± 0.07 mmol/L) were higher than in short dialysis (0.65 ± 0.05 mmol/L; $P < 0.001$). Phosphate levels during the measured rebound up to 60 minutes were significantly higher in the long dialysis group although the rate of rebound with time in the short dialysis treatments was greater (Fig. 15). Despite lower phosphate levels during dialysis the mass of phosphate removed during short dialysis was significantly less than during long dialysis (26.33 ± 4.38 vs. 30.89 ± 4.70 mmol; $P < 0.02$).

DISCUSSION

Phosphate kinetics remains a poorly studied area of hemodialysis research despite the ubiquity of hyperphosphatemia. Existing strategies to achieve phosphate control rely heavily on dietary restriction and the use of phosphate binders. The average daily intake of phosphate in a Western diet is 26 to 67 mmol with a "renal" diet expected to provide 18 to 36 mmol, with 10 to 30 mmol of this actually absorbed [9]. It is difficult to achieve a lower phosphate intake due to the necessity of maintaining adequate protein intake, so patients on hemodialysis are likely to remain in positive phosphate balance despite the use of phosphate binders. Utilizing high-flux membranes for hemodialysis or hemodiafiltration and increasing the surface area of the dialyzer membrane can remove phosphate more efficiently, although

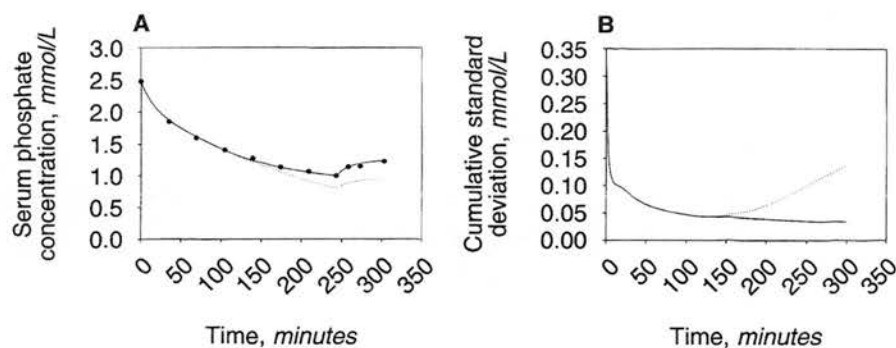


Fig. 12. (A) Representative treatment exhibiting three-pool kinetics. Symbols are: (●) extracellular phosphate data. Lines are: dotted, two-pool kinetics; solid, three-pool kinetics. (B) Cumulative standard deviation curves for panel A.

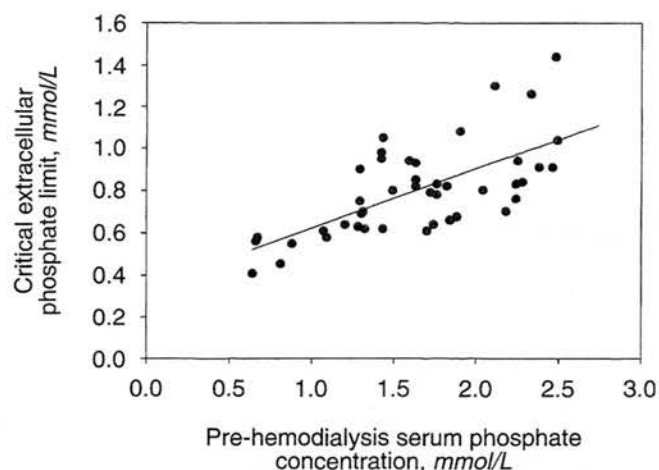


Fig. 13. Correlation between pre-dialysis serum phosphate concentration and critically low extracellular phosphate concentration according to the model ($r = 0.67$, $P < 0.0001$).

no improvement is seen in the pre-dialysis phosphate levels of patients dialyzed this way [10, 11]. Bicarbonate replacement fluid has not been shown to be superior to acetate in terms of phosphate removal [12].

It is known that phosphate removal does not follow the same diffusion kinetics described for urea [13, 14]. Regardless of the duration of dialysis a characteristic plateau phase is rapidly reached below which phosphate levels do not fall. The reasons for this are complex and relate to the mobilization of phosphate from unidentified stores during the dialysis procedure. Longer dialysis treatments remove a higher mass of phosphate, but also result in significantly higher post-dialysis phosphate concentrations according to our data. This is indicative of phosphate being mobilized to a greater extent in long dialysis. In the post-dialysis period there is a rebound phenomenon persisting for in excess of one hour. The rate of the rebound with time is significantly higher in the short treatment group, which may be due to the additive effect of re-equilibration of phosphate from peripheral compartments that have been poorly perfused during the short dialysis treatment.

