Mechanisms of progression in chronic nephritis.

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DEDICATION

I dedicate this thesis to all the rats that died from uraemia during the course of my experiments and in particular to H19 who like many others was wasted, uraemic and hypertensive ..... Without them this research would have been impossible.

I also dedicate it to my wife Penny for her continuous encouragement and her help throughout my research. Finally, I dedicate it to my little daughter Gemma, who hopefully will grow and live in a world where chronic renal failure is a treatable condition.

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PREFACE

This research started in 1979, when I was asked by Professor D.K. Peters and Dr. A.J. Rees to direct my attention to the factors involved in the progression of chronic renal failure with particular emphasis on the role of dietary protein on such progression. At the time, I agreed although my enthusiasm was restrained by the prospect of having to spend two to three years researching in a field of little interest away from the glamour and excitement of the immunopathology of renal diseases.

Indeed, as a medical student in the late Sixties and early Seventies I was fascinated and totally convinced, as many of my contemporaries, by the elegant findings of Dixon and his collaborators as well as Germuth and Rodrigues on the immunology of glomerular diseases. I believed, at that time, that immunology held the key to most unanswered questions in nephrology. This conviction was, to some extent, reinforced when I spent an elective period in 1973 at the Royal Postgraduate Medical School with Professors D.K. Peters and P.J. Lachmann.

Therefore, in the late Seventies, when I embarked on this research project, I was still to some extent under the immunology spell as most of the nephrology would have been for too long a time. Hence my disappointment at not being allocated a research project exploring yet another immune mechanism of renal injury. However, over the ensuing years and as my research progressed, and as echoes of similar research were reaching me from the United States I realised how important my work could be and how we were mislead

in assuming that acute and chronic nephritis were similar diseases and that the immune system had a preponderant role in both.

My research demonstrated in an experimental model of immune induced glomerulonephritis that progression to end stage renal failure occurred regardless of the surrounding host humoral immune response. Furthermore, progression with glomerular and tubulo-interstitial scarring could be prevented by dietary manipulations with both low and high protein diets equally protective; the first through the prevention of glomerulosclerosis and the latter through prevention of tubulo-interstitial scarring and calcifications.

In animals it seemed progression to end stage renal failure requires the combined destruction of both glomerular and tubular structures. The prevention of either was sufficient to slow the functional loss.

In man, my research established the predominant role of renal haemodynamics in the progression of chronic renal diseases, as for the first time the response to dietary protein restriction was shown to be a selective process. Chronic tubular disorders responded favorably to dietary protein restriction while glomerular and vascular nephropathies had a variable response. In these conditions the response to a low protein diet seemed to reflect the underlying haemodynamic pattern of the nephropathies.

From this research, I soon realised that my line of research was extremely valuable as it pioneered the exploration of a major clinical problem in nephrology ie. the progressive course to end

stage renal failure that seems to be the fate of most patients with chronic renal failure. Today in 1984, as I am writing the final lines of this thesis, the subject of progressive renal scarring has become a topical, even fashionable, one with an explosion of research and publications about it.

Over the last five years, nephrologists have finally realised that acute glomerulonephritis, a primary immune mediated disease, had little in common with its chronic counterpart, a sclerotic process, possibly haemodynamically mediated, akin to the natural ageing process of the kidneys.

Currently the role of dietary interventions as an investigative as well as therapeutic tool in experimental and clinical nephrology is becoming more and more prominent.

I hope that my contribution to the dietary revolution in nephrology will only start with the research presented in this thesis. Today, I am very grateful to both Professor D.K. Peters and Dr. A.J. Rees for having offered me the opportunity to be in the forefront of such an expanding and exciting area of nephrology.

ABSTRACT

The aim of this thesis was to explore some of the factors involved in the progression of chronic renal failure in experimental animals and in man. Dietary manipulations were used to indirectly explore some of the mechanisms of such progression to chronic renal failure.

In rats, using a new experimental approach, I found that the progression to chronic renal failure (CRF) of the immune mediated nephrotoxic nephritis (NTN) depended on the degree of renal damage or compensatory changes during the sub-acute stage of the disease. The role of the host humoral immunity had little effect on such progression.

In this model as well as in the sub-total nephrectomy model a low protein diet (LPD) prevented severe renal failure and protected against glomerulosclerosis. In NTN, a high protein diet (HPD) similarly provided some protection although through the prevention of renal tubulo-interstitial calcification and destruction. Such a beneficial effect was not reproduced by a urea induced osmotic diuresis or a DOCA and salt plasma volume expansion.

In man, a low protein diet slowed the rate of decline of patients with CRF. However the LPD beneficial effect was selective with a preferential action in patients with chronic tubulo-interstitial diseases.

Patients with hypertensive nephrosclerosis had a poor response to the diet while those with chronic glomerulonephritis responded variably. The response to a LPD correlated well with its effect on proteinuria as well as on renal blood flow.

From these data it appears that glomerular hyperfiltration might not be a universal phenomenon in CRF. Progression to renal failure occurring in ischaemic as well as hyperperfused glomeruli, with a maximal protection through dietary protein restriction in the latter group. Similarly, progression to end stage renal failure (ESRF) might require the combined destruction of glomerular and tubular structures with the prevention of either suffficient to delay the onset of ESRF.

CONTENTS

	Page
TITLE PAGE	1
DEDICATION	2
ACKNOWLEDGEMENTS	3
PREFACE	4
ABSTRACT	7
CONTENTS	9
AIMS OF RESEARCH	14
CHAPTER I	20
INTRODUCTION (General)	20
SECTION 1	
Chronic renal failure in man	20
I. The ageing kidney	20
II. The natural history of glomerulonephritis	22
III. Factors influencing progression	32
1. Genetic factors	32
2. Immunological factors	34
3. Haemodynamic factors	36
4. Dietary factors	41
SECTION 2	
Chronic renal failure in animals	47
I. The ageing kidney	47

		Page
II.	Experimental models	53
	1. Nephrotoxic serum nephritis (NTN)	58
	2. Autologous immune complex nephritis (AICN)	61
	3. Chronic pyelonephritis	62
	4. Remnant kidney model	63
	5. Diabetic nephropathy	65
III.	Factors influencing progression	67
	1. Pharmacological factors	69
	2. Hormonal and metabolic factors	75
	3. Haemodynamic factors	78
	4. Dietary factors	85

CHAPTER II 101

MATERIALS AND METHODS 101

Section 1

Animal Experimental Work 101

Animals 101

Preparation of rat glomerular basement membrane (GBM) 103

Production of rabbit anti rat nephrotoxic serum (NTS) 105

Immunisation procedures 105

Preparation of nephrotoxic globulin (NTG) 109

Testing the activity of the NTG 110

1. Indirect immunofluorescence (IF) 111

2. Quantitated estimation in vivo of NTG fixation on the GBM 111

3. Induction of nephritis (proteinuria) 114

Page

Models of nephrotoxic nephritis (NTN) 114

1. The standard model 115

2. The telescoped, accelerated model 115

Assessment of nephritis 116

1. Proteinuria (Biuret method) 116

2. Renal function 119

3. Autologous antibody measurement 119

4. Renal morphology 121

Renal biopsy procedure 121

Surgical procedures : 126

1. Unilateral nephrectomy (UNx) 126

2. Sub-total nephrectomy (SNx) 127

a. Left 2/3 nephrectomy 127

b. Right unilateral nephrectomy 127

3. Renal transplantation 129

a. Dissection of recipient rat 129

b. Dissection of donor kidney 131

c. Transplantation procedure 133

Experimental diets 124

Section 2

Human clinical investigations 134

Patients 134

Effects of low protein diet on chronic renal diseases 135

Effects of low protein diet on renal physiology 136

Experimental diets 138

Page

Statistical analysis of data 140

CHAPTER III 141

EXPERIMENTAL DATA 141

A. Results of Animal Experimentation 141

Section 1

Role of immune response in the progression of renal disease - nephrotoxic nephritis (NTN) 141

(a) Natural history of NTN 144

(b) Effect of late unilateral nephrectomy on the course of NTN 155

(c) The role of immune environment in the progression of NTN 163

Section 2

Effects of diet on the progression of chronic renal disease 176

(a) The sub-total nephrectomy model 176

Dietary protein 176

(b) Nephrotoxic nephritis 189

1. Acute effects of dietary manipulations 189

2. Chronic effects of dietary manipulations 206

(i) Dietary protein 206

1. The effect of early dietary protein introduction 210

2. The effect of late dietary protein introduction 216

Page

(ii) Dietary urea 226

(iii) Dietary salt and DOCA 235

Summary of Animal Experimentation 244

B. Results of Clinical Investigations in Man 247

Section 1

Role of dietary protein in the progression of chronic renal diseases 247

Section 2

Effect of dietary protein on renal physiology 255

CHAPTER IV

DISCUSSION (General) 265

REFERENCES 285

AIM OF RESEARCH

Over the last twenty-five years major research and investigations have concentrated on the mechanisms involved in the pathogenesis of acute glomerulonephritis (Dixon, 1968). We have seen a better understanding of the role of the immune system in glomerular diseases (Wilson & Dixon, 1981); Dixon as well as Germuth (1973) defined the respective roles of immune complexes and auto-antibodies in the induction of acute nephritides. The role of the complement system; its activation (Cochrane, 1969) as well as complement component deficiency (Agnello, 1978) were all incriminated in the production of acute glomerular injury.

Cellular immunity was discovered to be of importance in the induction and recovery of acute immune mediated experimental nephritides (Thomson, 1979); neutrophils (Cochrane, 1965), lymphocytes (Bhan, 1978) and more recently monocytes (Cotran, 1981) have all been considered the mediators of the acute and sub-acute inflammatory changes of glomerulonephritis. The coagulation system was thought to be of equal importance, as work pioneered by Vassalli and McCluskey (1971) went a long way in defining its role in the acute inflammatory process of experimental nephropathies.

From all these studies and many more our understanding of the factors involved in the induction of acute nephritis became clearer. Human glomerulonephritis like the animal counterpart is thought to be immune mediated. Immune complexes formed in the circulation (Germuth & Rodriguez, 1973) or in situ (Couser, 1980) along the glomerular basement membrane (GBM) are now considered of importance in the pathogenesis of many forms of acute glomerulonephritis, although major

doubts persist as to their real role in the disease process (Cameron, 1982). However in man, the role of planted exogenous (Wilson & Dixon, 1981) and endogenous (Agnello, 1976) antigens on the GBM and their induction of a phlogistic response remains to be better defined.

Auto-antibodies, and more specifically the ones directed at the glomerular basement membrane are likely to be pathogenic in man (Wilson, 1981); their presence in the circulation correlates with the severity of the disease activity, and their removal from the blood stream seems in most cases to improve the outcome of the disease (Rees, 1979).

The management of human glomerulonephritis has advanced along similar lines; with major attempts directed at the manipulation of the host immune response (Cameron, 1982). Immunosuppression became the cornerstone of therapy of acute glomerulonephritis (Cameron, 1971); Steroids (MRC, 1971), cyclophosphamide (Balslov, 1975), azathioprine (Sharpstone, 1969), chlorambucil (Grupe, 1976), nitrogen mustard (Baldwin, 1953), mercaptopurine (Shearn, 1965), have all been used in different disease entities and at different stages of the nephropathies. When conventional immunosuppression failed new approaches were designed such as plasmapheresis (Lockwood, 1979) and anti-lymphocyte therapy (Pirofsky, 1969). Anticoagulation received similar attention; heparin (Bone, 1975), warfarin (Kincaid-Smith, 1970), anti-platelets agents (Varenterghem, 1976), prostacycline (Webster, 1980) as well as defibrinating agents have all been applied to the management of acute kidney disease and failure.

In spite of all these advances in the understanding of the pathophysiology of acute glomerulonephritis and its management one frustrating and disturbing fact remained unchanged; acute glomerulonephritis in the majority of cases continued to progress sooner or later to end stage renal failure. The progression of nephropathies seemed a relentless, unstoppable, phenomenon (Cameron, 1979) with terminal renal failure a predictable event (Mitch, 1976). In spite of all the above mentioned advances the number of patients reaching and requiring dialysis continued to increase (EDTA, 1983). It seemed that our better understanding of acute glomerulonephritis had little impact on the incidence of chronic glomerulonephritis.

It appeared therefore that acute and chronic nephritides might be two separate entities with different pathogenic factors and requiring different therapeutic approaches. Treatment of chronic glomerulonephritis with immunosuppression might not only be pointless but was proved repeatedly to be a dangerous undertaking.

So, what is chronic glomerulonephritis ? Why does glomerular scarring progress often years after the acute, immune mediated, injury has subsided? What are the factors involved in such progression? Is the immune system a major determinant of progression of renal diseases as it is of their induction?

Could the chronic glomerular scarring and sclerotic process be slowed or even halted once started?

All these were the questions that I attempted and undertook to study and hopefully answer with the research involved in this thesis.

I undertook to study models of experimental chronic renal diseases in rats. In these models, some immune mediated and others not, I tried to define some of the factors involved in the progression of the disease to chronicity. I attempted, in an immune mediated model, to define and clarify the role of the persistent immune injury and inbalance in the perpetuation and progression of the glomerular injury. In this model as well as the others I tried to delineate some factors that could improve or accelerate the natural history of the nephropathies. By doing so, I hoped to be able to understand some of the pathophysiology of chronic glomerular disease. I used diet and in particular the manipulations of the dietary protein intake to alter the course of the experimental nephropathies. Such diets were previously used by others (Kleinknecht 1979) in similar experiments as will be reviewed in the introductory section, however I felt that in view of some shortcomings in previous experimental work I had to repeat and expand it. From my results in the experimental models of chronic renal failure, I drew some conclusions and advanced, some hypotheses as to the mechanisms involved in progressive renal scarring.

In the final stage of this research, I applied my newly acquired knowledge to the study of the progression of chronic renal diseases in man.

Here again, I used a similar approach, whereby I manipulated the dietary protein intake of patients with chronic renal failure and attempted to interpret the results of my observations. From these data, in man, I drew some conclusions as to the possible mechanism involved in the progression of various forms of chronic renal disease.

The research in this thesis was done over three years and consisted of different phases in succession. I used, in rats, the remnant kidney as well as the nephrotoxic nephritis (NTN) models of chronic renal failure. First, in the nephrotoxic nephritis model I defined the influence of the immune response on the longterm course of the disease. In this model, previous work has emphasised the dependency of the acute phase injury on the immune response (Dixon, 1961). However, little is known about the role of such immune response in the chronic phase of the disease.

The second phase of the research is directed at the exploration of the influences of non-immune related interventions on the course of the remnant kidney and nephrotoxic nephritis models. Emphasis in this phase was on the effect of dietary factors. The role of dietary protein in the progression of renal scarring following sub-total nephrectomy was studied. Similarly, the acute and chronic effects of dietary manipulations of protein, urea, as well as salt were explored in chronic nephrotoxic nephritis.

The third and final phase of the research was directed at the application in man of some of the animal experimentation findings; I studied the acute effects of low and high protein diets on

diseased kidneys in order to determine the short term influence of these diets on renal physiology. This was followed by an attempt to influence the longterm course of different forms of human nephropathies by a low protein diet. Although , previous workers have established that a low protein diet was beneficial in patients with chronic renal failure (Giordano, 1982), little was known of its differential effect on various forms of nephropathies.

I hope that this work will further our understanding of chronic renal diseases and will be the starting point for a rational therapeutic approach to sub-acute and chronic renal failure.

CHAPTER I

INTRODUCTION (GENERAL)

CHRONIC RENAL FAILURE IN MAN

I. The ageing kidney

Glomerulosclerosis in man is often the end result of a variety of insults; immune mediated glomerulonephritis, ischaemia, hypertensive nephrosclerosis, chronic tubulo-interstitial nephritides and ureteric obstruction all lead to end stage glomerular scarring with tuft obliteration and sclerosis (Baldwin, 1982). Before reviewing the factors involved in the progression of renal disease in man, it is important to review and appreciate that some of the changes induced by diseases of the kidneys can be seen in the ageing, normal, kidney, in the absence of intrinsic nephropathies.

The incidence of obliterated sclerosed glomeruli increases with age in the absence of underlying nephropathies. Glomerulosclerosis in men under the age of 40 was found to be a rare phenomenon in the absence of intrinsic renal disease. Over the age of 45 to 50 years the incidence of glomerulosclerosis exceeded 50% (Kaplan, 1975). This phenomenon of spontaneous, age related, glomerulosclerosis has recently been shown to increase in patients with unilateral renal agenesis, Kiprov et al (1982) in a recent review of 586 renal pathology specimens found unilateral renal agenesis in 5 of 29 patients with focal glomerulosclerosis; the incidence was greater than anticipated by chance alone. They also found focal glomerulosclerosis in 2 of 7 patients with unilateral renal agenesis (29%) among 9200 autopsies. No glomerular disease was found in 10 patients who died 8 to 46 years after having a unilateral nephrectomy in adulthood. This was in sharp contrast with the course of 7/8 patients with unilateral renal agenesis who developed proteinuria, glomerulosclerosis and renal failure.

The authors concluded that early reduction in renal mass, with subsequent nephron overload led to progressive glomerular scarring. Whether nephrosclerosis in these patients is the reflection of an acceleration of the ageing induced changes is open to speculation. Similarly, the renal function declines with age. Davies et al (1950) found a step-wise decrement in inulin clearance and renal blood flow after the fourth decade. Friedman et al (1972) studying renal function in 35 patients with an age range between 60 and 93 years found a reduction in glomerular filtration rate of 40 to 70% of normal in these patients. These authors suggested that the age related decline in renal function might be the consequence of progressive obstructive vascular changes resulting in glomerular ischaemia. However, McLachlan et al (1977) failed to show a correlation between glomerular involution in the secescent kidney and age related vascular changes.

It is apparent that glomerular scarring is not exclusively the result of preceeding glomerular injuries, but that glomerulosclerosis is an age related involutionary process. The understanding of injury related glomerulosclerosis might be greatly enhanced by the understanding of the factors involved in the age related changes in glomerular structure. Glomerulosclerosis might be the final pathway of diseased and senescent glomeruli. The underlying pathophysiology of ageing kidneys might shed some light on the mechanisms involved in the progression of acquired renal diseases.

It seems therefore that reduction in renal mass, as in unilateral renal agenesis, might accelerate the normally occurring changes in renal morphology. Before further exploring the factors operational in progressive renal scarring, spontaneous or acquired, it is important to review the natural history of some forms of chronic renal diseases.

II. The natural history of glomerulonephritis

In this section attempts are made to delineate some of the factors involved in the progression of chronic glomerular diseases. In order to do so, I will briefly review the natural course of three representative types of glomerulonephritis, namely : (1) membranous nephropathy, (2) focal and segmental glomerulosclerosis and (3) proliferative glomerulonephritis.

(1) Membranous glomerulopathy (MN)

Membranous nephropathy constitutes approximately 9 to 10% of human nephropathy presenting with nephrotic syndrome (Cameron, 1979). The etiology of this disease is unknown, however secondary forms are known to occur with different etiological factors such as infections (Glassock, 1980); bacterial, parasitic and viral, drugs (Fillastre, 1981) and tumours (Kaplan, 1976). The primary, idiopathic, form progresses to chronic renal failure.

Genetic predisposition to membranous nephropathy seems an important feature in acquiring the disease and to some extent in determining the rate of progression. This nephropathy occurs with a higher frequency in patients who are carriers of the major histocompatability complex antigens DR3 and B18 (Klouda, 1979).

Patients with membranous nephropathy and carriers of the complement haplotype Bf F_1 seem to have a more severe nephritis course and they are less likely to undergo spontaneous remissions (Cairns, 1980).

Otherwise, the prognosis of membranous nephropathy is influenced by the age of the onset of the disease; children have a better prognosis than adults. The incidence of renal failure is approximately 4% at 5 years in children compared to 20% over the same span in adults. Similarly, the rate of remission from the nephrotic syndrome is higher in children (35%) compared to 8-10% in adults (Cameron, 1970).

Patients with membranous nephropathy like others with mesangio-capillary GN (MCGN) and focal and segmental glomerulosclerosis (FSGS), with a nephrotic syndrome, have almost identical survival curves with 30 to 40% survival at 10 years (Cameron, 1979).

Proteinuria, and in particular the nephrotic syndrome, are important prognostic factors; patients who achieve any kind of remission, even partial, do not usually progress to renal failure (Idelson, 1977).

On the other hand, patients with persisting, non remitting, nephrotic syndrome invariably develop renal failure. The mechanisms involved in the progression of membranous nephropathy are unknown; genetic predisposition and persistent proteinuria are poor prognostic indicators. The role of the persistence of the initiating factors, and of subsequent haemodynamic adjustments as well as proteinuria, in the progression of the disease are all subject to speculation.

Steroid therapy, previously thought to be of little therapeutic value in membranous nephropathy (MRC, 1971), has recently been shown by the American collaborative study (1979) to slow the rate of progression of the disease. This study has been criticised, as the course of the nephropathy in the untreated group was exceptionally severe (Cameron, 1979b). Further studies are required in order to fully evaluate the role of steroid therapy in the treatment of membranous nephropathy.

Insights into the pathogenesis of renal diseases in man have often been obtained from experimental models in animals. The autologous immune complex nephropathy (AICN) in rats bear some similarities to (MN) in man. The understanding of the mechanisms operational in the progression of this animal model might give insights into the mechanisms of progression of its counterpart in man. AICN is induced in certain strains of rats by immunisation with autologous tubular brush border antigen (FxlA). Development of proteinuria in this model is associated with the presence of immune aggregates consisting of the FxlA antigen, autologous anti-FxlA antibody and complement along the subepithelial side of the glomerular basement membrane (Glassock, 1968).