Recognition of the departure of the phosphate data from standard two-pool kinetics has led to the hypothesis of a third pool of phosphate although its nature is not clear [13–17]. The existence of a third pool is consistent with work demonstrating the absence of a plateau phase when bovine blood is dialyzed in vitro [18]. Studies in erythrocytes demonstrate that intracellular to extracellular phosphate equilibrium was reached at a higher extracellular phosphate concentration in the erythrocytes of dialysis patients compared to controls, suggesting adaptation to the hyperphosphatemic environment [19]. This may explain the observation that the critically low point and target point are dependent on the pre-hemodialysis phosphate concentration. (Figs. 11B and 13). ^{32}P and NMR studies demonstrate glyco-phosphates in uremic erythrocytes not present in healthy controls that could conceivably act as a source of phosphate during dialysis [19, 20].

In studies of hemodialysis patients, Sugasaki described a single-pool extracellular compartment model and proposed phosphate generation from reserves, triggered by the reduction of phosphate during dialysis to a nadir unique to each patient. Their studies recognized the correlation of the phosphate nadir with the pre-dialysis serum phosphate concentration [20, 21]. Maasrani studied four pediatric patients over eight dialysis sessions and suggested that the plateau phase of phosphate kinetics was maintained by a time-dependent influx from the intracellular space [18]. These studies all described the influx of phosphate in terms of a linear or exponential increase in the rate of phosphate generation (or gain) that did not fully explain the observed data. Pogglitsch studied 28 hemodialysis patients and suggested that generation of phosphate was in response to falling extracellular phosphate levels [19]. The point at which phosphate generation was initiated was again observed to be dependent on the pre-dialysis phosphate concentration. This study suggested that additional phosphate was released into the extracellular space in proportion to the difference between the momentary phosphate concentration and a critical level (equivalent to the target concentration