Proteinuria becomes significant 9 to 10 weeks after the induction of the nephritis and increases thereafter, as renal function declines (Lim, 1973). During the acute stages of the nephropathy a severe reduction in single nephron glomerular filtration rate (SNGFR) occurs (Allison, 1974). The glomerular haemodynamic changes are heterogenous, however no hyperperfused-hyperfiltrating glomeruli have been observed in this model of experimental membranous nephropathy (Rocha, 1973).

Immunosuppression and anti-inflammatory agents have invariably failed to alter the course of the disease (Kupor, 1976) especially when applied late.

It seems therefore that membranous nephropathy in man and its counterpart the autologous immune complex nephropathy in rats are progressive diseases with severe proteinuria and progression to chronic renal failure.

(2) Focal and segmental glomerulosclerosis (FSGS)

Focal and segmental glomerulosclerosis like membranous nephropathy could be primary, with no known etiology or secondary to a variety of conditions.

As we have seen in the previous section this entity can be a normally occurring involutional change of senescence, with an increased percentage of glomeruli obliterated and sclerosed over the age of 50 (Kaplan, 1975). These morphological changes in man underly the known decline in renal function occurring with age (Friedman, 1972). On the other hand FSGS can be the final, common pathway to chronic renal failure of many nephropathies (Brenner, 1983); glomerular and tubular disorders evolve to chronic renal failure with an increasing percentage of glomeruli developing sclerotic changes (Kincaid-Smith, 1975).

Similarly, focal and segmental glomerulosclerosis occurs with an increased frequency in patients with unilateral renal agenesis (Kiprov, 1982); these patients develop in adulthood, proteinuria, hypertension and eventually chronic renal failure. Finally focal glomerulosclerosis has recently been reported following unilateral nephrectomy (Zuchelli, 1983).

The primary form of FSGS has a poor prognosis with survival rates of approximately 40-60% at 10 years (Cameron, 1979a).

The presence of renal failure when patients present with the nephropathy as well as the persistence of the nephrotic syndrome are poor prognostic factors (Idelson, 1977).

Brown and Cameron described a sub-group of patients with FSGS with a rapidly progressive course; these patients, all young, were severely nephrotic, hypertensive, had microscopic haematuria and extreme hypercholesterolaemia (Brown, 1978). This group of patients had recurrent profound hypovolaemic episodes secondary to the nephrotic syndrome and their proteinuria was refractory to treatment.

The management of FSGS remains hampered by our lack of knowledge of the pathogenesis of the disease and of the factors involved in its progression. Immunosuppressive and anti-inflammatory therapies have invariably failed to affect the course of the nephrotic syndrome and the disease (Cameron, 1979a).

Here again we turned to experimental models of glomerulosclerosis in order to improve our understanding of the entity. The experimental model, that bears similarities to FSGS is the remnant kidney model; animals, and in particular rats, submitted to a surgical reduction in renal mass develop focal and segmental glomerulosclerosis in the remnant kidney.

This experimental nephropathy, like the human counterpart, is characterised by proteinuria, hypertension and progressive renal

failure culminating in death from uraemia (Morrison, 1962). The renal morphology of the remnant kidney, like human FSGS, displays a spectrum of glomerular sclerotic lesions, tubular atrophy and tubuli-interstitial calcifications (Shinamura 1975). After acute reduction in functional renal mass, major morphological and functional adaptive changes occur in order to maintain the renal function (Hayslett, 1979). Haemodynamic changes occur in the remnant glomeruli with resulting glomerular hyperperfusion and hyperfiltration (Hostetter, 1981). The role of these changes in the progression of the disease and their implications in the understanding of chronic renal diseases in man will be discussed in a further section of this introductory chapter.

Therapeutic attempts to alter the course of experimental glomerulosclerosis have centered around dietary manipulations and their effects on the adaptive, progressive, morphological and haemodynamic functional changes (Brenner, 1982).

Human and experimental glomerulosclerosis are likely to be the final events in the natural history of many nephropathies with more than one pathway leading to these changes.

(3) Proliferative glomerulonephritis

The prototype of acute diffuse proliferative glomerulonephritis is the acute endo-capillary post streptococcal GN (PSGN).

The course and progression of this nephropathy has been the subject

of major investigations and controversies (Cameron, 1979).

The review of some of the data on the progression of PSGN will be of primary importance in the understanding of the chronic changes occurring in this nephropathy as well as other related diseases.

Acute post streptococcal glomerulonephritis, once thought to be a self-limited disease with no longterm sequelae, is currently believed to lead in a small but significant minority of patients to chronic renal failure (Baldwin, 1977).

The overall prognosis is affected by the patient's age at onset; pre-school children with the nephropathy fare much better than older ones and adults (Dodge, 1972). However, regardless of the age of onset, children with a nephrotic syndrome at presentation have a poor prognosis (Mota-Hernandes, 1976).

The controversy regarding the progression of PSGN started with Baldwin et al (1974) reporting a high incidence, 50% of histological progression after the acute episode. This observation, although not confirmed by others and with many shortcomings remains of major importance and relevance to the understanding of progression of acute immune mediated nephritis to chronicity and therefore deserves some attention.

In their first long follow-up observations of patients with PSGN these authors noted a high incidence, 50 to 60%, of glomerular sclerosis, a few years after the acute nephritis (Baldwin, 1974).

At this stage no correlation was observed between the morphological changes and renal function; the majority of patients had normal renal function.

The major histological finding at over 2 years after the onset of the disease was significant glomerulosclerosis with no further proliferative changes. The first having replaced the latter as the nephritis progressed to chronicity. Late, clinical relapses or progression, with proteinuria, hypertension and renal failure were noted in some patients (Schacht, 1976).

The factors involved in such progressive renal scarring after PSGN are poorly understood; the authors postulated a possible ischaemic etiology to the progressive glomerular sclerosis. They observed, major sclerotic alterations of the renal arterioles including the pre-glomerular ones. There was a close parallel between the vascular and glomerular sclerotic changes (Gallo, 1980). The group of patients with both vascular and glomerular sclerosis had a high incidence of systemic hypertension (Baldwin, 1982).

Major criticism (Kaplan & Vernier, 1978) has been directed to these observations by Baldwin and Gallo, as well as the concept of progression of acute post infectious glomerulonephritis. Studies from Trinidad (Nissenson, 1979), and South America (Rodrigues-Iturbe, 1976) failed, in sporadic and epidemic PSGN, to show evidence of long term progression.

Although the very nature of the long term follow-up of Baldwin's patients might have biased the results, as the sick continued to attend while the healthy dropped out. These author's major merit lies in drawing attention to the possibility of progression of immune mediated nephritides in man by mechanisms possibly unrelated to the initial immune insult (Baldwin, 1982).

They were among the first to introduce the concept of chronic haemodynamic, vascular and ischaemic changes replacing the acute immune ones with glomerulosclerosis resulting in chronic renal failure.

In animals, the nephrotoxic nephritis (NTN) model bears morphological and functional similarities to human acute proliferative glomerulonephritis.

In this model, after the injection of heterologous anti-glomerular basement membrane (α-GBM) antibodies, acute diffuse proliferative glomerular changes take place (Masugi, 1934). The nephropathy with proteinuria and renal functional impairment progresses to chronic renal failure with ensuing death from uraemia (Farr & Smadel,1939).

While the acute stages of NTN are characterised by glomerular proliferation and infiltration by leukocytes; neutrophils and macrophages, the chronic stages show predominantly sclerotic glomerular changes (Farr & Smadel, 1939). Various glomerular haemodynamic alterations have been observed during the acute phases of this experimental nephropathy (Blantz, 1979); in severe forms

glomerular vasoconstriction predominated (Blantz & Wilson, 1976).

In nephrotoxic nephritis, while immunosuppressive and anti-inflammatory agents seem to improve the acute nephritis (Kobayashi,1980) , they appear to be of limited therapeutic value when applied late in the course of the disease (Kurokawa, 1982). Progression of nephrotoxic-nephritis, in animals like its human counterpart, PSGN, remains a mystery.

The overall clinical experience of the natural history of glomerulo-nephritis seems to indicate that progression to renal scarring with glomerular sclerosis is an unpredictable and often relentless process.

The factors underlying such progression remain to be elucidated; the respective roles of the acute injury, the sub-acute compensatory and chronic sclerotic changes need further investigation.

Before reviewing experimental models of chronic renal failure in some detail and attempt to understand the factors involved in their progression, I would like, in the following section, to discuss the factors known to influence the course of human nephritides.

III. Factors influencing progression

In this section, I would like to review some of the factors that could predict the outcome of some forms of human glomerulonephritis. More important, I will attempt to discuss the factors that could influence the course of glomerulonephritis in man.

These factors are multiple and their effects are likely to be interdependent.

The progression of glomerulonephritis in man is thought to be affected by the following factors :

1. - genetic
2. - immunological
3. - haemodynamic
4. - dietary

1. Genetic factors

Until recently totally underestimated, the genetic predisposition to renal disease and glomerulonephritis in particular has become recently the subject of important and fruitful investigations.

Interest in HL-A association with disease entity has led to the recognition of some important immunogenetic association in glomerulonephritis.

(a) Anti-glomerular basement membrane disease (α-GBM) :

Rees et al were the first to notice a strong association between HLA-DRW2 and anti-GBM disease (1978).

These authors originally found 32 out of 36 caucasion patients with the disease to be carriers of the major histocompatibility antigen HLA-DRW2. HLA-DRW2 incidence in the normal population did not exceed 22 out of 113. This strong association was found to be only second to the one linking HLA-B27 and ankylosing spondylitis.

Later an increased incidence of HLA-B7 in this disease was observed; carriers of the antigens HLA-DRW2 and B7 had a poor prognosis with severe lung haemorrhage and renal impairment. The authors concluded that DRW2 was a marker for susceptibility to the anti-GBM disease, while B7 would influence the course and severity of this entity (Rees, 1981).

(b) Membranous nephropathy :

A strong association between DR3 and membranous nephropathy has been recognised (Klouda, 1979). More recently, further associations have been delineated with a strong one linking the disease and the haplotype HLA-DR3, B18 and BS F_1 (a rare complement haplotype encoded in the MHC) (Dyer, 1980). Patients carrying this complement haplotype seem to have a more severe nephritis with fewer and less likely remissions than patients who lack it (Cairns, 1980).

(c) Minimal change nephropathy (MCN) :

An association between MCN and HLA-B12 as well as DR7 has been reported (Alfiler, 1980).

(d) Mesangial IgA :

HLA-Bw35 has been detected with increased frequency in patients

The progression of some glomerulonephritis long after the acute immune mediated insult has subsided (Baldwin, 1982) lends support to the concept that immune factors are of secondary importance in the progression of renal diseases.

However, the role of the immune system in perpetuating an acute injury or in the progression of an acute nephritis cannot be totally ignored.

Chronic antigenic stimulation and abnormal immune response could lead to progressive renal disease in animals (Germuth & Rodrigues,1973)

In man, the role of continuous antigenic stimulation in the induction and perpetuation of glomerulonephritis is less well established. However, some examples are of significance; glomerulonephritis associated with certain infections, bacterial, viral and parasitic, tends to resolve without significant sequelae when the antigenic stimulus is withdrawn ie. the infection eradicated (Glassock, 1981).

Similarly, drug induced nephropathies, such as membranous GN secondary to gold (Van der Broek, 1966), penicillamine (Lance, 1978), mercury (Glassock, 1979), and captopril, regress after discontinuation of the offending agent (Fillastre, 1981).

Finally, continuous antigen stimulation with tumour antigens is believed to underly the spectrum of GN associated with neoplasiae (Kaplan, 1976). Regression of nephropathy is the rule when the cancer is treated and the putative tumour eradicated (Eagan & Lewis,1977).

The role of the immune response in the progression is further exemplified by the role of antibodies in the nephritis of Goodpasture's Syndrome; patients with anti-GBM disease and nephritis usually have circulating auto-antibodies directed against their glomerular basement membrane (Wilson, 1981). If treated early, the elimination of these auto-antibodies usually prevents the progression of the disease to chronicity (Lockwood, 1979). However these data are based on short follow-up studies. But it seems that in this model of human nephritis the induction and possibly the progression of the disease is dependent on the host immune response. Even so, in this nephropathy few patients have been known to progress to chronic renal failure well after the production of auto-antibodies has subsided (Rees, 1979).

From these data, it would seem that in man the immune system has a role in the induction of nephritides as well as to a lesser extent their progression.

More data are clearly needed as to the role of the host immune system in the perpetuation of the acute renal injury and the progression of the nephropathies. When available this information will rationalise our therapeutic approaches to chronic renal diseases.

3. Hemodynamic factors

Hemodynamic factors are likely to play a predominant role in the progression of human nephritis.

Three different mechanisms have been advanced and deserve separate attention :

a. Intra renal vascular changes (the hypoperfusion-ischaemia concept)

b. Intra renal hypertension (the hyperperfusion-hyperfiltration concept)

c. Systemic hypertension

(a) The hypoperfusion-ischaemia concept :

Many forms of glomerulonephritis, in man, show evidence of concomittant intra renal vascular changes (Kincaid-Smith, 1975). The vascular alterations with arteriolar sclerosis closely correlate with the severity of the glomerular sclerotic change (Gallo, 1978). This would imply a cause and effect relationship with ischaemia responsible for the glomerular scarring.

Such findings were clearly demonstrated in post streptococcal glomerulonephritis by Baldwin and Schacht (1976) and more recently in mesangial IgA nephropathy by Feiner et al (1980). These authors postulated that glomerular sclerotic changes are the result of glomerular ischaemia and hypoperfusion secondary to arteriolar narrowing. The presence, in their opinion, of ischaemic, collapsed, glomerular tufts rather than disorganised ones favoured ischaemia as the major factor in chronic glomerular damage (Gallo, 1978). Similar observations have been made in a variety of other glomerular diseases in which vascular sclerosis exceeded the damage that one would expect for the age of the patient and the duration or severity of hypertension. Kincaid-Smith reported that the incidence of renal arterial and arteriolar lesions ranged from 54% to 82% in normotensive patients under the age of 40 years who had various forms of glomerulonephritis (1975).

(b) The hyperperfusion-hyperfiltration concept

Recently attention has been directed to the role of glomerular hyperperfusion and intra renal hypertension in the progression of renal disorders (Hostetter, 1981a). Although substantial evidence in animal models of experimental renal failure attribute an important role to intraglomerular hypertension in the progression of renal diseases (Brenner, 1983), data in man are sparse.

In man, diabetic nephropathy is the main clinical entity where such phenomenon might be operational during the early stages of the disease (Viberti, 1979).

As early as 1959, Stalder and Schmid showed increased glomerular filtration in young diabetics. These early studies were recently confirmed by Mogensen (1976). This author showed as much as 40% increment in the glomerular filtration rate of new diagnosed, insulin-dependent, diabetics when compared to normal controls. These changes in GFR were acute and reversible and dependent on the acute metabolic changes; moderate hyperglycaemias increased the GFR, while strict diabetic control reduced the raised GFR to near normal. These functional changes of diabetic kidneys have morphological counterparts; Gundersen et al (1979) showed glomerular hypertrophy of diabetic kidneys. This was recently confirmed by Seya-Hansen et al (1980). Renal plasma flow is known to be increased in early diabetes and might contribute to the observed increments in glomerular filtration (Hostetter, 1982b).

The role of such hyperperfusion-hyperfiltration states in the long term attrition of diabetic glomeruli is current by being investigated.

In experimental models of diabetes in rats, these changes are thought to be major determinants of progressive renal scarring (Mauer, 1979). In rats, Hostetter et al (1981a). observed a dual haemodynamic response of glomeruli to hyperglycaemias; with moderate elevation of blood sugar, the single nephron glomerular filtration rate (SNGFR) was raised, while with severe hyperglycaemias a significant reduction in the SNGFR was noted.

These changes of hyperfiltration and hyperperfusion were accentuated by further reduction in renal mass through unilateral nephrectomy (Deen, 1974). Unilateral nephrectomy was observed to accelerate the rate of sclerotic changes in rat diabetic kidneys (Mauer, 1979). The mediators in experimental diabetes of the glomerular hyper-perfusion state are unknown; a marked reduction in glomerular capillary resistances is known to occur (Hostetter, 1981a) whether hormonal changes underly these changes is currently unknown. However, hormones such as growth hormone and glucagon, known to be increased in diabetes mellitus, have in the past been shown to increase renal blood flow (Corvilain, 1962). Their role in the pathyphysiology of diabetic nephrology remains to be explored.

Whether in man, intraglomerular hypertension is confined to diabetic nephropathy or whether it is a general phenomenon underlying compensatory changes following reduction of functional renal mass in other nephropathies is unknown (Hostetter, 1982b).

After all, glomeruli in early diabetic nephropathies are normal or increased in size unlike glomeruli of chronic glomerular disorders.

(c) Systemic hypertension

Although the role of systemic hypertension in the progression of chronic renal failure in animal experimental models has been well established (Baldwin, 1982b),such a role in the progression of nephritis in man is more difficult to establish.

Hypertension is invariably treated and controlled studies to assess the effect of untreated hypertension or the cause of renal disease in man would be unethical. However, recently Mogensen (1980) established, in diabetic nephropathy, an important role for hypertension in the progression of diabetic nephropathy. The author, in an overquoted study of six diabetic patients, showed that the rate of progression of the nephropathy in 5 out of 6 patients was slowed when hypertension was treated; anti-hypertensive treatment resulted in a considerable decrease in the progressive fall in GFR, from 1.24 ml/min/month before treatment to 0.45 ml/min/month during treatment. In this study a significant fall in albumin excretion occurred after anti-hypertensive therapy was initiated.

More recently, Parving et al (1983) showed that early and aggressive anti-hypertensive treatment reduced both albuminuria and the rate of decline in GFR in young patients with diabetic nephropathy. From these studies in diabetic nephropathy and others in mesangial IgA (Droz, 1976) and focal and glomerular sclerosis (Brown, 1978), hypertension seems to accelerate the rate of decline in renal function and bears a poor prognostic implication (Baldwin, 1982).

Haemodynamic factors seem to be of major importance in the progression of renal disease in man and might constitute the single common final pathway of nephropathies to end stage renal failure with severe vascular and glomerular scarring.

4. Dietary factors

Dietary therapy in renal disorders has been up to recently confined to the management of symptoms of uraemia in end stage renal failure (Giovanetti, 1964). Later, emphasis has been directed to the role of dietary therapy in the management of renal osteodystrophy (Johnson, 1975). More recently, however diet has been applied early in the course of renal diseases in order to influence the course of nephropathies (Giordano, 1982).

Johnson et al in 1975 studied 27 patients with chronic renal failure for an overall period of 2 years; these patients were kept on a moderate dietary protein restriction, aluminium hydroxide and calcium supplements. Renal osteodystrophy was prevented in these patients in spite of an apparent lack of effect on parathyroid hormone levels. Meanwhile, the major finding of this study was the observation that the progression of renal disease was significantly slowed by such diet. It is noteworthy that most patients in this study had creatinine levels of approximately 500umol/l at the onset of the dietary restriction, implying advanced renal failure.

A few years later, Maschio et al (1980) restricted the phosphate intake of patients with moderate renal insufficiency and noticed an improvement in their renal function; in this study patients with a serum creatinine of 300μmol/l at the onset had a slower progression

rate, whereas a second group of patients with serum creatinine of 200umol/l had an absolute halt to their disease progression for a 18 month period.

These data were confirmed by Hirata et al (1979). Both authors stressed the beneficial effect of phosphate restriction on the progression of renal disease, with emphasis on the additional advantage of early introduction of the dietary restrictions.

Heidland in 1978, reported a study of 8 patients with ESRD followed for one year; they were started on a 20 to 30g protein restricted diet with additional amino-acids for 6 months and this was followed by another 6 month period on the same low protein diet supplemented with ketoanalogous of amino-acids. On the ketoanalogous supplemented diet the serum phosphate levels decreased with subsequent reduction of the serum calcium or phosphate product. Renal function decline was slowed in this group. Walser et al (1973) failed to reproduce similar results with a ketoanalogue supplemented diet; phosphate levels remained unaffected and renal function continued to deteriorate at the same rate . However, when the diet was supplemented with aluminium hydroxide and the serum phosphate levels reduced, renal function improved. In both the Heidland and Walser studies the limited benefit of the diets is likely to be due to the late introduction of the dietary restriction.

Early institution of dietary therapy in chronic renal diseases is a prerequisite for successful dietary intervention. This was elegantly showed by Maschio et al (1982) when patients with different degrees of renal failure were followed for a period of 7 years on a diet proportionally restricted in protein and phosphate

the beneficial affect of the diet was maximal in the patients with mild renal failure.

Further studies from the same authors (1982) evaluated three groups of patients with chronic renal failure on protein and phosphate restrictions (Maschio, 1982).

In the first group (1); 25 patients had a mean serum creatinine of 218umol/l, in the second group (2); 20 patients with mean serum creatinine of 424umol/l, and in the third group (3); 30 patients with a mean serum creatinine of 228umol/l, the dietary protein and phosphate was restricted in the first 2 groups while the third was on a free diet. The restricted diet provided 40 Kcal/kg/day, 0.6gr/kg/day of protein, 700mg of phosphorus and 1000-1500mg of calcium/day. In comparison, the free diet provided 35Kcal/kg/day, 70gr of protein, 900mg of phosphorus and 800mg of calcium/24 hours. The slope of the reciprocal of serum creatinine was used to evaluate the rate of decline in renal function. The first two groups, on the restricted diets showed a marked improvement of their slopes, with Group 1 achieving the best result.

These authors concluded from their studies that moderate dietary restriction of protein and phosphorus should begin early in the course of renal diseases in order to prevent renal osteodystrophy and slow the progression of functional deterioration.

The respective roles of protein and phosphorus restrictions remain to be determined. As to the mechanisms of action of these diets; reduction in nitrogen load, prevention of hyperfiltration of remnant nephrons, reduction of parathyroid hormone secretion, maintenance of

a normal calcium x phosphate blood product, reduction of urea and phosphate load per residual nephron, might all contribute to the observed success of these diets in the management of early renal failure.

Having established, through many studies, that dietary protein and phosphorous restrictions are beneficial when introduced early in the course of renal disease, the question asked by many is how low should the protein intake be and what form of protein should the diet provide?

Bergström, who pioneered many studies on dietary therapy of patients with renal disease, showed in 1975 that a 20 gram protein diet supplemented by essential amino acids was beneficial and acceptable to most patients.

More recently, Kampf et al (1980) compared the rate of progression of renal failure of a group of 28 patients with a mean creatinine clearance of 13.2 $\pm$ 5.5 ml/min/1.73 m^2 over different periods of 3 months on variable types of protein intake. First, selective high biological value protein (40gr), then mixed poor protein (25gr) with additional essential amino acids (EAA) (6.66gr), followed by mixed poor protein (25gr) with additional ketoanalogues of amino acids (KA) and finally back on the first 40gr protein diet. In their experience, the mixed poor protein diet supplemented by ketoanalogue s proved inferior to both the standard and the EAA supplemented diets. However, the ketoanalogues diet, when used in patients with moderate renal impairment led to signs of malnutrition; such a diet should therefore be restricted to patients with advanced renal failure, creatinine clearance less than 10ml/min, where urea

reutilisation could take place and compensate for the low protein intake (Richards, 1975).

The observation of malnutrition with ketoanalogue supplementation of a mixed poor low protein diet raises the question of the minimum required level of safe protein intake in patients with renal failure.

In order to answer the question, it is essential to appreciate that uraemic patients will reutilise urea to synthesise protein, providing enough urea nitrogen was available metabolically (Richards, 1975). Urea nitrogen reutilisation is of great nutritional importance in the face of a very low protein intake providing sufficient calories are available to prevent catabolism (Giordano, 1982a).

Therefore, and with this notion in mind, a diet supplying 0.17 grams/kg/day of high biological value protein would be sufficient in advanced uraemia as long as the caloric intake approximates 52 to 60 Kcal/kg of body weight/day.

The overall concept of nutritional therapy in renal failure that emerges from most studies can be summarised as follows : dietary protein content should be modulated according to the degree of renal failure; with moderate protein restriction 0.5 to 0.6 gr/kg, 35 Kcal/kg, 900mg of phosphorus and with calcium supplementation at 1000 to 1500 mg/day, in patients with mild to moderate renal failure. Patients with creatinine clearance of less than 15ml/min would benefit from further restrictions; in these patients 0.17g/kg of protein, with a high caloric intake 52 Kcal/kg/day and 200 to 300mg

of phosphate, is sufficient as urea reutilisation would prevent hypercatabolism and malnutrition.

Dietary protein and phosphorus restriction is therefore well established as one of the cornerstones of the management of chronic renal failure.

The role of such diets is currently not restricted to its symptomatic relief of uremia but it is known to improve renal osteodystrophy and more significantly it offers a new scope in the management of progressive renal failure. Indeed such a diet, when introduced in time, can dramatically affect the course of chronic renal diseases. To understand the mechanism of action of these diets it is essential to have a better knowledge of the pathophysiology of the progression of chronic nephropathies.

Experimental models of chronic renal failure are currently fully explored in order to gain some insights into these mechanisms of evolution of renal and glomerular scarring.

CHRONIC RENAL FAILURE IN ANIMALS

I. The ageing kidney

Before discussion and reviewing the factors involved in the progressive scarring process of nephritic kidneys, it is importang to understand the ageing process of normal kidneys. It has long been known the ageing rats develop a progressive nephropathy (Elema, 1971). In this section I will discuss the histological and functional changes observed in the course of such nephropathy as well as the factors known to influence its rate of progression.

Ageing rats develop spontaneous glomerulosIcerosis (Guttman, 1960); this glomerulopathy is of focal and segmental nature not unlike its counterpart in man. In the rat insudation of hyaline material in the capillary loops occur, changes in epithelial cells are observed with fusion of podocytes, mesangial matrix increased and finally focal capsular adhesions are present. These changes are predominantly sclerotic in nature with no glomerular proliferation observed (Elema & Arends, 1975). At immunofluorescence, affected glomeruli display deposits of immunoglobulins (IgM) and complement (C3). The histopathologic features of spontaneous glomerulosclerosis in rats bear striking similarities to the ones described by Rich (1957) and more recently Habib in patients with focal glomerulosclerosis (1973).

Bolton and Sturgill(1980) studied the ultrastructural changes that occur in kidneys of ageing rats. They observed a progressive thickening of the glomerular basement membrane (GBM) of Sprague-Dawley rats; this process was segmental and irregular. Similarly epithelial cells underwent changes with age, with areas

of foot process obliteration noted at 6 months. This was a progressive phenomenon with increased areas of degeneration with age. Mesangial cells also changed; they expanded and contributed to the capillary lumen obliteration of ageing kidneys. Finally, endothelial cells displayed degenerative changes with microvillous formation. In general, tubular changes were found to parallel the glomerular alterations.

From these data, it became apparent that significant degenerative changes were present in all glomerular cell lines early in life, 3 to 6 months, and progressed steadily thereafter.

These morphological changes in the rat are accompanied by functional ones, abnormal proteinuria starts at 3 months and increases with time. Proteinuria follows the onset of epithelial foot process fusion (Bolton & Sturgill, 1980) and is unrelated to the structural thickening of the GBM. The GBM in the ageing rat retains its negative charge barrier (Couser & Stilmant, 1975).

These changes in rats occur spontaneously with age, however many factors are known to influence the rate of glomerulosclerosis of these kidneys. Male rats show more susceptibility to these morphological changes than their female counterpart (Blatherwick, 1937) Nephrosclerosis is affected by diet; diets high in protein (Lalich, 1970), amino acids (Lalich, 1971), and salt (Elema, 1971) have all been shown to accelerate the rate of glomerulosclerosis in the ageing rat.

Similarly, diffuse intercapillary glomerulosclerosis can be induced in rats by high sucrose feeding; Rosemann et al (1971) observed that rats fed a 72% carbohydrate (sucrose) diet developed diffuse glomerulosclerosis. Impairment of glucose tolerance was noted in the sucrose fed rats. The renal lesions were comparable to the ones developing spontaneously with age.

Dietary restrictions (33% and 46%) were shown to prolong the lifespan of rats (Berg & Simms 1971). However at all levels of food intake the incidence of renal lesions was higher in males compared to females (Simms, 1967). The severity reduced proportionally to the degree of dietary restriction. In another study by Rosen et al (1968) the effect of food restriction on the course of an accelerated model of glomerulosclerosis in rats was evaluated. Here again, food restriction retarded the onset of the glomerular changes induced by irradiation following unilateral nephrectomy. From this study, the authors concluded that growth retardation secondary to dietary restriction had a profound inhibitory effect on the progress of the glomerular sclerotic process. Glomerulosclerosis of ageing rats can be significantly accelerated by renal ablation and reduction of functional renal mass (Elema, 1971). Such an intervention, with reduction in renal mass, does in itself induce glomerular and tubular changes not unlike the ones occurring spontaneously with ageing. These morphological changes following renal ablation are associated with progressive renal functional decline (Morrison, 1962). The lesions following unilateral and sub-total nephrectomies are discussed in section .

Hormonal manipulations in rodents affect the onset and progression of spontaneous glomerulosclerosis; as previously stated these lesions are more common and severe in ageing male rats. In rabbits, cortisone administration leads to hyperglycaemia and hyperlipidaemia with focal and segmental glomerulosclerotic lesions similar to the ones observed in human diabetic nephropathy (Wilens & Stumpf, 1955).

Similarly, thyroid hormones seem to affect spontaneous and immune induced glomerulosclerosis. In rats, the supplementation of drinking water with thyroid hormones, led to the early onset of glomerulosclerotic lesions (Berg, 1966). However, it is important to appreciate that thyroid treated rats had a significant (50%) increase in their food consumption. The authors suggested that the increased metabolic rate induced by the thyroid hormones led to the acceleration of the normal glomerulosclerosis process of rat kidneys. Thyroidectomy was found to decrease the severity of glomerular lesions in the nephrotoxic nephritis model of immune induced glomerular scarring and chronic renal failure (Tomford, 1981).

Adrenalectomy, decreased the severity of proteinuria in rats with glomerulosclerosis (Addis, 1950). Similarly, the administration of mineralocorticoid (DOCA) accelerated the progression of the immune induced glomerulosclerosis of nephrotoxic nephritis rats (Knowlton, 1946).

Other factors, such as X-ray irradiation accelerate the course of intercapillary glomerulosclerosis in rodents (Guttman, 1960).

After whole body X-ray exposure of mice, intercapillary glomerulosclerosis was significantly increased. The effect of irradiation on the kidney was delayed with lesions appearing well after the exposure has ceased (Guttman, 1965). The authors postulated an immune mediated effect of irradiation on the kidney, as neo-natal thymectomy potentiated the effects of X-ray on glomerulosclerosis

Therefore it seems from these observations, that ageing rats develop glomerulosclerosis and that this renal ageing involutional phenomenon could be precipitated or accelerated by different surgical (Elema, 1971), dietary (Lalich, 1970), hormonal (Reiter, 1969) and immune (Guttman, 1965) manipulations.

This process of spontaneous glomerulosclerosis in ageing rats like the one occurring in man could give some insights into the mechanisms of acquired glomerulosclerosis and therefore further our understanding of progressive renal scarring following an acute nephritis.

The mechanisms involved in the sclerosis of ageing glomeruli in rats are not fully elucidated. However many hypothesis have been advanced to explain these morphological changes. First the overload, overwork theory (Couser, 1975), whereby high protein, amino acids, salt and solutes, intakes in general lead to functional overload of the nephrons with subsequent morphological damage. This overload could occur at normal level of solute intake when the nephron population is reduced by ageing leading to the overwork of the remnant functioning units. Second, the toxic

theory (Lalich, 1971), whereby the kidneys and glomeruli are directly damaged by toxic metabolites. A cumulative effect of these toxins would lead in the long term, with ageing, to glomerular attrition. Third, the immune or auto-allergic theory, whereby ageing is associated with an increased incidence of auto-immune manifestations leading to organ damage and dysfunction (Guttman & Bailey, 1965). The effects of whole body irradiation and neonatal thymectomy would support a role of the immune system in the pathogenesis and progression of spontaneous glomerulosclerosis. Finally, the haemodynamic theory, whereby glomerulosclerosis of ageing could be the consequence of renal haemodynamic adaptation with age (Brenner et al, 1982). Ad libitum feeding of rats, known to accelerate the age related glomerular changes (Berg & Simms, 1960), leads to increased glomerular blood flow and pressures. These haemodynamic alterations might therefore underly some of the changes observed in old rats.

It is likely that the pathogenesis of the glomerulosclerosis of ageing kidneys, like that of the ageing process in general, is multifactorial with the possible interaction of different influences on the kidney and body.

II. Experimental models

Chronic renal failure, in animals, does not develop in normal kidneys without a preceeding injury. Glomerulosclerosis and chronic renal failure are therefore the end result of an acute renal insult followed by perpetuating and aggravating influences that prevent morphological and functional recovery. On the other hand glomerulosclerosis of ageing is of little functional consequence, unless some factors intervene to accelerate its course and increase its expression. These factors in spontaneous glomerulosclerosis like their counterpart in injury induced glomerular scarring hold the clue to the understanding of the mechanisms involved in progression of renal diseases in animals as well as man.

In order to better understand the progression of chronic renal diseases in man, many experimental models have been designed in animals. Some of these models bear some similarities to human nephropathies, while the others, although unlike human diseases, are nevertheless useful in studying the pathogenesis of chronic renal failure.

During the past decade a variety of techniques, commonly used in basic physiology, have been applied to the study of renal pathophysiology and the progression of renal diseases. Recently, the pathophysiology of the glomerulus in health and disease has become a major field of investigations in nephrology. This was facilitated by the discovery by Thurrau of a strain of rats, Munich-Wistar, that possess superficial cortical glomeruli allowing direct access by micropuncture.

Before discussing the experimental models of chronic renal diseases, I would like to review some of the recent advances in glomerular physiology of the normal rat. Changes, in diseases, of these physiological parameters might be at the base of the ensuing structural changes of glomerulosclerosis.

Determinants of glomerular ultrafiltration in health :

In the rat, the glomerular filtration process is the result of a combination of several different variables :

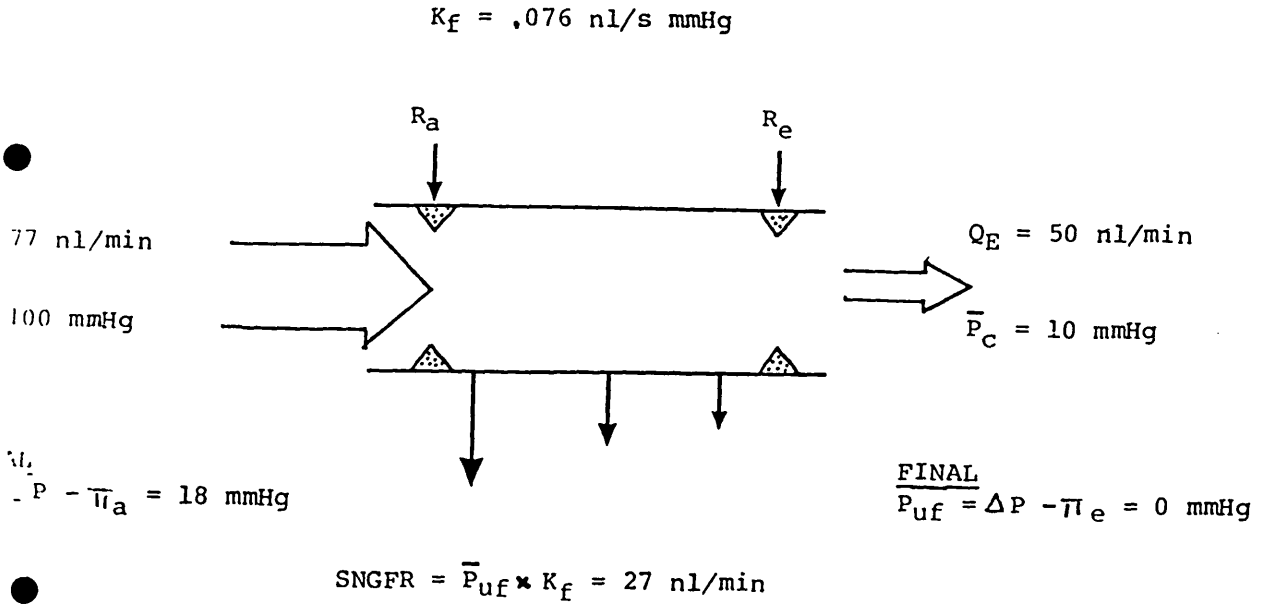
1. the glomerular capillary flow (Qa)
2. the transglomerular hydraulic pressure gradient ($\overline{\Delta P}$)
3. the initial plasma oncotic pressure (Πa)
4. the glomerular hydraulic permeability coefficient (Kf)

At the afferent end of the glomerular capillary bed, and with a mean arterial blood pressure of 100 mmHg, the glomerular capillary flow (Qa) is 77 nl/min. The hydraulic pressure generated by the arterial blood pressure (100 mmHg) drops to 45 mmHg on encountering the afferent arteriolar resistance (Ra) and then to 10-12 mmHg after passage through the glomerulus and the efferent arteriolar resistance (Re). The hydraulic pressure gradient ($\overline{\Delta P}$) between the glomerulus and the Bowman's urinary space, at the afferent end of the glomerulus approximates 35 mmHg and equals :

$\overline{\Delta P}$ = Intraglomerular pressure (PGc) - Intratubular pressure (PT) = 45 - 10 = 35 mmHg, this pressure gradient remains constant throughout the glomerular capillary. The net ultrafiltration force (PuF) in the glomerulus is the result of the difference between opposite driving forces; the hydraulic pressure gradient ($\overline{\Delta P}$) -

the glomerular oncotic pressure ($\overline{\Pi}$). The values for PuF varies continuously along the glomerulus due to the occurring ultrafiltration that starts at the afferent capillary end and the resulting steady increase of the oncotic pressure. Filtration ceases at a point along the efferent side where the oncotic pressure equals the pressure gradient. This state is known as filtration pressure equilibrium (FPE) with stoppage of any further filtration.

Figure 1



GLOMERULAR HAEMODYNAMICS

The single nephron glomerular filtration rate (SNGFR) equals PUF x Kf, where Kf (also known as LpA) is a derived value for the product of the intrinsic permeability of the capillary (K) and the total surface area available for filtration (S), ie. Kf = K x S.

In the studies pioneered by Brenner et al (1976) it appeared that changes in glomerular blood flow (Qa) are largely responsible for the autoregulation of GFR observed under states of varying arterial blood pressure and that changes in glomerular permeability coefficient (Kf) may significantly modulate the expected effect of isolated changes in oncotic pressure ($\overline{\Pi}a$), hydraulic pressure gradient ($\overline{\Delta P}$) and glomerular blood flow (Qa) in the SNGFR and vice versa. More recently, Schor, Ichikawa and Brenner (1981) have demonstrated the sensitivity of the ultrafiltration permeability coefficient (Kf) to a variety of factors including some hormones; A marked decrease in glomerular permeability coefficient was observed with infusion of angiotensin II, anti diuretic hormone (ADH), parathyroid hormone (PTH) and prostaglandins (PGE_2) (Schor, 1981).

Some of the changes they observed with angiotensin II and ADH could be mediated by the effect of these hormones on the mesangial contractile network (Ausiello, 1980) that regulates the spread of the glomerular basement membrane. On the other hand, some of the hormonal influences on the glomerular filtration rate and in particular the SNGFR, could be mediated by their actions on the pre and post glomerular capillary resistances (Blantz, 1976a)as well as on the walls of the periglomerular capillaries.

The role of cAMP as the mediator of the effects of some of these hormones on the glomerular capillary network is subject to current research (Dousa, 1980).

The understanding of the glomerular haemodynamic network is of importance in health and renal diseases. During an acute nephritis major haemodynamic changes occur in the glomerular capillary bed (Blantz, 1979), these alterations lead to further disturbances of renal blood flow that could result in the long term glomerular hyperperfusion or ischaemia both capable of inducing severe glomerular scarring (Brenner, 1982) and dysfunction.

II. Experimental models of chronic renal failure

I. Nephrotoxic serum nephritis (NTN, Masugi nephritis)

Since the late thirties it was apparent that a progressive nephropathy could be induced in rats by the injection of anti-kidney antibodies (Masugi, 1934). Rats injected with heterologous (rabbit) antibodies reacting with the glomerular basement membrane (GBM) develop an acute nephritis (Smadel & Farr, 1937) that, if severe enough, progressed to chronicity with renal failure and death from uraemia (Hackel, 1959). Within hours of the injection of such a heterologous anti-GBM antibody, rats develop a significant proteinuria (Feldman, 1963). This proteinuria in nephrotoxic serum nephritis (NTN) is characteristically divided into two phases; the first, immediate, and called the heterologous phase and the second delayed called the autologous phase (Hammer & Dixon, 1963). The heterologous phase is the result of the acute interaction between the injected antibody and the GBM. The autologous phase is the direct consequence of the interaction on the GBM of the heterologous antibody with the rat's autologous antibody response to it. The heterologous phase of NTN consists of an acute diffuse proliferative glomerular nephritis bearing striking similarities to human post streptococcal glomerulonephritis (Dixon, 1961). This early nephritis lasts 24 to 36 hours, with proteinuria decreasing within 24 hours from the first injection of the nephrotoxic antibody. The heterologous phase nephritis is replaced within 2 or 3 days by the autologous one that leads to chronic renal scarring and progresses to chronic renal failure (Unanue & Dixon, 1965).

While the early, heterologous, nephritis is characterised by a neutrophil infiltrate (Cochrane, 1965), the late, autologous phase shows an influx of mononuclear cells (Schreiner, 1978). The role of neutrophils and complement activation (Cochrane, 1965) are indispensable, in rats, to the development of the heterologous phase. Mononuclear cells on the other hand are important in the development of the autologous nephritis.

As the successive phases of injury subside, progressive glomerular and tubular scarring develop with ensuing severe glomerulosclerosis, tubular atrophy and nephrocalcinosis (Smadel & Farr, 1937). Proliferative changes, at the chronic stage, have been replaced by fibrosis and sclerotic ones (Fujimoto, 1980).