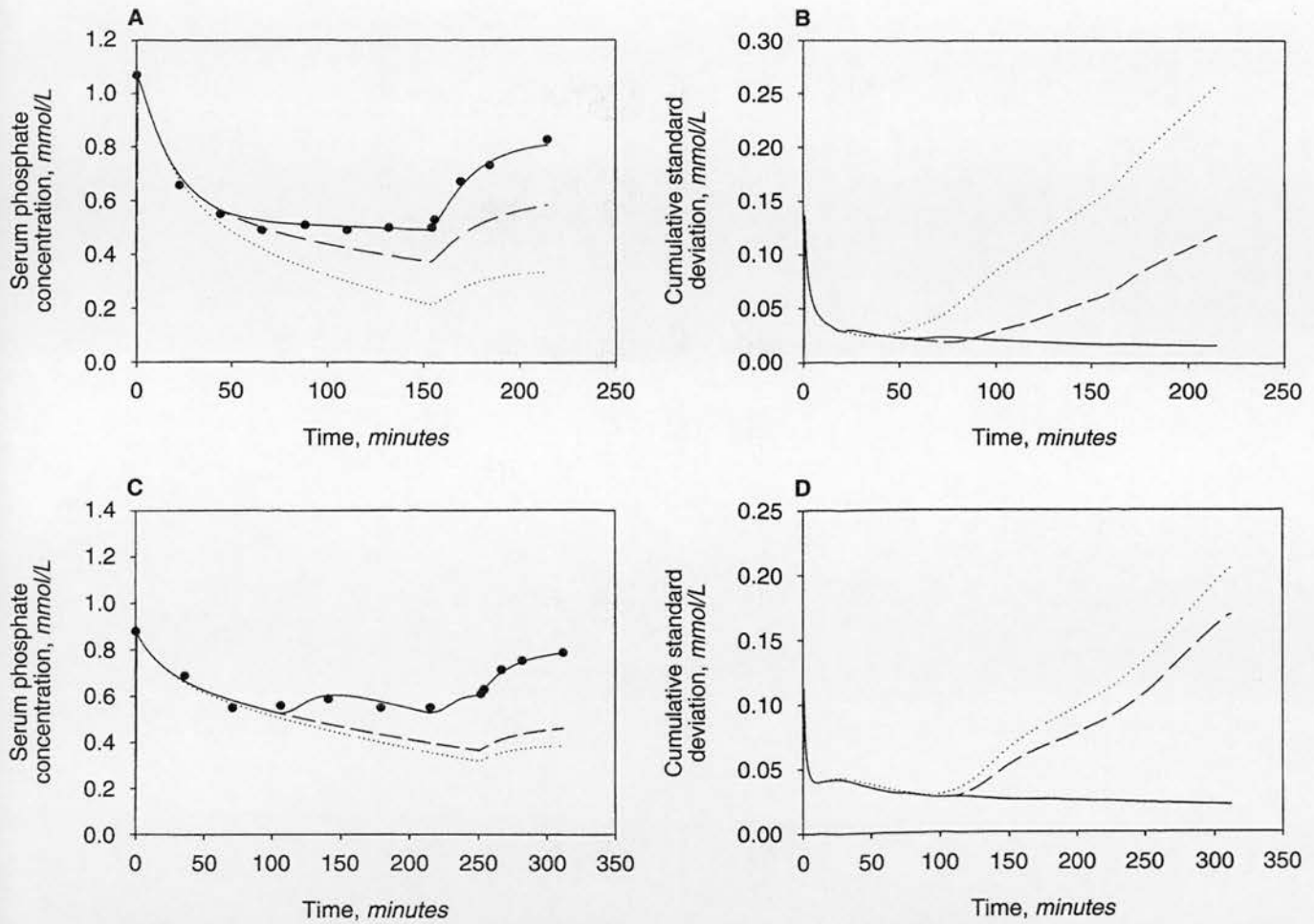


Fig. 14. Two representative treatments (A and B) exhibiting four-pool kinetics with corresponding cumulative standard deviation curves (C and D). (A) The pre-hemodialysis serum phosphate concentration is 1.07 mmol/L and there is a substantial contribution toward regulation from both the third and fourth pools. (C) The pre-hemodialysis serum phosphate concentration is 0.88 mmol/L and the contribution toward regulation from the third pool is minimal. This is presumably because this individual has little in the way of third-pool phosphate stores. Symbols are: (●) extracellular phosphate data; (dotted line) two-pool kinetics; (dashed line) three-pool kinetics; (solid line) four-pool kinetics.

Table 1. Comparison of cumulative standard deviation measurements for the fourteen treatments adequately explained by three-pool kinetics

	Model A (two pool)	Model B (three pool)
Long dialysis (8/29)	0.17 ± 0.06	0.03 ± 0.01 ^b
Short dialysis (6/29)	0.18 ± 0.13	0.04 ± 0.01 ^a

Values are mean ± 95% confidence intervals. Significance values are compared to model A (Student paired two sample for means).

^a $P < 0.05$, ^b $P < 0.01$

in our model). This process continued into the post-dialysis period [19].

Our model expands these ideas with the introduction of biphasic control involving sequential implementation of control mechanisms in response to changes in the prevailing phosphate concentration. (Figs. 1–7). In model A dynamic equilibrium between the intracellular and extra-

cellular compartments throughout the dialysis process is assumed, consistent with a two-pool model. Model B implements additional phosphate release from a third pool to maintain a pre-determined target phosphate concentration. Although the additional phosphate enters the extracellular space, which is consistent with Pogglitsch et al's model [19], we propose that it is changes in the intracellular phosphate concentration that trigger the control mechanism. In theory, the generation process from the third pool would be switched off once intracellular phosphate concentrations reach the target level, although this was not evident in the current data since the rebound clearly extended beyond our final measurement at 60 minutes post-dialysis. In model C, we additionally describe the existence of a fourth pool that is operational in the majority of treatments studied. There is a hysteresis element deduced from the discontinuities in the data that reflects a "switching on" effect that exists

Table 2. Comparison of cumulative standard deviation measurements for the 44 treatments explained by four-pool kinetics

	Model A (two pool)	Model B (three pool)	Model C (four pool)
Long dialysis (21/29)	0.24 ± 0.05	0.15 ± 0.05 ^a	0.03 ± 0.01 ^a
Short dialysis (23/29)	0.23 ± 0.03	0.11 ± 0.02 ^a	0.03 ± 0.01 ^a

Values are mean ± 95% confidence intervals. Significance values are compared to model A (Student paired two sample for means).
^aP < 0.001

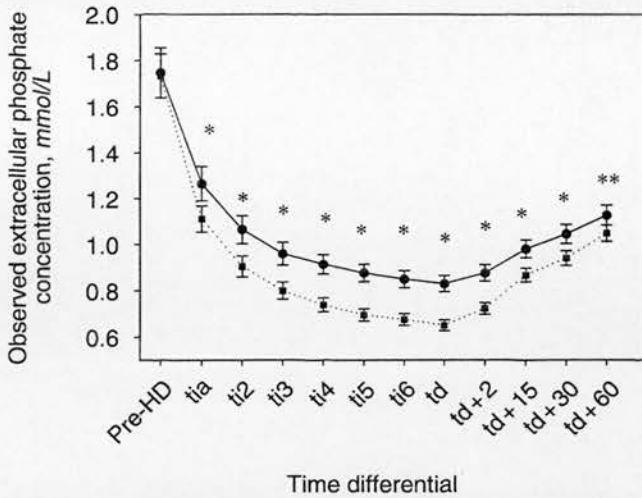


Fig. 15. Comparison of observed serum phosphate concentrations in long and short dialysis for all 58 treatments. Symbols are: (■) short dialysis; (●) long dialysis; *P < 0.001 and **P < 0.01, paired *t* test. Data are mean ± SEM values.

to protect against critically low intracellular phosphate levels. Where the critically low intracellular phosphate concentration is not encountered during dialysis (in 24% of patients in this study), the hysteresis element is not invoked and the three-pool model can adequately explain phosphate kinetics.

Four pools of phosphate in our model best describe the behavior of our data, and the kinetics of phosphate clearly involves more complex mechanisms than simple diffusion. The gut is an unlikely candidate for the third or fourth pools as all the patients we studied were fasting. Dialysis patients are likely to have pathological stores of phosphate possibly in bone as 86% of total body phosphate is in bone and approximately 250 mg phosphate is mobilized daily in the process of remodeling. The third-pool phase of phosphate regulation can potentially be explained by efflux from such stores, possibly from a pool of phosphate not yet incorporated into bony matrix. If so, this phase would only be sustained in individuals with excess total body phosphate. The recognition that most hemodialysis patients have an excess of stored phosphate presents a challenge to achieve better

Table 3. Intradialytic parameters

	Long	Short	P value
T _d min	240.7 ± 5.3	147.3 ± 6.5	<0.001
CPR %	4.3 ± 0.4	5.4 ± 0.8	<0.01
% Watson volume (V)	96.6 ± 3.2	68.0 ± 4.5	<0.001
K _d PO ₄ (model) mmol/min	158.4 ± 12.1	226.4 ± 13.1	<0.001
K _d PO ₄ (meas) mmol/min	171.8 ± 9.5	223.0 ± 17.0	<0.001
K _{ie} PO ₄ mL/min effective	351.0 ± 33.9	242.6 ± 23.2	<0.001
K _{ie} PO ₄ mL/min corrected for V	363.8 ± 32.7	363.8 ± 32.7	NS
K _d Urea(model) mmol/min	151.1 ± 9.3	214.8 ± 13.5	<0.001
K _d Urea(meas) mmol/min	185.6 ± 10.2	285.2 ± 15.2	<0.001
K _{ie} Urea mL/min effective	714.4 ± 46.7	513.2 ± 48.2	<0.001
K _{ie} Urea mL/min corrected for V	739.6 ± 38.7	699.1 ± 41.9	NS

Values are mean ± 95% confidence intervals. Abbreviations are: T_d, length of dialysis; CPR, cardiopulmonary recirculation; K_dPO₄(model), dialyzer clearance of phosphate used in model calculations; K_dPO₄(meas), dialyzer clearance of phosphate calculated during dialysis; K_{ie}PO₄, intracellular to extracellular transfer coefficient for phosphate; K_dUrea(model), dialyzer clearance of urea used in model calculations; K_dUrea(meas), dialyzer clearance of urea calculated during dialysis; K_{ie}Urea, intracellular-to-extracellular transfer coefficient for urea.

phosphate control in the pre-dialysis period. Earlier referral of patients with chronic renal failure would allow the timely introduction of dietary phosphate restriction and phosphate binders to prevent patients starting their dialysis careers in positive phosphate balance. Currently used phosphate binders may be inadequate for this task.

The observed time course of the hysteresis effect of our proposed model is difficult to explain if phosphate is released purely from a bone-related pool. The speed of the hysteresis response might imply phosphate release by a mechanism such as cell lysis, but the observation that the hysteresis can switch on and off repeatedly during a dialysis treatment is against this action. The more probable explanation is the existence of a fourth pool local to the intracellular space that provides emergency protection against life threatening, critically low intracellular phosphate concentrations. It is possible that glycoposphates as described in some *in vitro* studies [19, 20] could be involved in this short-term regulation mechanism. This mechanism is likely to represent physiological protection and also may operate in normal controls.

Our model sequentially implements the third and fourth pools, allowing all treatments in both the long and short dialysis groups to be explained by passive two-pool, three-pool, and emergency fourth-pool regulatory processes. This implies common mechanisms operating across all dialysis treatments and supports the physiological validity of the model. Phosphate generation from a third pool allows the removal of more phosphate during dialysis than is permitted by passive two-pool kinetics. This is theoretically beneficial for reducing the excessive phosphate stores that exist in chronic hemodialysis patients. However, the switch to fourth pool kinetics in the way we have described may give cause for concern. We have observed that the majority of dialysis patients in-

voke fourth pool kinetics in response to critically low phosphate concentrations. These low phosphate levels may contribute to dialysis-related symptoms and possibly to the excess morbidity and mortality seen in these patients in the longer term. Our study did not appear to result in major intradialytic symptoms, although this was not a primary end-point. This would be an interesting area for further study. To avoid the implementation of fourth pool kinetics it would be necessary to prevent phosphate concentrations reaching the critically low limit, which could be achieved by longer and slower treatments [9]. Daily dialysis currently appears to be the only technique capable of safely depleting excess phosphate stores and maintaining patients in optimal phosphate balance, although whether this will be practical for the majority remains doubtful.

ACKNOWLEDGMENT

This study was presented in poster format at The Renal Association Autumn meeting, Cambridge, September 2000.