Lubowitz et al (1974) studied the morphological and functional alterations in the sub-acute, chronic, phase of the nephritis (NTN). Rats were assessed 2 to 8 weeks after induction of a mixed passive and active autologous phase. Rats were injected with the heterologous anti-GBM antibody and the autologous phase was induced, passively, by the injection of pre-formed autologous antibodies. They observed moderate to severe histological lesions with marked heterogeneity of the affected glomeruli. This heterogeneity was reflected in the wide variations of SNGFR and tubular transit time. The authors noted that SNGFR and total kidney GFR fell proportionally and that in spite of marked variations in the SNGFR, the glomerulotubular balance was preserved in individual nephrons. Allison, Wilson and Gottschalk (1974) confirmed these observations in rats with moderate nephrotoxic nephritis. When studied 10 to 38 days after the induction of the nephritis, they observed a drop in the GFR proportional to

the decrease in filtration fraction (FF); renal blood flow remaining unchanged. These findings were similar to the ones known to occur in man (Brun, 1979).

Allison et al (1974) confirmed Lubowitz's observations of marked heterogeneity of the nephron population with SNGFR varying from 0 to 50 nl/min and proximal tubule pressure from 4 to 48 mmHg. Here again glomerulotubular balance was preserved. No supernephrons with increased filtration rate were observed in this experimental model of chronic renal failure.

More detailed haemodynamic studies in NTN were provided by Maddox et al (1975). Rats were studied 2 weeks after the induction of a mild NTN, with 40ug of rabbit kidney fixing antibody to rat GBM. In these rate, SNGFR remained unchanged in spite of a 30% reduction in the glomerular permeability coefficient (LpA). The observed increase in the effective filtration pressure (EFP) prevented the fall in SNGFR. This increased filtration pressure was brought about by the increase in glomerular plasma flow (Qa) and the glomerular capillary pressure (PGc). These changes were mediated by a fall in the afferent arterioles resistance (Ra). In this case, a mild volume expansion could explain most of these haemodynamic changes. In this mild nephritis model, the nephron population behaved homogeneously.

A more severe NTN was studied by Blantz and Wilson (1976); Rats were assessed during the acute phase, one week, after the injection of 225 to 450ug of anti-GBM antibody. SNGFR fell to approximately 45% of the control value. Glomerular permeability coefficient was

equally decreased as well as the glomerular plasma flow. These changes were brought about by a marked increase (50%) in afferent (Ra) as well as efferent (Re) arteriolar resistances. However, as the decrease in glomerular blood flow was offset by the simultaneous increase in the hydraulic pressure gradient from 35 to 50 mmHg, these authors observed that the reduction in SNGFR in this model was dependent on the fall in glomerular permeability.

Later studies by the same authors, showed the complement dependency of the acute vasoconstriction component compared to the complement independence of the reduction in GBM permeability (Blantz, 1978). Recently, Blantz et al (1981) demonstrated the role of the alpha-adrenergic system in the mediation of glomerulo vasoconstriction; α-adrenergic blockade, with phentolamine, prevented in severe nephrotoxic nephritis, the acute immune induced vasoconstriction and partially prevented the fall in glomerular permeability.

More recently Lianos et al (1983) attributed an important role to prostaglandins and in particular thromboxane (TxA_2) synthesis by the glomeruli in the acute vasoconstrictive response. They showed that selective inhibition of TxA_2 synthesis prevented the decrements in GFR and FF observed in NTN.

2. Autologous immune complex nephritis (AICN, Heymann's nephritis)

AICN is an experimental model of chronic glomerulonephritis in the rat which bears some similarities with human membranous nephropathy.

In this model the immunization of rats with a proximal tubule antigen ($RTE\alpha_5$, Fx1A) in complete Freund's adjuvant induced a glomerulo-nephritis with sub-epithelial immune deposits in the glomerular

basement membrane (GBM) (Heymann et al, 1959 and Edgington et al, 1967). The pathogenesis of this experimental model was originally thought to be mediated by the deposition in the GBM of circulating renal tubular antigen-antibody complexes (Glassock, et al, 1968). However, more recently it was observed that the injection of heterologous antibodies to Fx1A was followed by their immediate deposition and fixation to antigenic sites on the GBM (Couser et al, 1978 and Van Damme et al, 1978). These findings implied that the binding of antibodies to glomerular antigens were contributing to the pathogenesis of Heymann's nephritis.

In AICN, proteinuria develops slowly 4-5 weeks after the induction of the nephritis and progressively increases thereafter. During the early stages of the nephropathy, Allison et al (1974) observed a fall in glomerular filtration rate as well as filtration fraction with little change in renal plasma flow. There was a marked heterogeneity of SNGFR (5 to 93 nl/min) with complete glomerulo-tubular balance.

In this experimental model of chronic membranous nephropathy, the most consistent morphologic finding is a diffuse thickening of the GBM with epimembranous immune deposits. Mild mesangial cellular proliferation occurs occasionally in Heymann's nephritis (Klassen et al, 1971). The persistent proteinuria and nephrotic syndrome as well as the renal function and morphological changes in this model are so similar to membranous nephropathy in man making it an extremely relevant model of experimental chronic renal disease to study.

3. Chronic experimental pyelonephritis

Chronic bilateral pyelonephritis is induced in rats by the innoculation of the bladder with 10^8 organisms, proteus mirabilis (Bank & Aynedjian, 1966). Three to six weeks later the kidneys present histological alterations similar to the ones occurring in human chronic pyelonephritis.

Bank and Aynedjian (1966) observed histological abnormalities of a focal nature with patchy areas of relatively normal or hypertrophied tissue. Glomerular morphology seemed preserved, although the cortical nephrons, which were severely injured by the disease process did not contribute significantly to urine formation.

Total kidney GFR was reduced by 35% to 45%, however the SNGFR in the least affected areas was higher than normal. These supernephrons, with high filtration rates were possibly adapting for the loss of adjacent renal functional mass.

In this model of chronic tubulo-interstitial disease, the glomerulotubular balance was preserved in a manner similar to that observed in chronic experimental glomerulonephritis (Blantz, 1979).

Lubowitz et al (1969) confirmed these findings in experimental unilateral and bilateral pyelonephritis.

In these models the presence of supernephrons with high filtration rates and increased plasma flow might be the mechanism underlying functional adaptation to progressive reduction in renal mass. Whether these adaptive changes, like in the remnant kidney model

(Deen, 1974), lead to the progressive sclerosis of these adapting, compensating, glomeruli remains to be established.

4. The remnant kidney model

Following the surgical removal of a percentage of renal mass, immediate, significant, adaptive changes occur in the remnant kidney (Hayslett, 1979). Unilateral and sub-total nephrectomies are known to accelerate the spontaneous sclerotic lesions of ageing (Elema, 1971) as well as those of acquired experimental nephropathies. In animals the course of diabetic nephropathy (Steffes, 1978) can be accelerated by the concomittant reduction in renal mass. However, without preceeding renal injury, and in young rats, the resection of functional kidney tissue leads to the development of a progressive nephropathy (Morrison, 1962). In this renal disease, following unilateral or sub-total nephrectomies, proteinuria develops, followed by hypertension and progressive renal function decline (Morrison, 1966). Animals die a few months after the resection from renal failure. The morphological changes in the remnant kidney model are characterised by a severe progressive glomerulosclerosis with tubular atrophy and end stage renal nephrocalcinosis (Shimamura & Morrison, 1975).

Major renal functional adjustments occur immediately after the reduction in renal mass (Hayslett, 1979), and these changes are proportional to the extent of the renal tissue resected (Kaufman, 1975); for instance after unilateral nephrectomy (UNx), the intact kidney weight increases by 40% while its glomerular filtration rate shows an increment of 65% to 75% within weeks of the nephrectomy. Kaufman and Hayslett (1974)

established that following renal tissue removal the remnant kidney weight and function increased proportionally to the degree of functional tissue removal; the average value of mean glomerular filtration rate (MGFR) was 47.3nl/min in control rats and rose to 75.9nl/min after unilateral nephrectomy (UNx) and to 112nl/min following the removal of three quarters of renal mass.

Deen et al (1974) were able to study the haemodynamic adaptive changes following sub-total nephrectomy. They concluded that the observed increments in SNGFR was the result of the combined increase in glomerular blood flow and hydraulic pressure gradients in the remnant glomeruli. The increase in glomerular blood flow accounted for the majority (3/4) of the increase in filtration rate. In these studies, the GBM permeability coefficient (Kf) was not affected by the unilateral nephrectomy. The changes in renal blood flow were thought to be secondary to the marked alterations in glomerular capillary resistances; as both pre and post glomerular capillary resistances significantly decreased.

The remnant nephron population behaved in a homogeneous manner and glomerulo-tubular balance was preserved. It is conceivable that in these rats, following nephrectomy, blood volume expansion could account for some of the observed renal haemodynamic changes, including the drop in capillary resistances (Deen, 1974). In the last two models, chronic pyelonephritis and the remnant kidney, in the absence of initial glomerular damage, the remnant nephrons develop into hyperperfused-hyperfiltrating units.
These early adaptive changes in glomerular haemodynamics might be, in these two models, operational in the longterm pathogenesis of glomerulosclerosis leading to end stage renal failure.

5. Diabetic nephropathy

Experimental diabetes has been produced in rats by the administration of streptozotocin (Mauer, 1972), by pancreatectomy (Folgia, 1950) and by feeding high-sucrose diets to genetically predisposed strains (Cohen, 1972). Following approximately six months of diabetes the rats develop renal lesions suggestive of diabetic nephropathy (Lee, 1974). There is a progressive expansion and thickness of the mesangial cells and matrix. These changes are focal in nature at the early stages of the disease and later extend to become diffuse and lead to glomerulosclerosis. Concomittantly, hyaline "exudative" lesions develop within the glomeruli and progress to obliterate the glomerular capillary lumen.

In addition Cameron et al (1973) as well as Hägg (1974) described progressive GBM thickening in rats' diabetic nephropathy.

Although the glomerular pathology of diabetes in the rat does not mimic human changes as closely as do diabetic glomerular changes in the dog (Bloodworth, 1965) or monkey (Bloodworth, 1973), the similarities between rat and human diabetic glomerulopathy are striking (Mauer, 1972). Recently, Hostetter, Troy and Brenner (1981) have described the haemodynamic changes of glomeruli in diabetic nephropathy. These changes varied significantly according to the degree of hyperglycaemias; single nephron glomerular filtration rate (SNGFR) were significantly reduced (28nl/min) in severely hyperglycaemic rats compared to controls (49nl/min). Such reduction was mainly due to a fall in glomerular blood flow (Qa). In contrast, moderately hyperglycaemic rats had an increase of the SNGFR (69nl/min); this increment in filtration was attributed to the observed increase in blood flow (Qa). It is conceivable that in this group,

with moderate hyperglycaemias, the relative high blood volume (Hostetter, 1982b)in conjunction with decreased afferent and efferent glomerular resistances (Hostetter, 198la)led to the observed glomerular hyperfiltration. The mediators of these haemodynamic changes in diabetic rats are unknown, but a role of hormones such as insulin, glucagon and growth hormone cannot be excluded (Corvilain, 1962). In rats with diabetic nephropathy, the correction of the abnormal metabolic environment by pancreatic islet transplantation led to the regression of the renal morphological changes (Mauer, 1975).

The haemodynamic changes in diabetic rats bear some similarities to the ones observed in man, with hyperperfusion and hyperfiltration early in the course of the nephropathy possibly leading at the later stages to the well known glomerulosclerosis of diabetic kidneys.

Finally, the early hyperfiltration of diabetic kidneys alters the glomerular perm selectivity and could lead to proteinuria (albuminuria) as well as progressive mesangial overload, thought by some to be the forerunner of glomerulosclerosis (Couser & Stilmant, 1975).

III. Factors influencing progression

Glomeruli in experimental models of chronic renal failure respond to acute injury by hemodynamic compensatory and adaptive changes (Blantz, 1979). The role of these factors and others in the progression of acute glomerular injury to chronic scarring will be reviewed in this section.

However, before reviewing the factors that could modify the long term nephron response to injury, it would be useful to summarise the different influences coming into play after acute glomerular damage has occurred.

The predominent functional alterations are :

(1) alterations in renal blood flow (RPF) and glomerular filtration rate (GFR) (Maddox 1975).

(2) the loss of the ability to autoregulate RPF and GFR (Bennett & Glass ock, 1978).

(3) sodium retention and oedema formation (Godon 1972).

(4) systemic hypertension (Baldwin 1982b).

(5) proteinuria (Chang 1976).

All these acute changes could have important longterm consequences as they could contribute individually or combined in the perpetuation of the renal injury. Similarly, exogenous factors could influence the progression of the nephropathy by acting on these endogenous, intrinsic, renal changes.

The maintenance of the glomeruli plasma flow (Qa) after a glomerulonephritis depends on the fine balance between systemic blood pressure, extracellular volume (ECV) and the interplay of afferent and efferent glomerular arteriolar resistances subject to modulatory effects of local and systemic mediators.

It is likely, for instance, that during the acute phase of a glomerulonephritis, as the sodium retention increases the extracellular volume, there would be an increment in renal blood flow. This increase in glomerular blood flow brought about by decreased capillary resistances would maintain the GFR by compensating for the detrimental effect of the fall in the glomerular permeability.

Indeed, experimental data in rats have repeatedly shown that in mild acute glomerulonephritis the fall in Kf is accompanied by a rise in the net ultrafiltration force (Allison, 1974) and glomerular plasma flow (Maddox, 1975) resulting in an unchanged SNGFR However, with severe glomerular injury there is a marked increase in glomerular resistances and glomerular blood flow as well as filtration fall (Blantz & Wilson, 1976). It has been pointed out that the haemodynamic changes following glomerular injury could lead to progressive glomerulosclerosis.

Similarly, during an acute nephritis, sodium retention and hypertension could have a useful protective effect on renal function; sodium retention, volume expansion and intra renal hypertension could prevent significant falls in SNGFR (Arendhorst, 1980). However, chronic sodium retention and hypertension will lead to progressive arteriolar sclerosis (Wilson & Byrom, 1941).

Equally important, the severe proteinuria and hypoalbuminaemia occurring after severe glomerular injury could have important haemodynamic repercussions; as the afferent oncotic pressure (Πa) falls secondary to hypoalbuminaemia, the net ultrafiltration pressure increases as well as the SNGFR providing the glomerular permeability is not markedly reduced (Baylis, 1977). Here again, the contraction of prolonged hypoalbuminaemia, with oedema formation, will lead to the reduction of the effective extracellular volume and this will have opposite, detrimental, effects of glomerular filtration.

All these factors are likely to be interrelated with intrarenal hypertension influencing proteinuria which in turn could lead to further glomerular damage (Velosa, 1977) with resulting haemodynamic changes.

In the next section, I would like to review the effects of pharmacological, hormonal, haemodynamic and dietary factors on the pathophysiology of chronic experimental renal failure.

1. Pharmacological interventions

(a) Chronic immune mediated glomerulonephritis

The majority of research had addressed itself to alter the response of the kidney during the acute phase of nephrotoxic nephritis (NTN) and autologous immune complex nephritis (AICN). These two models of proliferative and membranous nephropathies have some similarities with their counterpart of human nephritides.

I will review in this section the pharmacological manoeuvres, that applied during the course of the nephritis, attempted to alter the natural history of the disease.

(1) Immunosuppressant and anti-inflammatory agents

The majority of immunosuppressive and anti-inflammatory agents have been tried with little success in these two experimental models.

In the autologous immune complex nephritis (AICN), Kupor and McPhaul (1976) and more recently Garovoy et al (1977) have tried immunosuppression with single and double drug treatment and found no beneficial effect.

On the other hand, a beneficial effect of the vasoactive antagonist cyproheptadine was reported on the proteinuria of AICN by Bolton et al (1974).

Fleuren et al (1980) have studied the effect of triple therapy with prednisolone, azathioprine and cyclophosphamide on the course of AICN. These authors found a decrease in the glomerular basement membrane's damage as well as the amount of deposited immune aggregates. However, these changes were only obtained when the therapeutic regimen were started early in the course of the nephritis. The authors concluded that, at the late stages of the disease, the role of immunological events are subsiding and might no longer account for the progressive scarring process.

In nephrotoxic nephritis (NTN) as early as 1954 Seegal et al reported that cortisone administration had no effect on the course of the disease, regardless of the time of its introduction. More recently, Kobayashi (1980) used methyl-prednisolone in the rabbit

rapidly progressive model of NTN; they noted some protection from glomerular damage in mild forms of the nephritis, and none in the severe, crescentic, variant. Kobayashi attributed the effect of methylprednisolone to the suppression of the autologous phase injury.

More recently, Kurokawa and Sakamoto (1983) have reassessed the role of steroid therapy in NTN. Prednisolone, which was given orally at doses of 3 and 5mg/kg daily from the day before nephrotoxic serum (NTN) injection, elevated the proteinuria in the heterologous phase, while inhibiting the development of proteinuria in the autologous phase. However, when given late, prednisolone (5mg/kg daily) was ineffective against established proteinuria in the autologous phase.

The role of the autologous immune response in the progression of NTN was evaluated by Shimizu et al (1974). It was observed that suppression of the autologous immune response, by induction of tolerance to the heterologous antibody, did not prevent progressive glomerulosclerosis. Tolerant and control rats had a similarly progressive chronic nephritis, providing the acute heterologous injury was severe enough.

These findings were recently confirmed by Saito et al (1983) who found little effect of immunosuppression by cyclophosphamide on the disease when the drug was introduced late in its course.

In general, it would seem that immunosuppression in experimental, immune mediated nephritis, has little influence on the chronic phases of the disease.

Similarly, non-steroidal anti-inflammatory agents were not successful when administered to alter the course of immune mediated nephritis. The role of the anti-inflammatory agents indomethacin and lysine acetyl salicylate on the course of NTN has been evaulated by Sessa et al (1978); the drugs were started 15 days after the onset of the nephritis in rats at a dose of 4mg/kg daily. Proteinuria was found to be reduced by indomethacin and to a lesser extent by salicylates. No morphological changes were observed. However, by transmission electron microscopy podocytes were found to be hypertrophied in both treated groups. Rats treated with indomethacin had additional ergoplastic inclusions in the GBM. The authors attributed the anti-proteinuric effects of indomethacin on its stimulation of GBM synthesis and the resulting decrease in its permeability.

Kurokawa and Sakamoto (1982) evaluated the effects of aspirin and indomethacin on nephrotoxic nephritis in the rat. They studied the prophylactic as well as the therapeutic effects of these drugs on the nephritis. Aspirin, 150 and 250mg/kg daily, when given prior to the injection of the nephrotoxic serum, suppressed the development of proteinuria in both the heterologous and autologous phases. However, when given late in the course of the disease, aspirin had no effect on the autologous phase proteinuria. In these experiments, the administration of indomethacin (3mg/kg daily) could not prevent the development of proteinuria in either the heterologous or the autologous phase.

(2) Anticoagulation

As with the immunosuppressant agents, anticoagulants have predominantly

been studied during the acute phase of immune nephritides. Heparin (Halpern, 1965), Warfarin (Vassali & McCluskey, 1964) and Ancrod (Naish, 1965) have all been reported to be more or less successful in altering the acute inflammatory process. In nephrotoxic nephritis anticoagulation at the time of induction of the disease was found to improve the acute phase of the nephropathy (Halpern, 1965). However, Border et al (1975) using heparin in NTN, in rabbits, showed no demonstrable therapeutic effect. These authors concluded that fibrin deposition in the glomeruli could occur independently of the systemic coagulation process. Naish et al (1972, 1975) showed a protective effect of defibrination of Ancrod on the acute phase of NTN. The role of anticoagulation was recently reviewed by Sano et al (1981) in AICN. Glomerulosclerosis was found to be reduced in rats treated for 4 months with heparin, warfarin or dypiridamole.

(b) The remnant kidney model

Few data are available on the effects of pharmacological interventions on this model of chronic renal failure.

Purkerson et al (1976) studied the effects of heparin administration on the course of the nephropathy developing in rats following five-sixths nephrectomy. Heparin was started after the renal infarction was completed and rats were followed for 28 days. Heparin prevented glomerular capillary occlusion, lowered the blood pressure to near normal and prevented azotemia. The authors postulated that progressive glomerulosclerosis was mediated by thrombotic mechanisms prevented by heparin administration. Pursueing the same therapeutic rationale,

Zimmerman et al (1981) studied rats, after a similar degree of renal mass reduction, for 5 weeks on aspirin (ASA) and dypiridamole, they reported that ASA as well as dypiridamole reduced the blood pressure, improved renal function and decreased the severity of morphological changes. These authors attributed the protective effects of these drugs to their anti platelet aggregation activities.

White and Grollman (1964) have shown that immunosuppressive manipulations of rats with partial renal infarcts prevented the associated hypertension.

(c) Diabetic nephropathy

Immunopathologic studies of rats made diabetic by alloxan or streptozotocin have demonstrated progressive localisation of IgG, IgM and C3 within the mesangium (Mauer, 1972).

Following the transplantation of diabetic kidneys to normal rats, mesangium IgG and C3 mesangium deposits completely disappeared (Lee, 1974).

The role of these immune deposits in the pathogenesis of diabetic nephropathy is not known, but it is thought that they represent non specific fixation and traping of serum proteins (Mauer, 1972). Glycosylated proteins might adhere more readily to the glomerular basement membrane.

Treatment with cyclophosphamide or neo natal thymectomy has little effect on the development of these immunohistochemical lesions (Hägg, 1974).

2. Hormonal and metabolic manipulations

(a) Chronic immune glomerulonephritis

The use and effects of mineralocorticoids in particular deoxycorticosterone acetate (DOCA) have been extensively studied in the acute phases of Masugi nephritis (NTN). Knowlton et al (1976) showed that the administration of DOCA and salt during the induction of the nephritis, intensified the nephritic process.