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APPENDIX

Model A: Passive diffusion

The mass of PO₄ in the intracellular and extracellular compartments is:

$$M_i = C_i V_i \text{ and } M_e = C_e V_e \quad (\text{Eq. 3})$$

where C_i, C_e and V_i, V_e represent the concentrations and volumes of the intracellular and extracellular spaces, respectively. The rate of change of mass in the extracellular compartment depends on the fluxes F₁, the diffusive flux across the dialyzer membrane F_d and the convective component F_c as depicted in Figure 1.

$$\frac{dM_e}{dt} = F_1 - F_d - F_c \text{ and } \frac{dM_i}{dt} = -F_1 \quad (\text{Eq. 4})$$

Assuming passive diffusion across the cell membrane then

$$\frac{dM_e}{dt} = \frac{d(C_e V_e)}{dt} = (C_i - C_e)K_{ie} - C_e K_d \quad (\text{Eq. 5})$$

where K_{ie} is the mass transfer coefficient of the cell membrane with respect to phosphate and K_d is the dialyzer clearance.

Since both the volume and concentration of the extracellular compartment are changing, then

$$\frac{d(C_e V_e)}{dt} = C_e \frac{dV_e}{dt} + V_e \frac{dC_e}{dt} = (C_i - C_e)K_{ie} - C_e K_d \quad (\text{Eq. 6})$$

and

$$\frac{dV_e}{dt} = Q_u \quad (\text{Eq. 7})$$

so

$$V_e \frac{dC_e}{dt} = (C_i - C_e)K_{ie} - C_e K_d - C_e Q_u \text{ and } V_e(t) = V_e(0) - \int_0^t Q_u dt \quad (\text{Eq. 8})$$

Therefore,

$$\frac{dC_e}{dt} = \frac{(C_i - C_e)K_{ie} - C_e K_d - C_e Q_u}{V_e(t)} \quad (\text{Eq. 9})$$

Similarly for the intracellular space,

$$\frac{dM_i}{dt} = \frac{d(C_i V_i)}{dt} = (C_i - C_e)K_{ie} \quad (\text{Eq. 10})$$

so

$$\frac{dC_i}{dt} = \frac{(C_i - C_e)K_{ie}}{V_i} \quad (\text{Eq. 11})$$

Model B: Passive diffusion + regulated intracellular PO₄

The derivation of model B is identical to Model A with the addition of flux F₃. F₃ represents a source of PO₄ from the third pool that feeds into the extracellular space.

As a first approximation it is assumed that the flux F₃ is proportional to the difference between the intracellular concentration of phosphate and a desired set point, C_{iset}. Hence, an expression for flux F₃ may be written as:

$$F_3 = (C_i - C_{iset}) \cdot G_3 \quad (\text{Eq. 12})$$

The constant of proportionality is given by the gain G₃, which governs the rate at which phosphate is liberated from the third pool. The differential equation describing the extracellular compartment is thus:

$$\frac{dC_e}{dt} = \frac{(C_i - C_e)K_{ie} - C_e K_d - C_e Q_u + F_3}{V_e(t)} \quad (\text{Eq. 13})$$

The kinetics of the intracellular compartment are identical to that of Model A above.

Model C: Passive diffusion, intracellular regulation + immediate action

Model C considers an additional mechanism that is invoked when phosphate falls below a critical limit. Under this condition, phosphate is liberated immediately at a fixed rate F₄, from a fourth pool contained within the intracellular environment. A more sophisticated mechanism is beyond limits of that which could be deduced from the data available.

The hysteresis element has the properties:

$$\text{IF } C_i < C_{i\text{crit}} \text{ AND } F_4 \text{ off, THEN } F_4 \text{ on} \quad (\text{Eq. 14})$$

$$\text{IF } C_i < C_{i\text{HighLimit}} \text{ AND } F_4 \text{ on, THEN } F_4 \text{ off} \quad (\text{Eq. 15})$$

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Dialysis quality and quantity: How much and how often?

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Abstract

Hemodialysis is accepted as standard therapy for end-stage renal failure but despite four decades of experience the morbidity and mortality associated with the treatment remains unacceptably high. Quality of dialysis is traditionally measured with reference to urea clearance but it is becoming increasingly apparent that other solutes across the range of molecular size are also important. More prolonged or more frequent therapy may improve dialysis delivery and enhance survival in patients with end-stage renal disease.

Key words: Hemodialysis, adequacy, frequency

Dialysis permits the patient to be fully rehabilitated, to have a satisfactory nutritional intake and a sufficient production of red blood cells, to maintain normal blood pressure values and to prevent the development of neuropathy.¹

INTRODUCTION

The definition of adequate dialysis proposed by de Palma in 1971 is still highly relevant but remains a distant goal. Understandably, the pioneers of dialysis were more concerned with preserving life than with reducing morbidity, but from the time de Palma detailed his objectives to the present day, it has remained clear that dialysis therapy is suboptimal when measured against these standards. The functions of the healthy kidney include removal of metabolic waste products, maintenance of water, electrolyte and acid-base homeostasis, blood pressure regulation, mineral and bone homeostasis, and regulation of erythropoiesis. Excretory function is replaceable by dialysis, albeit only partially and nonselectively and maintenance of acid-base, fluid and electrolyte, and mineral homeostasis remain approximate and difficult to individualize. Dialysis cannot replace other functions, only some of which can be delivered by other means e.g., administration of vitamin D analogs and erythropoietin. Of the

myriad of possibilities, the adequacy of dialysis therapy is traditionally based on the clearance of urea from the blood, because blood urea levels are easy to measure and are related to protein catabolism. However, the need to consider dialysis adequacy in its more global context is increasingly being recognized.

The concept of adequacy

Wolf (1951) provided the first description of hemodialysis (HD) adequacy. Focusing on the performance of the membrane, he described the concept of dialysance and noted varying dialysance values for different solutes.² Michaels (1966) provided further assessment of dialyzer function with his analysis of the mass transfer coefficient.³ The first attempt to correlate dialyzer function and patient outcome appeared with the square meter/hour theory (Babb, 1971).⁴ The following year, the same group proposed the "middle molecule hypothesis,"⁵ suggesting that adequate removal of larger molecules, which diffuse more slowly across a membrane, requires dialysis sessions of longer duration and that accumulation of these "middle molecules" causes some of the toxic effects of renal failure.

The National Co-operative Dialysis Study (NCDS) and urea kinetic modeling

In 1974, Gotch and Sargent proposed urea kinetic modeling (UKM) as a technique for monitoring HD adequacy.

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The method was used in the protocol of the NCDS. This landmark study had a 2×2 factorial design with patients randomized to a low or high time-averaged urea (TAC_{urea}) to examine the effect of small solute clearance, and a long (4.5 hr) or short (3 hr) dialysis treatment as a surrogate for middle molecule clearance. The results (1983)⁶ showed better outcomes at lower TAC_{urea} values but suggested no significant benefit from longer treatment times. This may have hastened the move toward shorter treatment times in the United States. The study could be criticized on several counts. It was short (24–48 weeks), there were small numbers (165), all patients were < 60, and none were diabetic. In addition the high TAC_{urea} arm of the study was terminated early due to excess mortality. In 1985, Gotch and Sargent reanalyzed the NCDS data⁷ and quoted the first marker of “adequate” HD on the basis of the parameter Kt/V , where K is the dialyzer clearance, t is the time on dialysis, and V is the urea distribution volume. Their analysis suggested that improvements in outcome continued up to a Kt/V of 1.0 and patients with a $Kt/V < 0.8$ did poorly. This was the first time that body mass (expressed as V), and hence nutritional status was related to outcome. Keshaviah (1993) fitted an exponential curve to the same data, suggesting continuing benefit at $Kt/V > 0.8$.⁸ The use of UKM to prescribe dialysis dose allows assessment of dialysis efficiency by comparing the prescribed dose with the delivered dose, correlation with markers of nutritional status, and individualization of treatments on the basis of body mass. Problems may arise from the use of potentially inaccurate estimates of dialyzer clearance (K) and the use of anthropometric estimates of V . Because of this, several other measures of dialysis adequacy have been used.

Urea reduction ratio (URR)

In 1991, Lowrie and Lew advocated the use of the URR to simplify the monitoring of dialysis adequacy.⁹ A URR of 63% gives essentially the same amount of dialysis as a single pool Kt/V of 1.2 and the 2 parameters are often quoted together. While this simplifies the practicalities, the URR gives no information about nutritional status, cannot compare the prescribed dose with the delivered dose, takes no account of the effects of ultrafiltration and is difficult to adjust when off-target.

Two-pool UKM and equilibrated Kt/V

The single-pool Kt/V is only a valid mathematical description of solute clearance from a homogenous pool. The multicompartmental nature of the circulation during

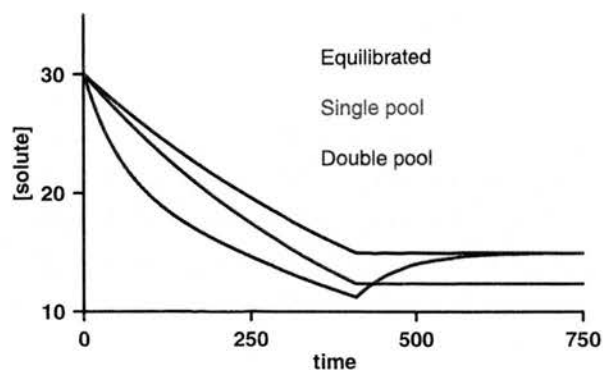


Figure 1 Small solute kinetics during hemodialysis.

dialysis is demonstrated by the rebound increase in solute concentration after the cessation of dialysis. The rebound has several components: in the presence of access recirculation, reequilibration takes several seconds to complete as blood mixes in the vascular access; cardio-pulmonary recirculation lasts for around 2 min; reequilibration between body compartments can take several hours, depending on the solute, but for urea, it takes around 30 to 40 min. Failure to account for the rebound in dose estimation may result in overestimation of delivered dose. Formal estimation of 2-pool Kt/V takes into account rebound, but is cumbersome for use in routine practice and requires analytical software to implement. The equilibrated Kt/V (eKt/V —Figure 1) is a compromise. It can be inferred from the single-pool estimate by one of several accepted methods.^{10–12} For any given dialysis treatment, the eKt/V will be lower than the corresponding single-pool Kt/V .