Similarly, Yokoyama (1957) and Huang et al (1963) demonstrated, in animals given ACTH during the induction of the nephritis, a detrimental effect on proteinuria and on the progression of the disease.

Lippman (1954) observed the sex dependent effect of adrenal steroids in nephritic rats; cortisone, hydrocortisone and DOCA all had detrimental effects on the nephritis in male rats, with females being protected from the hormonal deleterious influence.

Adrenalectomy of the male rat reduced the proteinuria of nephrotoxic nephritis (Lippman, 1954).

In all these previously quoted experiments steroids were administered early in the course of NTN, when rats were already fluid overloaded and avidly retaining sodium (Bennet & Glassock, 1978).

Both knowlton (1946) and Nishimori (1960) observed the development of severe hypertension in nephritic rats following adrenalectomy.

Whether the effect of steroid hormones on the course of nephrotoxic nephritis is dependent on their anti-inflammatory, salt retaining or other metabolic effects is not fully established, however a multi-factorial action is likely to take place.

The role of the animals metabolic rate and thyroid hormone status on the course of immune nephritis has not received in the past the attention it probably deserves. It has been shown that spontaneous, age related, glomerulosclerosis was adversely affected by the administration of thyroid hormones (Berg, 1966). However, it was not until recently that Tomford et al (1981) evaluated the effects of thyroparathyroidectomy on the course of nephrotoxic nephritis; thyroparathyroidectomy had a significant protective effect against deterioration of renal function in this model, it prevented renal functional decline, parenchymal calcifications and the concomittant severe morphological changes. Parathyroidectomy, in the same study, had little effect on the course of the disease. These authors showed a close correlation between proteinuria and hypertriglyceridaemia. Thyroidectomy almost abolished hypertri-glyceredaemia of NTN.

Sex hormones strongly influence the nature and progression of renal disease in rats. Normal as well as nephritic male rats have higher levels of protein excretion than females (Addis, 1950). Lippman et al (1954) showed a pronounced sex differences in rats, to the injection of nephrotoxic globulin (NTG); male rats had a more severe nephritis. The same authors have showed the deleterious influence of steroid administration to male, nephritic, rats.

(b) The remnant kidney

Endocrine manipulations in this model, similarly to the ones described with the immune mediated nephritides, have concentrated on the pituitary, adrenal and thyroid axis.

It was shown that hypophysectomy prevented the longterm conpensatory growth of the remnant kidney following contralateral nephrectomy (Reiter, 1969).

Astarabadi (1962) has confirmed these findings by showing renal compensatory hypertrophy did not occur in the absence of the pituitary gland. Similarly, Fagelman (1966) postulated that the compensatory growth is dependent on the pituitary effect on a renotropic factor. This renotropic factor although not produced by the pituitary gland required its presence for its activation. Growth hormone substitution corrected the decline in body weight following hypophysectomy without entirely correcting the renal weight.

Adrenalectomy, on the other hand, did not affect the increase in kidney weight after unilateral nephrectomy (Reiter, 1969). Anti-diuretic hormone with and without additional saline infusion increased the mitotic index of the remnant kidney (Reiter, 1969). Similarly DOCA increased thymidine incorporation in the remnant kidney, while aldosterone increased its protein synthesis (Castles & Williamson, 1965).

Sex hormones had an important influence on kidneys after unilateral nephrectomy (UNx); as the course of post-UNx nephropathy was more severe in male rats (Halliburton, 1967) and castration in mice was

shown to reduce the size and weight of the kidney (Fadial, 1967). On the other hand, the administration of androgens increases the amino-acid incorporation of the kidneys as well as their rate of protein synthesis (Kassenaar, 1962). Male mice receiving a single injection of oestrogen showed a sharp decrease in kidney size relative to body weight (Shimkin, 1963).

3. Hypertension

(a) Chronic immune nephritis

Hypertension in the context of chronic experimental renal diseases should be divided into intrarenal and systemic hypertension.

Intrarenal hypertension, is the consequence of the fall in the glomerular arteriolar resistances leading to increased glomerular blood flow and filtration pressure (Hostetter, 1982b). The role of such hypertension in the progression of experimental renal disease remains speculative (Brenner, 1982), On the other hand, systemic hypertension often accompanies experimental models of chronic renal failure. Early on in the course of nephritis, there is a marked fluid and salt retention that often culminates in systemic hypertension (Bennett, 1978). Raised blood pressure was previously thought to have a detrimental effect on glomeruli, through its occlusive effects on blood vessels in general (Wilson & Byron, 1941) and pre glomerular arterioles in particular (Azar, 1977). More recently, however, Azar et al, 1978 defined some haemodynamic changes in the kidneys of hypertensive rats. These authors showed a different response of the glomerular capillary bed to hypertension between normal and diseased kidneys. In the two kidneys, post-salt model, Holtzman rats responded to hypertension by increasing

the glomerular capillary pressure, single nephron glomerular filtration (SNGFR) and glomerular blood flow (Qa). In this model no change in glomerular arteriolar resistances was observed. Therefore, in the presence of systemic hypertension and lack of response from the glomerular capillary resistances, the intra glomerular pressures increased (Azar, 1978). On the other hand, in the one kidney-post-salt model, the adaptive haemodynamic changes post nephrectomy overode the effects of hypertension on the glomerular microvasculature; there was marked decrease, in this model, of afferent and efferent glomerular resistances leading to an increase in the glomerular plasma flow (Qa) and SNGFR (Azar, 1977). Because vascular resistances were decreased, hypertensive rats has significantly higher blood flow and filtration rates than the one kidney normotensive rats, or the two-kidney hypertensive ones (Azar et al, 1976).

Berececk et al (1980) showed an increase in the renal vascular bed resistances and vascular reactivity following DOCA and salt administration. These changes were present before the onset of systemic hypertension. In this study, the authors were able to demonstrate an increased reactivity of the renal capillaries in response to nor-adrenaline, angiotensin II and vasopressin infusions.

From these different studies it becomes apparent that the kidney response to systemic hypertension can vary according to the degree of the underlying renal damage. Moreso, it is likely that systemic hypertension as well as intrarenal hypertension can accelerate, through these haemodynamic changes, the course of the original nephritis (Baldwin, 1982b).

(b) Chronic immune nephritis

In experimental animals the course of the nephritis is aggrevated by the concomittant presence of hypertension. Similarly nephritis in rats potentiates the hypertensive response to deoxycorticosterone acetate (DOCA) and salt administration.

In nephrotoxic nephritis as well as in autologous immune complex nephropathy (AICN), hypertension increases the magnitude and severity of proteinuria, accelerates the decline in renal function and exacerbates the morphological renal changes (Baldwin, 1982b) Tikkanen et al (1980) observed severe hypertension developing in AICN when rats were treated with DOCA and salt; nephritic rats were otherwise normotensive and DOCA and salt induced only mild hypertension in normal control rats. Hypertension in nephritic rats increased the proteinuria without a significant deleterious effect on their renal function over the 16 weeks observation period The mechanisms through which an underlying nephritis predisposes and potentiates hypertension remain poorly understood. Neither fluid and salt retention, nor the renin-angiotensin system seem to fully explain the observed changes. It would seem more likely that nephritis alters the vascular bed reactivity making animals more prone to the development of hypertension. The same authors showed that nephritis induced in spontaneously hypertensive rats (SHR) had a similar enhancing effect on the development of hypertension (Tikkanen, 1981a). Here again a permissive effect on proteinuria was induced by hypertension. Once more no function or morphological renal changes induced by hypertension were demonstrated. Tikkanen speculated on the role of the immune system

in the immune mediated nephritis on the course of hypertension in these two experimental models (Tikkanen, 1981b). Motoki et al (1978) reported similar data on the progression of NTN in normal and SHR. In hypertensive rats the nephritis progressed while it resolved spontaneously in control nephritic rats. In this study, renal function and morphology were more severely affected in the hypertensive nephritic rats compared to the nephritic normotensive ones. The same authors have shown similar enhancing effects of nephrotoxic nephritis on the hypertension of the Goldblatt model (Masuyama, 1977).

From these many studies, it seems that the course of both autologous immune complex and nephrotoxic nephritis can be aggrevated by the superimposition of systemic hypertension.

(c) The remnant kidney model

In the remnant kidney model, hyperperfusion is currently thought to be the major factor in the progression to chronic renal failure with glomerulosclerosis (Hostetter, 1981b).

Prevention of the intrarenal hypertension, in this model, seems to protect from the severe glomerular structural changes. Hostetter et al (1981b) have succeeded in preventing intra-renal hypertension in nephrectomised rats by feeding them a low protein diet. The low protein diet, with its angiotensin like effect, prevented the acute fall in glomerular arteriolar resistances, thereby protecting the glomeruli from the nephrotoxic effects of hyperperfusion. From these studies, they concluded that intra-renal hypertension, with hyperfiltration and hyperperfusion, was an important pathway to end stage renal failure and glomerulosclerosis

after sub-total nephrectomy. They postulated that this might be the final common pathway to glomerular scarring in all nephropathies after an initial reduction of a critical amount of functional renal mass. However, the role of intra renal hypertension in other models of experimental renal failure remains to be fully determined.

Systemic hypertension is known to develop following unilateral and sub-total nephrectomies (Chann, 1932). Its role in the perpetuation of the glomerular damage and its progression is not well defined. Purkerson et al (1976) treated rats following a five-sixths nephrectomy with anti hypertensive agents. Anti hypertensive medication decreased the animal's blood pressure and the severity of the glomerular occlusion although the character of the pathologic lesions was not altered and renal failure not prevented.

In the same experiment, heparin treated sub-total nephrectomised rats did not develop hypertension. The authors concluded that the observed prevention of renal vasculature occlusion in the heparin treated group was due to the anti thrombotic effect of the drug. However, since these early studies, Wilson et al (1982) showed an effect of heparin on hypertension; with decreased blood pressure and related morphological changes in spontaneous hypertensive rats treated with this drug. Therefore, the beneficial effect of heparin on the remnant kidneys of rats in the Purkerson experiments could to some extent be directly related to the prevention of hypertension. More recently, Kaysen and Kropp (1983) using rats following seven-eights nephrectomy studied the effect of dietary supplementation with tryptophan; they showed that although tryptophan inclusion in the diet reduced the proteinuria and prevented the onset of hypertension, renal failure

developed as well as the expected histological changes. They concluded that proteinuria as well as the hypertension known to occur following sub-total nephrectomy do not influence significantly the course of the progressive nephropathy.

These data from Purkerson (1976) and Kaysen (1983) seem to attribute a secondary role to hypertension in the progression of renal disease after the reduction in renal mass.

This might be in keeping with Azar et al (1977) observations, where in this model, hypertension has little impact on renal haemodynamics. The remnant kidney model might therefore behave differently from the models of chronic renal failure where the two kidneys are involved.

(d) Diabetic nephropathy

As previously reviewed in section , intra renal hypertension seems to predominate in the early stages of diabetic nephropathy in animals (Mauer, 1979) and man (Gunderson, 1979). Kidneys of diabetic rats are increased in size (Seyer-Hansen, 1980), have an increased glomerular filtration rate as well as renal blood flow (Hostetter, 1981a).

The mediators of these changes in glomerular haemodynamics with diabetes and hyperglycaemias are the subject of current research, the role of the expanded intravascular blood volume in early diabetes and with moderate hyperglycaemias has been advanced as one of the factors responsible for the observed hyperperfusion and hyperfiltration of diabetic kidneys (Morgensen, 1971).

Growth hormone, glucagon and other vasoactive hormones are increased with hyperglycaemias (Lundbeck, 1976). Similarly, changes in the responsiveness of vascular beds to angiotensin II and catecholamines have been demonstrated in diabetes (Christlieb, 1976).

In order to further the hypothesis that intra renal hypertension influences the development and progression of experimental diabetic nephropathy, Mauer et al (1979) studied the effects of unilateral nephrectomy on the renal pathology of diabetic rats, this procedure accelerated the development of glomerular lesions in the diabetic rats.

The same authors studied the effects of systemic hypertension on the course of experimental diabetic nephropathy. They induced unilateral renal artery stenosis and hypertension and observed a marked increase in the diabetic lesions of the unclipped kidney. The contralateral clipped kidney was protected to some extent from severe morphological changes. The authors concluded that the alterations in glomerular haemodynamics induced by unilateral nephrectomy and hypertension led to the acceleration of the lesions of diabetic nephropathy (Mauer, 1979).

It is interesting however, that the incidence of hyaline nodular glomerular lesions was not affected by the occurrence of hypertension.

From these studies, it would appear that both intra renal and systemic hypertensions affect the course of experimental diabetic nephropathy; the haemodynamic changes induced by hypertension could well be a primordial factor in the pathogenesis and progression of

4. Dietary factors

The role of dietary manipulations in the progression of renal diseases has recently received renewed interest. Excess dietary intake in general has been found detrimental on the course of spontaneous renal disease of ageing (Kennedy, 1957). Similarly, decreased food intake was shown to prolong life spans of rats and improve the age related changes in the kidneys (Berg, 1960). I will review in this section the main studies on dietary interventions aimed at affecting the course of experimental renal disease. From some of these studies insights into the mechanisms of progression of renal disease were obtained.

(1) Dietary protein

As early as 1939, Farr demonstrated a beneficial effect of protein restriction on the course of nephrotoxic nephritis in rats. In the seventies, protein and caloric restriction were found equally beneficial on the course of the SLE-like disease affecting NZB/W mice (Fernandes, 1976); survival in these mice was prolonged as the onset of the renal lesions were delayed by the food restriction. More recently, as attention and research have been intensified in the field of nutrition in chronic renal failure the role of dietary protein was fully explored.

Uraemic rats were found to decrease their overall food intake (Chantler, 1974) and more significantly they were found to utilise dietary protein less efficiently (Pennisi, 1978). Wang et al (1976) fed uraemic rats diets containing 5%, 15% and 23% protein and observed a decreased protein efficiency ratio in these rats compared

to normal controls. These data suggested that growth and anabolic processes were depressed in uraemia.

Uraemic animals fed the highest protein content (23%) had the lowest level of food consumption. Uraemic rats at all levels of food intake have abnormal utilisation of their protein diet; these rats have abnormal levels of amino acids in their blood with low tyrosine levels as well as tyrosine/phenylalanine ratio. The low levels of tyrosine in uraemia are thought to be secondary to the increased activity of hepatic tyrosine aminotransferase (Shear, 1973). Wang et al (1976) showed that survival in uraemic rats was inversely related to their protein intake.

More recently, Pennisi et al (1978) demonstrated that optimal growth in uraemic rats can be achieved by diets reduced in its protein content, providing an adequate amount and quality of amino acids was provided.

In all these studies cited the emphasis was on protein intake and metabolism in uraemic rats with little attention directed at the effects of protein intake on renal function.

It was Kleinknecht et al (1979) who explored extensively the role of dietary protein on the progression of renal failure in rats. These workers studied rats after sub-total nephrectomy on different levels of protein intake (14%, 27% and 37%). These French authors showed decreased growth of uraemic rats compared to control rats.

Growth, in normal rats, was proportional to the dietary protein intake, while uraemic rats on a high protein diet had the lowest growth rate. Anaemia and stunting was therefore maximum in uraemic rats on the 37% protein diet. In normal rats, growth was retarded on the 7% protein diet and addition of essential amino acids and histidine improved their growth rate.

In these experiments, animals fed a high protein diet had a rapid decline of their renal function and died from renal failure. It is interesting to note however that in the high protein group the degree of nephrocalcinosis was markedly reduced after sub-total nephrectomy. Conclusions regarding the nephrotoxicity of high protein diet cannot be made on the basis of these experiments as protein supplementation was based on fish flour. Such a diet is equally rich in salt, calcium and phosphate. However data concerning growth and protein intake although accurate only applied to weanling, growing, rats. As it is known that protein and amino acid requirements vary between young and adult rats (Matsuro, 1976).

Saluski and Kleinknecht (1981) stressed the difficulty of achieving an optimal diet for growing uraemic rats. As diets restricted in protein (7.5%) and supplemented with essential amino acids (EAA) 1% did achieve normal growth, but were as nephrotoxic as the one containing 14% protein. Rats on the low (7.5%) protein, non supplemented, diet had the lowest growth, but their life span was prolonged due to the decreased severity of their glomeruloscerotic lesions. In these experiments the diets were matched in their phosphorus content.

More and more evidence has accumulated to indicate that in the sub-total model of chronic renal failure in rats protein restriction prevents rapid deterioration in renal function and slows the rate of scarring of the remnant kidney (Laovari, 1982). Deen et al (1974) established that these remnant kidneys are hyperperfused and hyperfiltrated, with low glomerular arteriolar resistances. The effect of a low (6%) protein diet was studied by these authors. They observed that, in rats following an eleven-twelths nephrectomy, a low protein diet significantly altered the course of the ensuing nephropathy.

Such a diet had dramatic effects on the adaptive haemodynamic changes- it increased glomerular arteriolar resistances and subsequently decreased glomerular blood flow and hydraulic pressure (Hostetter, 1981b). Glomerular filtration in this group, on the low protein diet, one week after surgery was similar to control, non nephrectomised rats on a normal diet. Single nephron glomerular filtration rate (SNGFR) on the low protein diet did not show the two-fold increase in the nephrectomised controls. On the normal diet, the nephrectomised rats displayed a wide range of morphological changes; including attenuation of glomerular epithelial cells with the subsequent appearance of cellular blebs. The low protein diet group had milder histological abnormalities with fewer than 10% of the glomeruli showing the epithelial blebs. The authors concluded that hyperperfusion and hyperfiltration which seem to be the immediate compensatory mechanism following nephrectomy led to the progressive damage of the remnant nephrons. Similarly, they suggested that a low protein diet by attenuating these compensatory changes prevented the progressive glomerular scarring.

Finally, the overall reduction in food intake, 8 grams/24 hours, protected rats irradiated following unilateral nephrectomy from the accelerated changes in the kidneys (Rosen, 1968). In this study rats were malnourished and renal protection was achieved at the expense of growth retardation.

Similarly rats overfeeding is known to accelerate the sclerotic process of normal, ageing, kidneys (Berg, 1960). Rats overeating due to genetic predisposition (Shimamura, 1982) or to hypothalamic lesions all have increased incidence of spontaneous glomerulosclerosis (Kennedy, 1960).

A high protein intake has been known to increase the severity of age related glomerulosclerosis in rats (Lalich, 1971). However, it took as long as 2 years (600 days) of 30% and 50% protein diets to induce a significant glomerulosclerosis in normal, non nephrectomised, rats.

Lalich et al (1971) claimed that after uninephrectomy rats eating a diet providing 40% protein had appreciable glomerular and tubular lesions within 150 days.

Unfortunately, although repeatedly quoted this study did not control for caloric, salt, calcium or phosphate intakes. As any of these substances when in excess could produce accelerated glomerular scarring after reduction in renal mass.

More recently, the same authors in a more comprehensive study evaluated the effect of a high salt (3,6%) and high protein intake on the renal ultrastructure and function of uninephrectomised rats (Lalich, 1975). They noted a high salt and protein intake significantly increased the urine volume and solute excretion. The supplemented diet increased the urea level of the experimental rats and accelerated the glomerulosclerotic lesions. They concluded that an increase in the total amount of solute excreted in the urine was sufficient in promoting glomerulosclerosis in these rats. However, in this study as in previous ones, conclusions concerning the nephrotoxicity of high protein diets cannot be reached as changes in the dietary mineral content was not excluded.

It becomes apparent from the majority of early studies on the role of high protein diet in the progression of renal disease, that they ignored the relative importance of high phosphate intake on the renal lesions (Lalich, 1975). Moreso, as high protein diets are low in their carbohydrate and fat contents, the effects of these deficiencies on renal function cannot be excluded. We will see later in this section, that diets with variable fat contents affect renal function in some other models of renal failure.

More important, the nephrotoxicity of high protein diets does not seem to be universal, as studies by Bovee et al (1979) in dogs failed to show a significant difference between nephrectomised animals on normal and high protein diets. These authors studied dogs with 75% nephrectomies achieved either through surgical ligation or after surgical ligation with additional bacterial innoculation of the remnant kidney. These dogs were fed diets

containing 19%, 27% and 56% protein. Animals were studied over 4 years. In this model the high protein diet group had increased glomerular filtration rate and renal blood flow when compared to controls on a normal diet. Although kidneys from the animals on the high protein intake showed more glomerulosclerosis, there was no correlation between these morphological changes and renal function.

From these studies reviewed in this section, the body of experimental evidence seems to indicate that food, and in particular protein, restriction protects ageing and diseased kidneys from further deterioration. On the other hand, overfeeding and excess protein and solute excretions might be nephrotoxic although further control studies are required.

(2) Dietary phosphate

Phosphate toxicity on normal and diseased kidneys have been established since the thirties; the elegant studies of McKay and Oliver (1933) have shown, in rats, that excess phosphate intake (10%) led to calcium phosphate deposition in the renal tubules.

More recently, Ibels et al (1978) demonstrated a protective effect of phosphate restriction on the progression of renal failure, after sub-total nephrectomy, in uraemic rats. In their study, a phosphate deficient diet (0.04%) was compared to normal diet (phosphate : 0.5%). Albuminium hydroxide was added to the diet in an additional experimental group. Rats were started on the diet 6 weeks prior to seven-eighths nephrectomy and followed for a total of 12 weeks. Animals fed the

diet low in phosphate had the better course; proteinuria was decreased, renal function preserved and survival improved. Animals fed the normal diet showed severe tubular calcium deposition and atrophy as well as glomerular and interstitial changes. At the end of the observation period, rats restricted in phosphate had near normal kidneys.