National Institutes of Health (NIH) consensus conference and the evolution of guidelines

In 1993, an NIH Consensus conference¹³ was convened in response to the high mortality among U.S. dialysis patients. Among the conclusions was that this excess mortality may be at least partly explained by suboptimal HD dosing related to problems with vascular access, nonadherence to dialysis prescription, and under-prescription. The recommendation was made that “until randomized controlled trials have been completed, delivered HD dose [should] at least equal a measured fractional urea clearance Kt/V value of 1.2 (single pool).” This afterwards found echoes in Renal Association Clinical Practice Guideline on Adequacy of Hemodialysis (1996), and the K/DOQI (1997) and subsequent guidelines.

The HEMO study

A number of uncontrolled studies had suggested benefits from delivered doses in excess of 1.2 spKt/V. Japanese Registry data demonstrated reduced mortality with increasing Kt/V up to a level 1.8.¹⁴ It was suggested that the improved outcomes in Tassin were at least partly attributable to a higher dose (mean 1.67 spKt/V).¹⁵ In an attempt to provide clarity, NIH commissioned the HEMO study. One-thousand eight-hundred and forty-six prevalent dialysis patients were recruited into a 2 × 2 prospective randomized study of standard-dose (eKt/V 1.05) vs. high dose (eKt/V 1.45) groups (to assess the effects of small solute clearance) and low-flux vs. high-flux membranes (to assess the effects of middle molecule clearance) and followed them for a mean of 2.84 years. No significant difference was found between the dose groups with respect to all-cause mortality, though in subgroup analysis, women in the high-dose group had a lower mortality ($p=0.02$) than those in the standard-dose group.¹⁶ Differences in body size could not be implicated,¹⁷ but suspicion of a denominator effect remains. Likewise, there was no significant difference between mortality in the high-flux and low-flux groups, although in subgroup analysis, in the group of patients who had dialyzed for over 3.7 years before inclusion in the trial, there was a 36% reduction in mortality ($p=0.001$). The effect did not seem to relate to differences in residual renal function. In addition high serum β_2 -microglobulin levels predicted mortality. Design issues included the exclusion of heavy patients, overrepresentation of African Americans, and lack of account for dialyzer reuse and water quality.¹⁸ Nevertheless, the HEMO study appears to have defined the practical limits of the adequacy targets for conventional thrice-weekly HD. So why has mortality among HD patients remained so high? Why is survival after transplantation progressively compromised by increasing dialysis vintage before transplantation?¹⁹ These and similar issues suggest that we have much to learn still about what constitutes adequate dialysis. In this context, the HEMO study has served to fuel numerous debates: are we using the appropriate dosing parameters? Are current dosing targets applicable across all patient subgroups and under all circumstances? What is the relative importance of session time and frequency? Is it justifiable to continue using low-flux membranes in the light of potential benefits of high-flux ones? Some of these issues are further discussed below.

A denominator effect?

The use of V to normalize dialysis dose (Kt) has been questioned.^{20,21} V is an independent predictor of survival

across a large range of delivered dialysis doses, an effect that has been attributed to malnutrition in a high-risk group of patients with a low body mass.^{21–24} The dialysis dose (Kt) required to achieve the target Kt/V is less in patients with a low body mass. Malnourished patients, who have an inappropriately low V, are therefore likely to be underdialyzed. There may be more fundamental issues. Dialysis attempts to replicate renal excretion of metabolic waste products, whose generation is proportional to the metabolic rate. Basal metabolic rate is proportional to M^a , where M is the body mass and a the mass index, which, at around 0.67 to 0.75, implies that smaller patients have proportionally higher metabolic rates and hence require proportionally more dialysis than their larger counterparts. Normalizing by M^a (or V^a) or by body surface area (BSA), would abolish this effect. It is standard practice to normalize the glomerular filtration rate (GFR) for BSA. Normalizing dialyzer clearance by the same factor would seem to be a logical extrapolation. Following the same reasoning, the generation of metabolic waste relates not only to an individual's basal metabolic rate but also to their degree of physical activity and catabolic state. Very active or catabolic individuals may well require more dialysis. Finally, V is frequently estimated anthropometrically usually by the Watson equations, which are different for men and women, such that women of a particular height and weight have a smaller V than similarly sized males. Hence, women will tend to be prescribed a lower Kt to deliver the same Kt/V, which risks underdialysis in some. The use of V estimated by formal UKM is not subject to the same criticism.