These authors postulated that an increased phosphate load per residual nephron could promote renal calcium phosphate deposition and subsequent dysfunction. Equally relevant, in their opinion, was the effect of a low phosphate diet on circulating parathyroid hormone levels, as low levels might prevent nephrocalcinosis. However, this recent study had major shortcomings; the first and major criticism being the lack of data on the animal's food consumption, as a phosphate depleted diet is known to decrease food intake (Laouari, 1982). The beneficial effect of phosphate restriction, in this study, could be the mere reflection of a reduction in food intake. Moreso, details were not provided regarding the rats weights and rate of growth on the different experimental diets. Finally, the experimental diets were started six weeks prior to the nephrectomies and their maximal beneficial effect might have occurred during the early, compensatory post nephrectomy phase. Therefore, the study might be less relevant to the study of progression of chronic renal disease. In another study, the effect of diet containing 3% phosphate and supplemented with disodium ethane-1-hydroxy-diphosphonate (EHDP) was studied. As the renal functional mass was reduced, the nephrotoxicity of phosphorus was greatly increased. Nephrocalcinosis was less severe with reduced phosphate intake as well as in rats supplemented with

diphosphonates in spite of a high phosphate intake. In this experiment, the diets were started during the early post-nephrectomy phase. The mechanisms of phosphate toxicity was again thought to be nephron overload and stimulation of parathyroid hormone secretion (Haut, 1980). The protective effect of diphosphonate against phosphate nephrotoxicity was one of the main findings of this study. Renal calcification could apparently be prevented by agents inhibiting calcium phosphate crystallisation.

Prevention of phosphate induced renal damage, in sub-total nephrectomy by another crystallisation inhibitor was later reported by Gimenez et al (1982). These workers showed that the injection of 3-phospho-citric acid to nephrectomised rats had a striking protective effect against renal calcifications. Animals were randomised on three different regimes : normal phosphate intake, 3.6mmol/day (1%), high phosphate intake, 12mmol/day (2.2%) and high phosphate (2.2%) with additional 50umols of phosphocitrate per day. These diets were introduced, late, two weeks after completion of the nephrectomies. Renal function deteriorated in the high phosphate (2.2%) group with marked renal calcifications and tubular atrophy. Animals fed the 1% phosphate diet as well as the diet with daily phosphocitrate supplements had a milder course with no noticable decline in their renal function. Renal calcification was eight fold increased on the high phosphate diet. No correlation was found between serum levels of calcium and phosphate and the extent of nephrocalcinosis. This work, although original and interesting had some shortcomings; in particular the lack of controls, as the injection of rats with acid could affect the acid-base balance and consequently the urine calcium excretion

(Gimenez, 1982). Urine acidification in itself is known to increase urine calcium excretion and could alter the calcium phosphate product (Whiting, 1980). Therefore it remains to be determined if the observed beneficial effect of phosphocitrate is a specific one.

Karlinsky et al (1980) studied the effect of dietary phosphate restriction on the course of the immune mediated nephrotoxic nephritis (NTN). They compared a phosphate restricted diet (0.04%) with a normal diet providing 0.5% of phosphorus. Rats on the phosphorus restricted diet had an overall better course. Renal function in this group was preserved and survival was prolonged. There was no significant differences in proteinuria between the two groups. An unexpected and unexplained finding, in this study, was the observation of increased kidney phosphorus concentration in the phosphorus restricted nephrotoxic nephritis group as compared with control animals on normal and low-phosphorus diet. The mechanism of the protection provided by the low phosphorus diet in nephrotoxic nephritis remains to be determined.

Therefore, the overall body of experimental evidence suggests that decreased phosphate consumption and the reduction of phosphate load per functioning nephron prevents nephrocalcinosis in experimental models of uraemia (Alfrey & Tomford, 1983).

Meanwhile, some doubts remain as to the relative beneficial effects of low phosphorus and protein diets. Laouari et al (1982) showed that, when rats were pair fed, the overall reduction in food intake was more important in the prevention of renal disease

progression than phosphate restriction. These authors concluded that a phosphorus restriction sufficient to cause moderate hypophosphataemia, disappearance of phosphaturia and to prevent hyperparathyroidism without causing anorexia, had little if no beneficial effect on renal deterioration (Laovari, 1982).

(3) Dietary carbohydrates

Few experiments dealt with the effects of dietary carbohydrate on renal function and morphology in animals. However in 1971 Cohen et al observed that rats fed a high sucrose diet had an increased incidence of age related glomerulosclerosis. In their study, rats fed a high sucrose diet (72%) for a period varying between 9 and 18 months developed diffuse intercapillary glomerulosclerosis. Control rats fed a high starch diet failed to develop significant renal pathology. Rats fed a sucrose-rich diet has an increase in kidney weight and morphological changes observed at light microscopy. These histological changes consisted of an increase in mesangial cellularity and matrix as well as various degrees of glomerulosclerosis. Similarly, focal tubular atrophy and dilatation was observed. At electron microscopy, these rats kidneys showed mesangial and epithelial cell cytoplasmic inclusions consisting mainly of lipid droplets. These rats has impaired glucose tolerance and are likely to have been hyperlipidaemic; liver lipid synthesis is known to increase in rats fed a high sucrose diet (Cohen & Teitelbaum, 1968).

It is therefore difficult to know if the observed nephrotoxicity of high sucrose diets is a direct effect of carboydrates on the kidneys or if it is merely the result of the secondary hyperlipidaemia.

More recently, Boot-Handford and Heath (1981) have confirmed these findings in the rat and attributed the nephrotoxicity of sucrose to the fructose moiety.

Rats fed a high sucrose as well as those on a high fructose diet developed lesions of glomerulosclerosis within six months. On the other hand rats fed starch, a high glucose diet or a diet with mixed refined and unprocessed carbohydrates failed to develop similar renal lesions. The nephrotoxicity of sucrose and fructose was associated with an increased kidney weight concomittantly with an increment in serum fructose level as well as renal tissue concentration of fructose-1-phosphate.

It would seem therefore that carbohydrate and in particular dietary sucrose and fructose are nephrotoxic in rats when consumed in excess over a long period of time.

Further studies are required to evaluate the nephrotoxicity of carbohydrate on already damaged kidneys, with reduced nephron mass.

More important however would be to dissect the respective roles of hyperglycaemia and hyperlipidaemia in the nephrotoxicity of carbohydrates. The lesions described by Rosenmann et al (1981) in sucrose-fed rats were very suggestive of lipid nephrotoxicity.

(4) Dietary lipids

Less attention has been paid to the role of dietary fat in the progression of renal disease than to dietary protein and phosphorus. However, Fernandes et al (1973) showed that reduction of dietary fat prolonged life and decreased the auto-immune manifestations normally observed in the NZB/W mice. These mice, particularly females, develop a few weeks after birth an autoimmune disease similar to human systemic lupus erythematosus (SLE). Death, in these animals is a consequence of renal failure, as nephritis represents an early and progressive manifestation of the disease (Lambert & Dixon, 1968). When these animals were fed a diet rich in fat albeit low in protein it resulted in an acceleration of the disease process (Fernandes, 1973).

More recently, the role of dietary fat in the progression of renal disease has been evaluated. Hurd et al (1981) studied the effects of different fatty acids rich and deficient diets on the survival and renal involvement in the NZB/W F1 hydrid. Their work was based on the assumption that prostaglandins, in particular PGE; was protective in this model; animals given PGE_1 had a prolonged survival and milder immune mediated nephritis (Zurier, 1977). Hurd et al showed that feeding BW mice with a diet containing 28% coconut oil, deficient in essential fatty acid (EFAD), prolonged mouse survival.

On the EFAD diet the auto-immune manifestations were decreased and the severity of the glomerulonephritis was attenuated. No similar effect was observed on a diet, supplemented with sunflower oil 20%, and known to be rich in essential fatty acids. It was assumed from these studies that the EFAD diet beneficial effect was mediated by its non specific anti-inflammatory action (Bonta, 1977).

A similar EFAD diet was studied in rats with nephrotoxic nephritis. Dubois et al (1982) found a detrimental effect of such a diet on the course of NTN.

In nephrotoxic nephritis, Tomford et al (1981) showed that thyroparathyroidectomy (TPTx) improved the overall prognosis of the disease. A main consequence of TPTx was the reduction in serum triglyceride levels with the concomittant fall in proteinuria and improved nephritis outcome.

Prickett et al (1981) studied in the SLE like disease of mice the effects of a diet rich in polyunsaturated fatty acid. They compared the effects of a diet low (0.05%) and high (14.4%) in eicosapentoic acid (EPA) on renal involvement and survival. The diet, high in EPA, protected the mice from the development of the life threatening glomerulonephritis. Simultaneously, there was a marked reduction in auto-antibody levels. This observation remains unexplained although it is likely that such a diet alters the immune response of these animals, therefore minimising the spectrum of autoallergic manifestations.

Finally, Barcelli et al (1982) showed a beneficial effect of alterations in dietary linoleic acid on the course of chronic renal failure in the sub-total nephrectomy model. These authors observed that a diet high in linoleic acid (27%) had a protective effect against proteinuria and renal failure following renal mass reduction. Similarly, they noted that such a diet increased the prostaglandin E2/thromboxane ratio of the renal cortex of remnant kidneys. They postulated that changes in prostaglandin formation, secondary to the diet, could affect the development of chronic renal failure in this model.

All these studies and others emphasise the need for further evaluation of the role of lipids and fatty acids on the progression of experimental immune mediated models of chronic renal failure. These diets have anti-inflammatory (Bonta, 1977), anti platelets (Sanders, 1983) as well as metabolic (Saynor, 1981) and immune effects (Mertin & Hunt, 1976) that could all combine to affect the progression of nephritis.

From all the data reviewed in this introductory section it becomes apparent that progression of chronic renal failure is likely to be a multifactorial process.

However, many questions remain unanswered including :

1. The mechanisms underlying the progression of severe immune mediated glomerulonephritis ?

2. The role of the immune system in the progression of immune mediated nephritides ?

3. The role of dietary protein manipulations on the course of glomerular and non glomerular disorders in experimental animals.

4. The relevance of the experimental data to human nephritides ?

5. The effects of acute and chronic dietary protein manipulations on different nephropathies in man.

The purpose of this thesis is to address and hopefully answer some of these questions.

CHAPTER II

MATERIALS AND METHODS

I. Animal experimental work

Animals

All animal, experimental, work was done on rats. Sprague Dawley and Lewis rats were the strains used. Adult rats were used throughout the study; age at onset of experiments : 8-10 weeks, weight : 200-300gr, Male rats were used in preference to females, in view of their predisposition to more severe nephritis.

Only in transplantation experiments were highly inbred, Lewis, syngeneic rats used in order to minimise the extent of graft rejection on the course of the nephritis.

Rats were housed by groups of up to six to a cage. They were transferred once weekly to individual metabolic cages for 24 hour urine collections. Rats were conditioned to the environment for 2 weeks before they were used for experiments.

The metabolic cages were supplied with a tunnel feeder and a urine-faeces separator to avoid contamination of urine by food and faeces respectively.

Animals were fed, 20 grams/day, commercial laboratory diet (Purina) unless stated otherwise in the experimental protocol and had free access to tap water.

Rabbits, New Zealand white, were used for immunisation and antibody production purposes. Male as well as female rabbits were used, weighing 2 to 3 kg.

Rats were bled from the median tail artery under light ether anaesthesiae. A 23 gauge butterfly needle was used for all bleeding, blood sampling, and injection procedures.

Rat immunisation, when required, was achieved by the injection into the hind foot pads of the antigen emulsified in Freund's complete adjuvant (CFA).

Rabbits were immunised by the injection of the antigen into their femoral muscle. Rabbits were bled from the ear veins.

Rats that showed any sign of a life threatening condition, such as respiratory infection, unrelated to the underlying experiment, were sacrificed and excluded from the experimental data.

Rats developing symptoms and signs of uremia (end stage renal failure) were equally sacrificed. In these rats with experimental end stage renal failure, time of sacrifice was considered the time of experimental death. Death from uremia was confirmed by blood creatinine level and histological evaluation of the kidneys.

Different steps of the induction of nephrotoxic nephritis will now be discussed. Nephritis was induced in rats by the injection of heterologous rabbit anti-glomerular basement membrane antibodies. Therefore rat glomerular basement membrane (GBM) had to be prepared. Rabbits were immunised with the new GBM preparation. The anti-GBM antibody produced was tested in vitro and in vivo before used in experiments on the progression of renal disease.

Preparation of rat glomerular basement membrane (GBM) (Krakower & Greenspon, 1951)

- Each preparation of GBM required the use of 50 rats

- Rats were anaesthetised with ether and 4 to 5 mls of blood collected from the aorta, in order to obtain red blood cells for antibody absorption studies.

- Rats were sacrificed and the kidneys removed and kept on ice until stored at $-20^{o}C$.

- For GBM preparation, the kidneys were decapsulated and the renal cortex was dissected with a blade.

- Renal cortex was kept and the remaining kidneys were discarded

- Cortical tissue was pulped (squashed) and then pushed through successive sieves to isolate the glomeruli.

- The cortical pulp was first pushed through a 106um sieve (Endecotts Ltd.) allowing the glomeruli to be filtered and contaminating material to be excluded.

- The suspension containing the glomeruli was collected through rinsing the sieve with cold phosphate buffered saline (PBS).

- This step was repeated once.

- The glomerular suspension was then put through a smaller diameter 63um sieve. This mesh size retained the glomeruli.

- Glomeruli were rinsed off the sieve mesh with cold IM NaCl.

- The purity of the glomerular suspension was assessed by light microscopy examination; a suspension containing 90% to 95% glomeruli was considered satisfactory. Otherwise the final step of the preparation was repeated.

- The glomerular suspension in 1M NaCl was sonicated 3 x 10 seconds at $4^{o}C$. This was followed by microscopical examination to assess the efficiency of the procedure.

- The suspension was centrifuged for 15 minutes at 120g, this step was repeated three times, with collection of the final precipitate (GBM) and its resuspension in water. The supernatant was discarded.

- This final suspension was lyophilised.

- Dry GBM was weighed and stored at $-20^{o}c$.

Production of rabbit anti rat nephrotoxic serum (NTS)

New Zealand, white rabbits, weight : 2 to 3kg, were immunised with the rat GBM in order to obtain nephrotoxic serum (NTS) containing high titres of rabbit anti rat glomerular basement membrane antibodies (α-GBM Ab).

Preparation of the antigen (GBM) :

10mg of dry GBM was suspended in 1ml of PBS.

This suspension was mixed volume/volume with Complete Freund's adjuvant (CFA) (Difco, Laboratories Inc., Detroit, Michigan)

The suspension was emulsified in CFA by repeated sonication : 30 seconds x3.

Immunisation procedure

Day 0 : Rabbits were injected with 10mg of GBM in CFA into their femoral muscle

Days 7,15,21 : Repeated boosting with 1mg of GBM in Incomplete Freund's adjuvant (IFA)

Days 36,51 : Same as above.

- Two weeks after the last booster injection the rabbits were bled and 10mls of serum tested from each, for antibody activity.

- Three rabbits were usually immunised simultaneously.

Testing of the nephrotoxic serum

Testing the activity of the putative rabbit anti rat glomerular basement membrane antibodies was done by indirect immunofluorescence (IF).

- Normal rat kidneys snap frozen in liquid nitrogen and stored at -70°C were used.

- 4 u sections were cut with a cryostat (Slee) at -20°C.

- The sections were fixed on a microscope slide with cold air.

- Different dilutions : 1/500, 1/1000, 1/5000, of the NTS were prepared in PBS.

- The rat kidney sections were first washed with cold PBS for 10 minutes x3.

- The washed sections were incubated with 10ul of the different dilutions of the NTS for 20 minutes at room temperature.

- The sections were washed with PBS : 10 minutes x3

- Fluoresceinated isothiocyanate (FITC) conjugated sheep anti-rabbit gamma globulin (Miles Laboratories), 10ul of a 1/8 dilution in PBS was added to the kidney sections and left to incubate at room temperature for 20 mins.

- Sections were thoroughly washed in PBS : 10 minutes x3

- Sections were dried and mounted with 90% glycerine in PBS for examination under ultraviolet light microscopy.

- Positive immunofluorescence staining of the glomerular basement membrane at a NTS of 1/1000 or less was considered satisfactory. Staining occurred along the TBM as well as the GBM.

- Low titres of nephrotoxic serum; negative at 1/1000, was discarded and the rabbits further boosted twice at fortnightly intervals.

Preparation of the nephrotoxic serum

- High titre nephrotoxic serum was decomplemented : 30 minutes at 56°C.

- The decomplemented serum was adsorbed with normal rat serum and packed red blood cells (RBC's); NTS was mixed with rats RBC's volume/volume and left at room temperature for 3 hours and at 4°C for 12 hours.

- After adsorption NTS was tested on a haemagglutination plate as well as by Ouchterlony (Laurell, 1966) in order to detect remaining anti-RBC's and anti rat serum protein respectively.

- In the absence of significant RBC's haemagglutination, the NTS was considered satisfactory.

The NTS was ultracentrifuged, before use, at 40,000 rpm for 30 minutes in order to eliminate large immune aggregates.

After centrifugation, the NTS was ready for use as it is or the globulin fraction was precipitated by saturated ammonium sulphate and the nephrotoxic globulin fraction was used.

Haemagglutination procedure

- In order to detect antibodies (IgM) directed against [illegible] contaminating nephrotoxic serum we tested this serum before use in haemagglutination plates.

- A V shaped 96 wells macrotitre plate was used.

- Individual wells were filled with rat RBC's [illegible] in PBS.

- Two-fold dilutions of NTS was added to individual wells.

- The microtitre plate was left at room temperature for [illegible] then at 4°C overnight before reading.

- In case of positive haemagglutination, the NTS was reabsorbed with rat RBC's ; 3 hours at room temperature and overnight at 4°C.

Preparation of nephrotoxic globulin (NTG)

Nephrotoxic globulin was prepared from NTS by saturated ammonium sulphate (SAS) precipitation (Pennel, 1960).

- Saturated ammonium sulphate was prepared : 500 grams of ammonium sulphate in 500ml of distilled water, mixed in a water bath at 50°C for 2 hours and left overnight at room temperature. The PH of the final solution was adjusted to 7.2.
- Nephrotoxic serum was diluted 1:2 with phosphate buffered saline (PBS)
- NTS (diluted) was mixed with ammonium sulphate to give a final concentration of 45% SAS (volume/volume).
- The mixture was stirred for 30 minutes at room temperature and left for 1 hour to floculate at 4°C.
- The mixture was centrifuged at 8000 rpm for 15 minutes at 4°C.
- The ammonium sulphate precipitate was resuspended in cold PBS and the supernatant discarded.
- A second precipitation with ammonium sulphate was repeated, followed by centrifugation.
- A third and final precipitation with a decreased concentration 40% of ammonium sulphate was done, followed by centrifugation.

- The final precipitate was suspended in 1/10 of the original NTS volume of PBS.

- The solution containing the nephrotoxic globulin fraction (NTG) was dialysed against 5 litres of cold PBS (4°C) for 24 hours in order to dialyse off the ammonium sulphate.

- Sodium azide was added to the first 5 litres of dialysate (PBS), and excluded from the last ones in order to prevent the contamination of the NTG.

- After thorough dialysis the solution was centrifuged and contaminants discarded.

- The final protein concentration of the NTG was estimated by spectrophotometry at 280nm with an extinction coefficient of 1.2.

- The protein content varied between 15gr and 20gr/litre.

Testing the activity of the nephrotoxic globulin (NTG)

The activity of the NTG fraction was assessed by three different methods.

1. Indirect immunofluorescence

2. Quantitated fixation, in vivo, to the glomerular basement membrane

3. Induction of nephritis

1. Indirect immunofluorescence (IF)(Bentner, 1970).

- The same procedure of IF as the one used to estimate the activity of the NTS was used

- Positive sections showed a linear distribution of the NTG along the glomerular and tubular basement membranes.

2. Quantitated estimation (in vivo) of NTG fixation on the GBM

This step required the double radiolabelling of the nephrotoxic globulin (NTG) with iodine-125 and of rat's serum globulin with iodine-131. (McConahey & Dixon, 1966).

The rat globulin fraction was prepared by 50% ammonium sulphate precipitation in a manner similar to the one previously described for NTG.

Radiolabelling (I^{125} - I^{131})

- Solutions required :

(a) chloramine T : 2.5 mg/ml water

(b) sodium metabisulphite : 2.5mg/ml water

Method

- A final concentration of 2mg/ml of NTG was used : 100ul

- I^{131} : 1 uCi per ug of protein : 200 uCi/200ug protein (1 uCi/mg).

- Chloramine T (CT) was added : 25ul of the 2.5mg/ml solution, and mixed, on ice, for 60 seconds.

- Sodium metabisulphite : 50ul of the 2.5mg/ml solution was added to stop the reaction.

All the reactions took place on an ice tray in order to keep the temperature at $4^{o}C$.

- The efficiency of labelling varied between 50% and 60%, (TCA precipitation).

- The specific activity of labelling varied between : 0.4 to 0.5 uCi/ug of protein.

The separation of free iodine from protein bound iodine was achieved by filtration of the radiolabelled solution on a pre-swollen sephadex-25 column. This was followed by the dialysis against PBS of the protein bound fraction in order to eliminate residual free activity. At this stage $\geq$95% of radioiodine was protein bound.

By this method both the nephrotoxic globulin (NTG) and the rat serum globulin fraction (RγG) were trace labelled with I^{125}-NTG and I^{131}-RγG.

The determination of the in vivo binding of the NTG was an important step to appreciate the potency of the anti-GBM antibody and to therefore estimate the amount fixed on rat kidneys during the subsequent experiments.

- Rats were injected with 1ml of both radiolabelled NTG and RγG/rat (protein conc : 15-20 mg/ml).

- The radioactivity ratio between the I^{125}-NTG/I^{131}-RγG was 10/1.

- After the injection into 5 rats, the animals were put in individual metabolic cages for 24 hours.

- At 24 hours, the urine collection was assessed for proteinuria.

- Rats were bled, killed and the kidneys were removed.

- Radioactivity for both serum and kidneys was counted on a double channel gamma counter (LKB). The final count was adjusted for I^{131}/I^{125} crossover and background counting.

- The specific binding of the injected I^{125} labelled NTG to the kidney was calculated using the formula :

$$\text{Kidney } I^{125} - \text{Kidney } I^{131} \times \frac{\text{Serum } I^{125}}{\text{Serum } I^{131}}$$

- The percentage of binding varied between 0,75 to 1%/kidney; 70ug to 310ug of kidney fixing antibody/kidney.

3. Induction of nephritis (proteinuria)

- Rats (4 in each group) were injected with different amounts of nephrotoxic sum globulin in order to determine the minimum dose required to induce proteinuria.

- The doses used were : 0.5, 1, 1.5, 2 mls

- Proteinuria was estimated before and 24 hours after the injection.

- Proteinuria, in male rats, before the injection was in the normal range : $\leqslant$ 20mg/24 hours.

- In the majority of cases (rats weighing : 200 grams) 1ml of NTG (20mg) was found to be sufficient to induce a significant proteinuria: $\geqslant$50mg/24 hours; Proteinuria was induced by $\geqslant$100 ug of kidney fixing antibodies/kidney.

Models of nephrotoxic nephritis (NTN)

Two models of nephrotoxic nephritis were used throughout the experiments :

1. the standard nephrotoxic nephritis model
2. the telescoped, accelerated, NTN model

The standard NTN model was used sporadically, however most experiments on progression of renal disease were done with the accelerated variants of NTN.

1. The standard model (Hammer & Dixon, 1963)

- Rats were put in individual metabolic cages once in the week preceeding the induction of the nephritis to determine the baseline proteinuria and renal function

- Animals were injected with 2mls (30-35mg) of nephrotoxic globulin into the median tail artery under general ether anaesthesia

- Following the injections, rats were kept for 24 hours in individual metabolic cages to assess the severity of the heterologous phase.

2. The telescoped, accelerated model (Unanue & Dixon, 1965)

In this variant of NTN rats were pre-immunised with rabbit gamma globulin (Rγ G) before the injection of the rabbit nephrotoxic globulin (NTG).

- On day -5 - Pre-immunisation of rats by foot padding with 1mg of rabbit γ globulin (0.05ml) in complete Freund's adjuvant (CFA) (0.05ml).

  Each rat was therefore injected with 0.1ml containing 1mg of RγG.

- Day 0 : Rats were injected with 2mls (30-35mg) of nephrotoxic globulin or serum into their median tail artery under light general ether anaesthesia.

Assessment of nephritis

- The progression of the NTN was done on a weekly basis.

- For this purpose the rats were put once a week in individual metabolic cages.

- Rats weight, proteinuria and renal function were assessed serially at weekly intervals.

- Bleeding was performed under light ether anaesthesia from the median tail artery.

- Renal morphology was examined at different intervals and at time of death or end of the experiments according to individual experimental protocols.

- We measured the levels of circulating rat anti-rabbit gamma globulin antibodies as an indicator of the host autologous immune response.

1. Proteinuria :

- Proteinuria was measured using the biuret method (Gornall, 1949)

- Estimation of proteinuria was done on 24 hr urine collections

- Urine was centrifuged in order to eliminate contaminants.

- 1ml of urine was precipitated with 1ml of cold 20% trichloroacetic acid. (TCA)

- Urine was left, after mixing with TCA, at $4^{o}C$ for 3 hours.

- This was followed by centrifugation at 3000 rpm for 15 minutes.

- The supernatant was discarded and the pellet drained.

- The pellet, with the precipitated protein, was resuspended in 0.5ml of 1M sodium hydroxide (NaOH).

- 1.5ml of distilled water (H20) was added.

- 2ml of Biuret reagent was added.

- The final solution was thoroughly mixed and left to stand at room temperature for 30 minutes when stable colour was obtained.

- A blank tube as well as a standard were prepared :

  Blank: 0.5ml (NaOH) + 1.5mls (H20) + 2mls Biuret reagent

  Standard: Bovine serum albumin (BSA) was used : 5mg/ml solution 0.5ml (BSA) + 1.5mls (H20) + 2mls Biuret reagent.

- After 30 minutes at room temperature, the colour of the reaction stabilised and samples were read in a colorimeter at 540 nm (peak wave length for BSA).

- The test result was calculated according to the formula :

$$\text{Urine protein (mg/ml)} = \text{Test OD} \div \text{Standard OD} \times \text{Standard protein concentration (5)}$$

- The proteinuria (mg/ml) was multiplied by the 24 hr urine volume and reported as mg/day.

- Normal proteinuria, in adult male rats, did not exceed 20mg/24 hrs.

Albumin measurement in serum and urine

- Rocket electrophoresis was used to measure serum and urine levels of protein (Laurell, 1966).

- Rabbit anti rat serum albumin antibodies were incorporated into 1% agarose in glycine-barbitone-tris EDTA buffer (GBT), pH 8.5 - 8.6.

- The final antibody concentration was 2% (volume/volume).

- Plates were poured, thickness 1.5mm, and 2mm wells were punctured at the base of the plate.

- 2ul of test samples at dilutions of 1/50 for serum and 1/10 for urine and standard (RSA) were put in individual wells.

- Electrophoresis was achieved at 120 volts for 4 to 6 hours using a water cooler or at 40 volts overnight.

- Gels were washed in PBS, pressed and dried

- Gels were stained in Comassie blue 0.5% in acetic acid methanol water.

- Gels were destained with destaining buffer (acetic acid methanol).

- The height of the electrophoretic rockets was measured and compared to a standard solution of rat serum albumin (Sigma) at different dilutions.

2. Renal function

Renal function in experimental rats was determined by serum and urine creatinine measurements.

Both serum and urine creatinine were measured by standard auto-analyser techniques, based on the Jaffé reaction.

Normal serum creatinine varied with the animal's age and size; however the normal range was between 30 and 60umol/l. Creatinine clearance varied equally with animal's age and size; the normal range was between 1 to 4 ml/min/kg of body weight.

3. Autologous antibody measurement

The rat, autologous, anti-rabbit gamma globulin antibodies were measured by a solid phase radioimmunoassay. (Voller, 1976).

- 96 wells mictrotitre plates were used (Dynatec).

- Microtitre wells were coated with 100ul of rabbit gamma globulin (IgG) : 50ug/ml in PBS or complement fixation diluent (CFD).

- Wells were incubated with Rγ G for 3 hours at 37°C.

- The wells were washed with CFD-Tween (0.05%) x3.

- Three different dilutions (1/500, 1/1000, 1/5000) of the test serum in CFD-Tween were added to the wells : 100ul.

- The putative serum was incubated for 1 hour at 37°C.

- Plates were washed with CFD-Tween x3.

- Iodinated ^{125}-rabbit anti rat IgG was added and incubated for 1 hour at 37°C.

- Wash with CFD-Tween x3.

- Wells were counted for radioactivity in a double channel gamma counter (LKB).

- Results were expressed as percentage binding of a known highly positive sample, obtained by immunisation and repeatedly boosting of a rat with rabbit gamma globulin.

- All samples were tested in triplicate.

- The coefficient of variation of the test did not exceed 5%.

4. Renal morphology

Material was obtained for histological studies by open biopsies of the kidney.

Renal biopsy procedure :

- Animals were anaesthetised with ether.

- The left flank was shaved

- A small, 2 to 3cm, incision was made along the left flank through which the kidney is exposed.

- A small wedge biopsy of renal cortex was taken with a blade. Gentle pressure was exerted for a few seconds on the bleeding point.

- The deep, muscle, layer was sutured with catgut.

- The superficial, skin, layer was sutured with silk (mersilk 4/0).

- Animals were left lying on their left flank for a few minutes until recovery from the anaesthesia.

- The open biopsy procedure lasted between 10 and 15 minutes, with a minimum mortality rate.

At time of death, kidneys were removed, weighed and processed for histological examination.

Renal material was divided into three : 1) fixed in 10% buffered formalin for light microscopy examination, 2) fixed in gluteraldehyde for electron microscopy and 3) snap frozen in liquid nitrogen for immunofluorescence. Light microscopy sections were stained with hematoxin-eosin and periodic acid Schiff reagent.

An arbitrary scoring system was defined for glomerular sclerosis and tubular atrophy :

- Glomerulosclerosis score :

1. glomerular basement membrane thickening and mild cellular proliferation (glomerular cell count >50, without crescent formation)

2. Focal and/or segmental glomerular hyalinosis

3. severe focal and/or segmental glomerular fibrosis

4. Diffuse glomerular sclerosis/fibrosis

- Tubular atrophy score :

1. Absent tubular atrophy

2. Mild tubular atrophy

3. Moderate tubular atrophy

4. Severe tubular atrophy

- The presence and severity of intra renal calcifications were assessed on the renal sections by light microscopy (H&E staining).

Induction of hypertension

- The model used to induce hypertension in rats was the DOCA and salt one. (Selye, 1943)

- Deocycorticosterone acetate (DOCA) (Sigma) was injected subcutaneously into rats at a dosage of 2.5mg thrice weekly.

- Sodium chloride (NaCl) was added to the rats drinking water to a final concentration of 0.9%, while potassium chloride (KCl) was added to a final concentration of 0.2%.

- Systolic blood pressure measurements were made in conscious rats by a tail cuff method using a doppler ultrasonic flow-meter to detect the pulse. (Bunag, 1973).

- Rats were not anaesthetised and the tails were pre-warmed before measurements (5 minutes at 37°C).

- In normal unanaesthetised rats systolic blood pressure did not exceed 120 mmHg.

Experimental diets

The low and high protein diets used throughout these experiments were matched for their caloric, calcium and phosphorus contents and only differed in their protein and starch contents.

Casein was the major protein constituent of the diets and varied in concentration from 8% to 78%. The calcium concentration was 0.85% and that of phosphorus was 0.67%. The low protein diet was supplemented with calcium phosphate in order to match the phosphorus content of the high protein diet. The caloric content of the diets was 2680 ME Kcal/kg. Rats were provided with 20 grams of diet/rat/day. Food was supplied in a single bowl for all the rats in individual cages, and the food consumption was assumed to be even between all the rats in the cage. The food bowls were weighed when the rats were presented with their daily allowance and 24 hours later in order to determine the amount of food consumed.

The details of the different diets are shown in Table (1).
(the diet was provided by Labsure Company).

TABLE 1

EXPERIMENTAL DIETS

Ingredients	Low protein diet %	Medium protein diet %	High protein diet %
Maize starch	62,5	53,5	-
Corn oil	3	3	3
Salkafloc	17	15,3	5
Casein	9	20	85
Minerals	3	3	3
Chalk/limestone	0,1	0,3	1,7
Di calcium phosphate	3,4	2,9	-
Calculated analysis	Percentage composition		
Protein %	8	18	78
Calcium	0,85	0,85	0,85
Phosphorus	0,67	0,67	0,67
ME Kcal/kg	2680	2680	2680

Surgical procedures

(1) Unilateral nephrectomy (Figure 2)

- The procedure was performed under general ether anaesthesiae.

1 - The rat's left flank was washed and shaved

- A 2 to 3cm lombotomy incision was performed.

2 - The left kidney was exposed through the incision.

3 - The renal capsule was removed and the adrenal gland dissected off the kidney upper pole.

4 - The left kidney was pulled on its vascular axis.

- A clip was applied to the vascular pedicle of the left kidney.

5 - The renal vascular pedicle was ligated with silk proximal to the clip.

6 - The pedicle was cut distal to the clip and the ligature.

- The kidney was removed.

- The clip was released.

2. Sub-total nephrectomy (Figure 3)

Two step sub-total (5/6) nephrectomies were performed. A - left two-thirds (2/3) nephrectomy followed a week later by B - right unilateral nephrectomy.

(a) Left two-thirds (2/3) nephrectomy :

- Same anaesthesia as previously was used.

1 - 2 Rat's left flanks were washed and shaved.

3 - Kidney was exposed as for unilateral nephrectomy and the adrenal gland was detached.

4 - The upper and lower poles were ligated with silk.

5 - Following ligation the two poles were resected distal to the ligatures.

6 - Pressure was applied on the bleeding surfaces until stopped.

(b) Right unilateral nephrectomy :

The method for right nephrectomy was similar to the one described above for left nephrectomy.

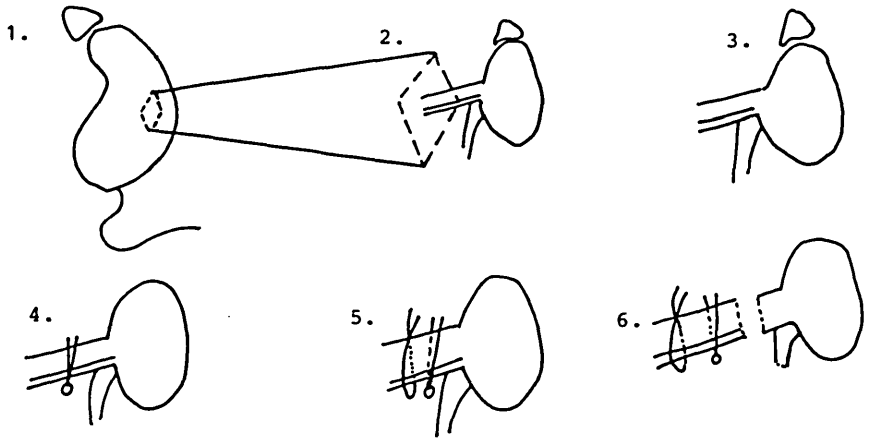


Figure 2 : Unilateral nephrectomy

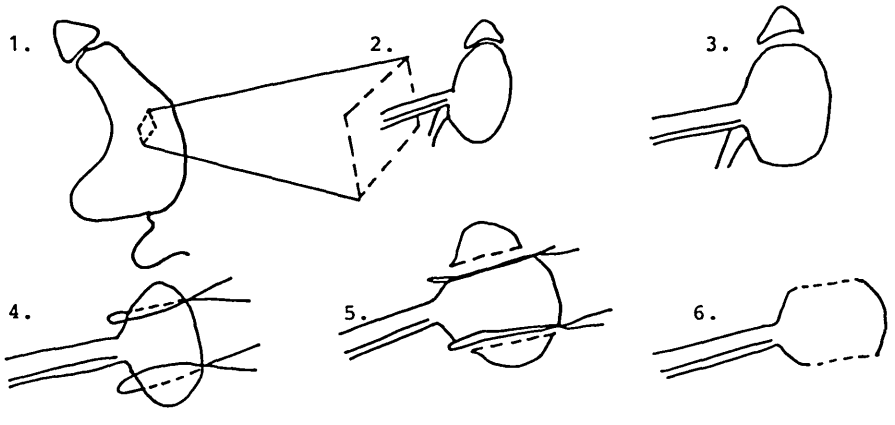


Figure 3 : Sub-total nephrectomy

3. Renal transplantation (Figure 4)

All the transplantation stages were done with a dissecting microscope (Zeiss).

(a) Dissection of recipient rat :

1. Anaesthesia was induced with chloral hydrate (0,8ml) injected intraperitoneally and maintained with ether.

2. Incision : a median, laparotomy, incision was made.

3. The liver was moved upward and protected with gauze.

4. The stomach and the small bowel were displaced upward and to the left in order to expose the left kidney.

5. Left kidney is exposed.

6. Peri-nephric fat is dissected off the surface of the kidney.

7. The renal capsule is dissected and removed.

8. The left adrenal gland is dissected off the upper pole of the kidney.

9. The ureter is dissected with removal of the periureteric fat.

10. The renal vascular pedicle is dissected with careful separation of the renal artery from the renal vein.

11. Arterial collateral vessels emerging from the renal artery dissected and ligated with mersilk (6-0).

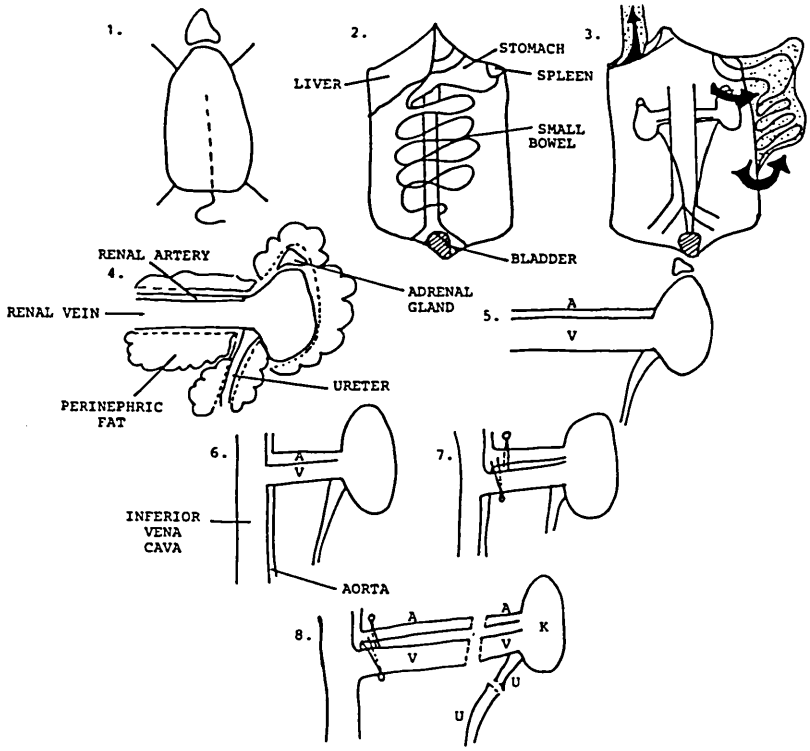


Figure 4 : Renal transplantation
Dissection of the recipient rat

12. Clips are applied distally near the renal vascular pole.

13. The renal artery and vein are cut proximal to the kidney in order to have a long arterial and venous stump for anastomosis to donor vessels.

14. The ureter is cut proximal to the kidney.

(b) Dissection of the donor kidney (Figure 5)

i. Left kidney

- The steps from 1 to 12 as in dissection of recipient rat.

13. The renal artery and vein are cut near the clips and distal to the kidneys in order to have a long vascular pedicle attached to the donor kidney.

14. The ureter is cut distally to the kidney.

ii. Right kidney (Figure 6)

The dissection of the right kidney is more difficult than the left in view of the short vascular pedicle and the position of the right artery pertaining to the renal vein. Similarly, the right vein is short and required, sometimes, the dissection of the inferior vena cava (ivc) and the removal of an ivc cuff to anastomose to the recipient's renal vein.

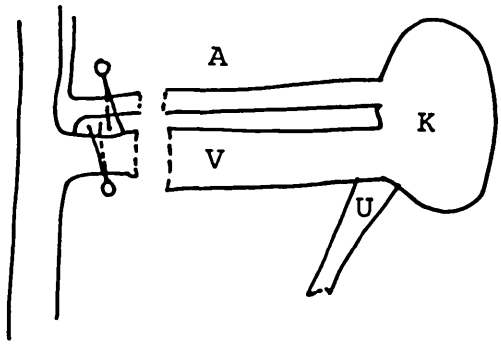


Figure 5 : Dissection of the donor left kidney

A : renal artery, V : renal vein,

K : kidney, U : ureter

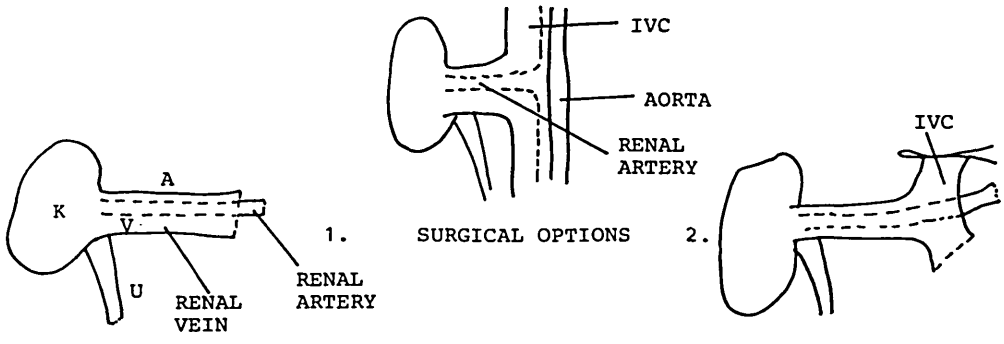


Figure 6 : Dissection of the donor right kidney,

IVC : Inferior vena cava

(c) Transplantation procedure

- Donor kidney was placed in the recipients left renal fossa.