Flux, convection and Hemodiafiltration

Native renal function is mainly a convective process that is tempered by specific absorption mechanisms and is continuous in nature. The ideal renal replacement therapy should therefore mimic this as closely as possible. Hemodiafiltration is a step closer to this ideal. This was first described in the 1970s as a technique using high-flux membranes and combined convective and diffusive transport. This allowed rates of removal of low-molecular-weight solutes similar to those achieved in conventional HD but much augmented clearance of "middle molecules."²⁵ HDF requires the replacement of large volumes of filtered fluid with a substitution fluid, free from chemical and bacteriological contamination. With the availability of systems allowing online sterile fluid production, the technique is now economically viable. Subsequent experience with online HDF has suggested potential benefits including reduced serum β_2 -microglob-

ulin levels and an associated delay in the clinical manifestations of dialysis-related amyloidosis, improved cardiovascular stability on dialysis,²⁶ and possibly improved survival.²⁷

Treatment time

Following the NCDS, less importance was attributed to treatment time as an independent outcome determinant. This fuelled a progressive reduction in treatment times particularly in the U.S.A., which, as alluded to earlier, was subsequently identified as a factor in the excess mortality of U.S. dialysis patients.¹³ Treatment time, although not a primary intervention, did not emerge as an independent determinant of outcome in the HEMO study. However, there are indications that treatment time plays a role in determining outcome independent of Kt/V and ultrafiltration rate.²⁸

Treatment frequency

The current norm of thrice-weekly scheduling was borne of pragmatism rather than evidence. Indeed, there is evidence of an increased rate of sudden and cardiovascular death after the “long gap” (Friday to Monday and Saturday to Tuesday) in USRDS data.²⁹ There is increasing interest in more frequent treatment and increasing evidence that daily dialysis, short-hours, and particularly long-hours nocturnal dialysis improves small and “middle-molecule” solute concentrations, blood pressure, and general well being.³⁰ It also appears that such patients experience a minimal “dialysis hangover” in stark contrast to those dialyzed thrice weekly.³¹ New approaches are required to allow comparison of the dialysis doses delivered by different frequency schedules and indeed by different modalities. The currently favored option is the standard Kt/V,³² which is based on the notion that outcomes are similar in standard thrice weekly HD and peritoneal dialysis because of similar mean peak plasma urea levels. For intermittent treatments, this quantity can be viewed as their weekly continuous equivalent. By increasing the frequency of dialysis, it is possible to increase the weekly standard Kt/V to a far greater extent than by increasing the adequacy of the individual dialysis session (Figure 2). There is a great need for controlled data relating to dialysis frequency, and the National Institute of Health have recently initiated 2 studies to assess the impact of more frequent scheduling on outcome.

How much is enough?

At present, there is a general consensus in the guidelines. Kidney Disease Outcomes Quality Initiative (K-DOQI)

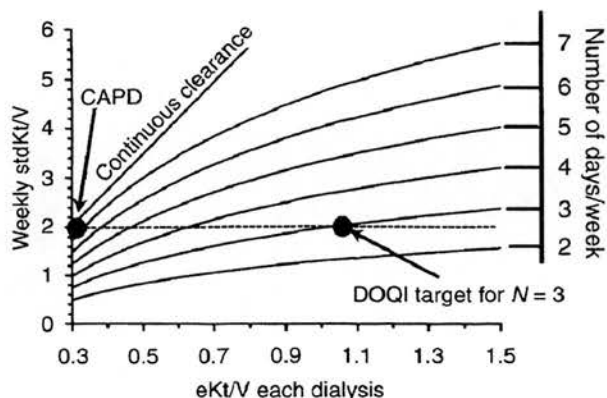


Figure 2 Relationship of weekly standard Kt/V to equilibrated Kt/V per dialysis session and frequency of dialysis sessions. (Adapted with permission from Gotch FA: The current place of urea kinetic modelling with respect to different dialysis modalities. *Nephrol Dial Transplant* 1998; 13(Suppl 6): 10–14.)

(2006) recommends a minimum single-pool Kt/V of 1.2, with a target of 1.4; the U.K. guidelines recommend an equilibrated Kt/V of 1.2 (spKt/V 1.3), with similar recommendations from the European Best Practice guidelines (2002). On the basis of the HEMO study, it would seem appropriate to prescribe increased doses in women who are not doing well on standard doses, and to use high-flux membranes in patients destined for long-term treatment. Although not yet compelling, the case is building up for the use of high-flux membranes as a standard. In spite of this consensus, mortality among HD patients remains high, and because we appear to have exhausted the benefits of increasing dialysis dose on a per-treatment basis using a thrice-weekly schedule, we now need to explore the potential benefits of increased treatment frequency. Certainly, the logic of thrice-weekly treatments with their long weekend gap needs to be questioned in patients with little residual function. Alternate-day treatment might be a more reasonable baseline. Increased-frequency center-based treatment is likely to be impractical and significant expansion of home-based therapy needs to be explored. But this will not suit all. Perhaps, the traditional “one size fits all” approach to dialysis therapy needs to be revised.

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