- The donor's and recipient's vascular pedicles are placed opposite each other.

- The arterial anastomosis was first done :

  - end to end anastomosis
  - using Mersilk 6-0 (ethicon)
  - the anastomosis of the posterior wall was done first and the anterior wall next.
  - the arterial anastomosis was discontinuous

the venous anastomosis followed :

  - end to end anastomosis
  - using mersilk 6-0 (ethicon)
  - continuous suturing
  - here again, the venous posterior wall was anastomed first.

- The venous clip was released before the arterial one.

- Arterial anastomatic leak lasts for a few minutes and can be controlled by intermittent reapplication of the clip.

- Ureteral anastomosis is simililarly end to end.

- Warm ischemia time seldom exceeded 45 minutes.

II. Human clinical investigations

Role of dietary protein in the progression of chronic renal disease

This study was divided into three experimental subsets.

Experiment I

This was a retrospective study where we attempted to determine the factors that influence the progression of chronic renal diseases (CRD). We studied 58 patients with chronic renal failure (CRF); 32 males and 26 females, with an average age of 50 years (range 18 to 66 years). These patients had variable degrees of renal functional impairment at the onset of the observation : serum creatinine 351 ± 20umol/l (mean ± SD). They were studied over a period of 33 ± 21 months (mean ± SD) : range 6 to 90 months. The group consisted of 26 patients with chronic glomerulonephritis (CGN), 15 patients with chronic tubulo-interstitial nephritis (CTIN); predominantly chronic pyelonephritis (CPN) with some analgesic nephropathies (AN), 9 patients with hypertensive nephrosclerosis (HNS), 7 patients with polycystic renal disease (PKD) and 2 patients with diabetic nephropathy (DN).

Chronic glomerulonephritis was diagnosed in most cases by histology, analgesic, diabetic and hypertensive nephropathies by history and chronic pyelonephritis as well as polycystic renal diseases radiologically. It is clear that some of the patients classified as hypertensive nephrosclerosis could have an underlying glomerulonephritis, however, the predominant feature of this group is a longstanding hypertension with or without mild proteinuria (<1g/24 hours).

We determine the rate of decline in renal function in these patients using the reciprocal of serum creatinine (1/serum creatinine) (Mitch, 1976). Individual reciprocal of serum creatinine slopes were correlated with observation means of blood pressure (systolic and diastolic), means of serum calcium and phosphorus as well as means of proteinuria.

Experiment II: Effect of LPD on chronic renal diseases.

The second phase of this study was a prospective one aimed at determining the response of different nephropathies to a low protein, low phosphate, diet, with emphasis on the factors that influence such a response.

We studied 39 patients; 22 males and 17 females with an average age of 59 years. At the onset of the study the mean serum creatinine was 341 $\pm$ 28umol/l. The group included 14 patients with CGN, 10 patients with CITN, 9 patients with HNS, 4 patients with PKD and 2 patients with DN.

Experimental protocol

Patients were studied over a 6 month period on a normal diet and then put for another 6 month period on a low protein diet.

Diet

The protein intake prescribed was 0,5g/kg of body weight/day. The diet was equally low in phosphate (700mg/day) and calcium (350mg/day). A high calorie intake (2,500 - 3,000 Kcal/day) was achieved through carbohydrate and polyunsaturated fat supplementation. The details

of the actual dietary intake of the patients during the low protein period of the study are shown in Table 2.

Assessment of protection of chronic renal failure

All the patients were followed monthly as Outpatients. The patients dietary intake was assessed by an interview as well as dietary questionnaires. Patients were examined monthly with their body weight and blood pressure recorded. Serial monthly estimations of serum urea, creatinine, electrolytes, calcium, phosphorus, as well as protein and albumin were made. Simultaneously, creatinine clearance and proteinuria were estimated. Nitrogen balance as well as dietary protein intake were assessed by calculating the urea nitrogen appearance (Grodstein,1980), using the following formula :

UNA (grams/day) = urinary urea N (grams/day)

+ dialysate urea N (grams/day) +

$[(SUN_f - SUN_i \text{ gm/l}) \times Bw_i \text{ (kg)} \times 0.60 + (Bw_f - Bw_i) \times SUN_f \times 1]$ *

Experiment III: Effects of dietary protein on renal physiology.

The final phase of our study explored the short term effects of dietary protein loading and restriction on the renal function of patients with chronic renal failure. We studied 12 patients, 11 of whom were included in the previous (phase 2) prospective study. They included 7 males and 5 females with an average age of 52 years (range 45 - 65 years). Renal function was severely impaired in this group at the beginning of the study; creatinine clearance 27 $\pm$ 3.6ml/min (mean $\pm$ SEM).

*Nitrogen balance was calculated from : N intake - UNA

Experimental protocol

Patients, previously on a low protein diet, were started on a high protein diet (HPD) for 2 weeks and then switched to a low protein diet (LPD) for another 2 week period.

Experimental data

The low protein diet in this short term experiment differed from the one used in the long term study in that it was supplemented with sodium chloride, calcium and phosphate in order to match the high protein dietary content. On the LPD the mean protein intake was 46 ± 3g/day, sodium 126 ± 11mmol/day, calcium 1343 ± 62mg/day, phosphorus 1453 ± 55mg/day. The diets therefore only differed in their protein (129 ± 5.6g/24 h on the HPD) and carbohydrate contents. The two diets were isocaloric (2,353 ± 128 Kcal/day) (Table 3).

Assessments

Patients were tested on days 0 (LPD), 15 (HPD) and 30 (LPD). The following tests were done : full blood counts, serum urea, creatinine, electrolytes, calcium, phosphorous and bicarbonate. Glomerular filtration rate (GFR) as measured by endogenous creatinine as well as chromium ^{51} EDTA clearances. Effective renal plasma flow (ERPF) was estimated by the clearance of I^{131} hippuran using the single shot method.

The single shot technique without simultaneous urine collection was used for both clearances; the two doses were injected rapidly with sampling at 5, 10, 15, 50, 45 and 60 minutes for the calculation

of the clearance of radio-iodinated hippuran and at 1, 2, 3 and 4 hours for the estimation of the Cr^{51}-EDTA clearance. The dose injected was: Cr^{51}-EDTA : 100 uCi/m^2 and I^{131}-Hippuran : 15 uCi/m^2.

The clearance methods of single dose radionuclides were based on the disappearance of the injected substance from the plasma, its redistribution between intra- and extravascular compartments and finally its complete and exclusive elimination by the kidneys (Sapirstein, 1955). We used the two compartment open mamillary system assuming a rapidly reached equilibrium between the extra- and intravascular compartments with the injected substance cleared from the intravascular compartment into the urine.

A double exponential analysis of plasma disappearance curve was used to estimate the clearance; the basic, computer assisted, mathematical principles were outlined by Sapirstein in 1955.

A good correlation has been observed between the clearances of single shot Cr^{51}-EDTA and that of inulin (Favre, 1978) although some doubts remain as to the accuracy, in renal failure, of the estimation of ERPF by a single injection of hippuran. Vitye and Lebel in 1969 and Donath in 1971 have found this method reliable and reproducible. However, in the absence of basic condition for the estimation of ERPF for individual patients, it is useless to attempt to determine the reproducibility of such clearance (Reubi, 1960). In our hands, we observed an internal consistency in our estimated hippuran clearances in man as patients who increased their clearances on a high protein diet were the same ones whose clearance fell when started on a low protein diet. Patients who failed to respond to the diets kept their hippuran clearance within a standard deviation of 8 ± 1.6 %.

TABLE 2

LONG-TERM EXPERIMENT

Estimated daily consumption (Mean ± SEM)

LOW PROTEIN DIET

Protein (Gram)	34 ± 4 *
Fat (Gram)	65 ± 6
Carbohydrate (Gram)	400 ± 15
Na (mmol)	85 ± 10
K (mmol)	70 ± 8
Ca (mg)	1100 ± 128 **
Pi (mg)	700 ± 45
Energy (Kcal)	2000 ± 175

* Mean Protein intake was : 0.52±0.15 g/Kg/day

** Calcium dietary intake (350 ± 25mg/24 hours) was supplemented by calcium carbonate in the majority of the patients in order to increase the daily calcium consumption to 1000-1500 mg.

TABLE 3

SHORT-TERM EXPERIMENT

Estimated daily consumption (Mean ± SEM)

Ingredients	Low protein diet	High protein diet
Protein (grams)	46 ± 3	129 ± 5.6
Fat (grams)	68 ± 5	81 ± 7
Carbohydrate (grams)	300 ± 25	285 ± 27
Na (mmol)	126 ± 11	115 ± 15
K (mmol)	78 ± 10	85 ± 10
Ca (mg)	1343 ± 62	1405 ± 75
Pi (mg)	1453 ± 55	1550 ± 35
Energy (Kcal)	2353 ± 128	2235 ± 154

Statistical analysis of results

Results were expressed as mean and standard error of the mean (SEM) unless stated otherwise. The Mann-Whitney U test was used to estimate the significance of differences between the experimental groups at specific points. The relation between variables was measured by linear regression analysis using the least squares method. The significance of differences between the slopes was estimated by one way analysis of variance (Snedocor, 1956). Survival was calculated by the life table method and difference between groups by log rank test (Peto, 1977). Paired t-test was applied to compare data from individual patients. A probability of 5% was used as the criterion of significance.

CHAPTER III

EXPERIMENTAL DATA

A. RESULTS OF ANIMAL EXPERIMENTATION

I. Role of the immune response in the progression of chronic renal disease - nephrotoxic serum nephritis

Introduction

Nephrotoxic serum nephritis (NTN) is an experimental model of immune mediated nephritis (Masugi, 1934). The nephritis is induced in animals by the injection of heterologous anti-kidney antibodies, more specifically anti-glomerular basement membrane (GBM), antibodies (Hammer & Dixon, 1963). This is followed, in rats, by the development of a diffuse proliferative nephritis not unlike its human counterpart. The disease, if severe enough, progresses to chronicity with end stage renal failure and death of uraemia (Smadel & Farr, 1937).

The acute stages of NTN have been extensively investigated over the last 10 to 15 years (Fujimoto, 1980). The acute injury in this model is usually divided into two separate phases, the early heterologous and the late autologous phase (Unanue & Dixon, 1965). These two phases previously described in the introductory section are immune mediated. The autologous phase has been repeatedly shown to progress to chronic renal failure (Tomford, 1981) and more recently it has been demonstrated by Shimizu et al, 1974 that the heterologous phase, if severe enough, can do likewise. Therefore, this nephropathy which is immune mediated in its acute phase and progresses to uraemia is an ideal model to study and relate to acute immune mediated humal glomerulonephritides.

Although major advances have been achieved in the understanding of the acute stages of the disease, little is known on the factors involved in its progression through the chronic stages.

The knowledge, acquired in the past (Dixon, 1968), about the immune mechanisms involved in the pathogenesis of acute NTN has led to the acquisition of useful insights into the pathogenesis of human immune mediated nephritis.

The respective roles of antibody fixation on the basement membrane (Unanue & Dixon, 1965), the cellular infiltrates (Shigematsu, 1970), the complement (Cochrane, 1965) as well as the coagulation systems (Vassalli & McCluskey, 1964), in the genesis of the acute injury of this experimental nephropathy all led to speculation on their respective roles in the pathogenesis of human nephritis.

From this study, I hope that a similar impact can be achieved, through the understanding of chronic nephrotoxic nephritis, on the comprehension of chronic human glomerulonephritis.

In man, the study of the influences determining the progression of chronic renal failure, after an immune mediated injury, has been hampered by the lack of measurable parameters of the immune response that would correlate with the induction or the progression of these nephritis. Anti-glomerular basement membrane nephritis is an example that has been recently, extensively investigated (Rees, 1979). In this human model, nephritis is induced by the anti-GBM, auto-antibody, that fixes to the GBM and induces a severe, often crescentic nephritis, in many ways analogous to

nephrotoxic nephritis. The auto-immune response is primordial to the induction of the disease and correlates with the disease activity (Wilson & Dixon, 1973). The circulating anti-GBM antibodies can now be measured in patient's plasma and therefore their precise contribution to the acute and chronic phases of the disease process could be evaluated. Although their role during this acute phase is currently beyond any doubt (Wilson, 1981), their contribution to the progression of the nephropathy remains to be determined. Few examples of disease progression, well after the auto-antibody has been eliminated from the circulation, point to their secondary role during the chronic phase of the nephritis (Rees, 1979). Similarly, nephrotoxic nephritis, in rats, is characterised by the presence in the rat's serum of circulating autologous antibodies to the heterologous ones fixed on the basement membrane (Unanue & Dixon, 1965). The contribution of these antibodies to the development and progression of the nephritis will be crucial in the understanding of the nature of the chronic disease.

In order to establish this role, I had to, first, study and determine the natural course of nephrotoxic nephritis. Second, to design an in vitro test to measure and titrate the circulating autologous antibodies. Finally, I had to use a new rat transplantation model, to enable me to subtract the nephritic kidney, during the sub-acute phase, from its original immune environment and transplant it into a new host. This new host's immune response could be manipulated in such a way as to determine the influence of the autologous antibody on the progression of the nephritic process of the transplanted kidney.

(a) Natural history of chronic nephrotoxic nephritis (NTN)

It is important to understand the detailed course of nephrotoxic nephritis as our first step in studying the factors involved in its progression.

I would like to report the course of the accelerated variant of NTN in 9 Sprague-Dawley adult rats. It is important, to emphasise the observed inter-experiment and inter animal variations in the severity of NTN. These variations were not due to difference in rat strain, size, age or weight and could not be attributed to variation in the quantity or quality of the nephrotoxic antibody. However, the rats I report and describe in this experiment are fairly representative of the entire population studied. Nephritis was induced in these rats by the injection of 2mls of rabbit nephrotoxic serum (NTS). These rats were previously immunised, 5 days prior to the injection of the NTS, with 1mg of rabbit gamma globulin (RɣG) in complete Freund's adjuvant (CFA). Serial weekly measurements were made of rats weight, serum and urine creatinine, serum autologous rat anti-RɣG antibody levels as well as 24 hour urine protein excretion. Rat kidneys were biopsied at day 30 for morphological assessment and later at time of death.

Proteinuria

In these animals proteinuria developed immediately (2 to 4 hours) after the injection of the nephrotoxic serum. However the protein excretion increased progressively during the first 3 to 5 days, in order to peak at day 5 at a level of 120 $\pm$ 8mg/24 hours. After day 7, and as the mixed heterologous-autologous acute phase was subsiding the level of proteinuria decreased slightly and stabilised

at 118 ± 12mg/24 hours. This degree of proteinuria remained more or less unchanged up to day 30. At this stage, a third phase followed as proteinuria started to rise and increased significantly in most animals as they developed renal failure; it reached 350 ± 25mg on day 60 and 380 ± 15mg on day 75.

It seemed therefore, from the pattern of protein excretion, that the course of nephrotoxic nephritis could be divided into three separate phases; an acute phase over the first week, a sub-acute phase following and lasting to day 30 and finally a phase of accelerated proteinuria between days 30 and time of death. (Figure 7).

Renal function

Renal function declined in these rats during the days following the injection of the nephrotoxic serum; serum creatinine rose from 50 ± 6umol/l at the beginning of the experiment to 85 ± 12umol/l on day 5. A second phase followed the early acute one. During this sub-acute phase, the renal function progressively returned to near normal by the end of the first week and were maintained at this level up to day 30. The third and final phase followed, with an accelerated decline in renal function starting on day 30. On day 50, serum creatinine was increased to 125 ± 12umol/l and reached 350 ± 35umol/l on day 70.

From a renal function aspect, it is conceivable that the initial decline in renal function is due to the direct injury secondary to the fixation of the anti-GBM antibody and the subsequent inflammatory response (Shigematsu,1970). The following subacute-chronic phase

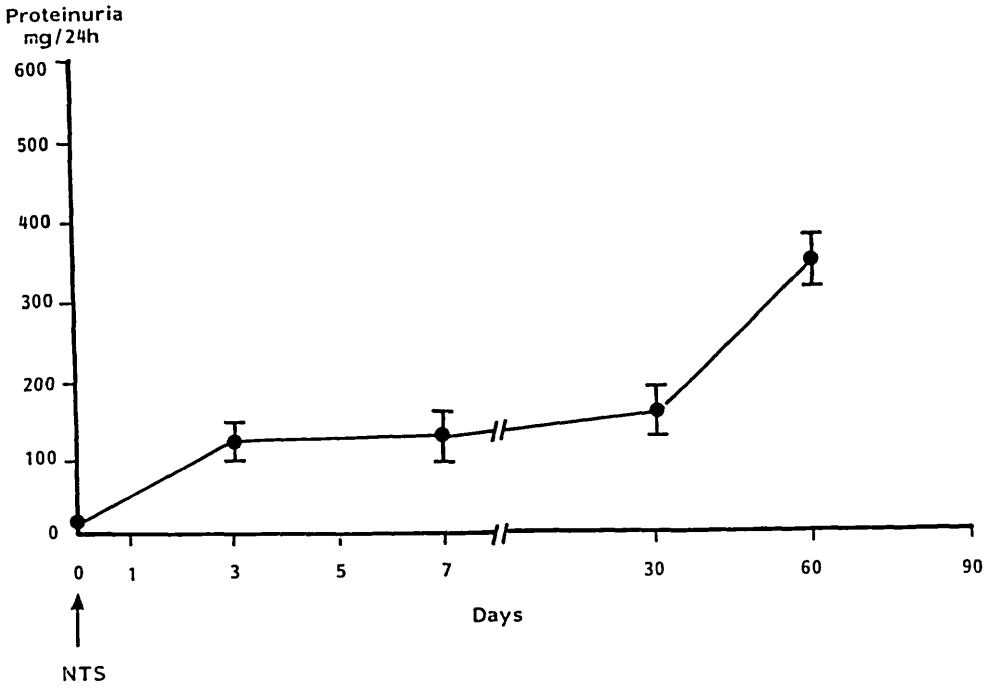


Figure 7 : Proteinuria in nephrotoxic nephritis

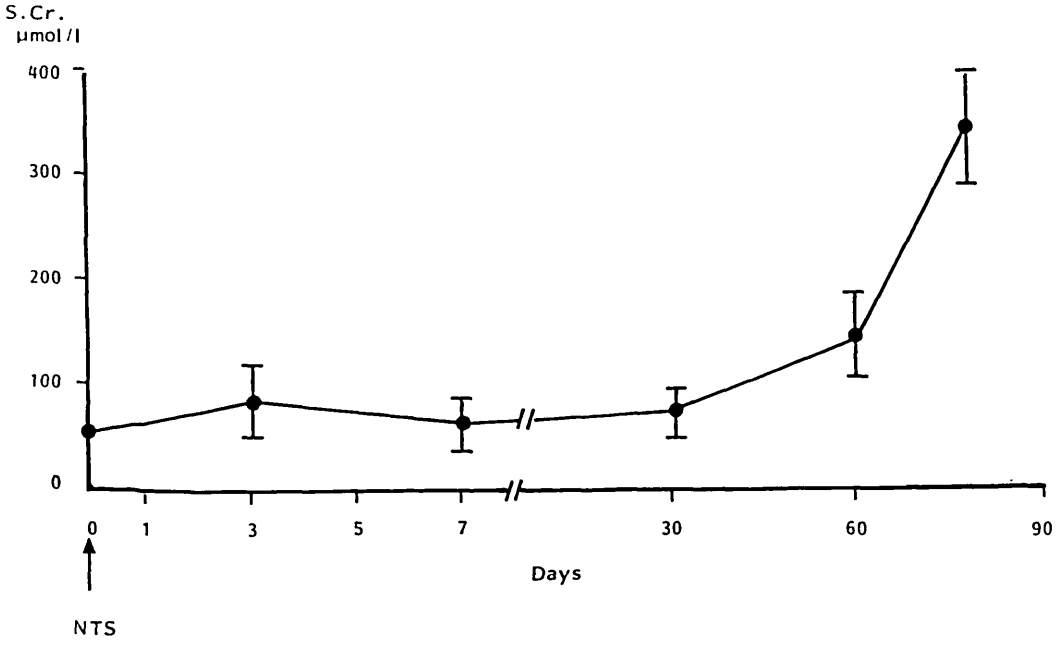


Figure 8 : Renal function in nephrotoxic nephritis

lasting for 21 days, with recovery from severe renal failure and their maintenance at a stable level, might represent the phase of compensatory changes leading to the renal failure (Brenner, 1982). The final phase is the one of end stage renal disease leading to death from uraemia. (Figure 8).

Autologous antibody response

Rat, anti rabbit gamma globulin, antibody response was evaluated using the solid phase radioimmunoassay described in the Materials and Methods section.

Within 5 days of the immunisation of the rats with rabbit gamma globulin (RγG), they developed detectable circulating antibodies to RγG (40 ± 15%). The autologous antibody levels increased progressively and peaked around days 45 to 60 at (65 ± 18%). A decline in these antibody titres followed. However, they remained raised until the rats died from renal failure. (Figure 9).

Survival

All animals survived the early acute phase and the following sub-acute one up to day 30. From day 30 on, there was a progressive decline in rat's survival with 8 out of 9 rats dying from renal failure by day 90. (Figure 10).

Histology

The renal morphology during the acute stage of nephrotoxic nephritis, days 3 to 7, showed a diffuse proliferative nephritis with a glomerular cellular infiltrate. These early changes are replaced by hyalinotic and sclerotic changes during the later changes.

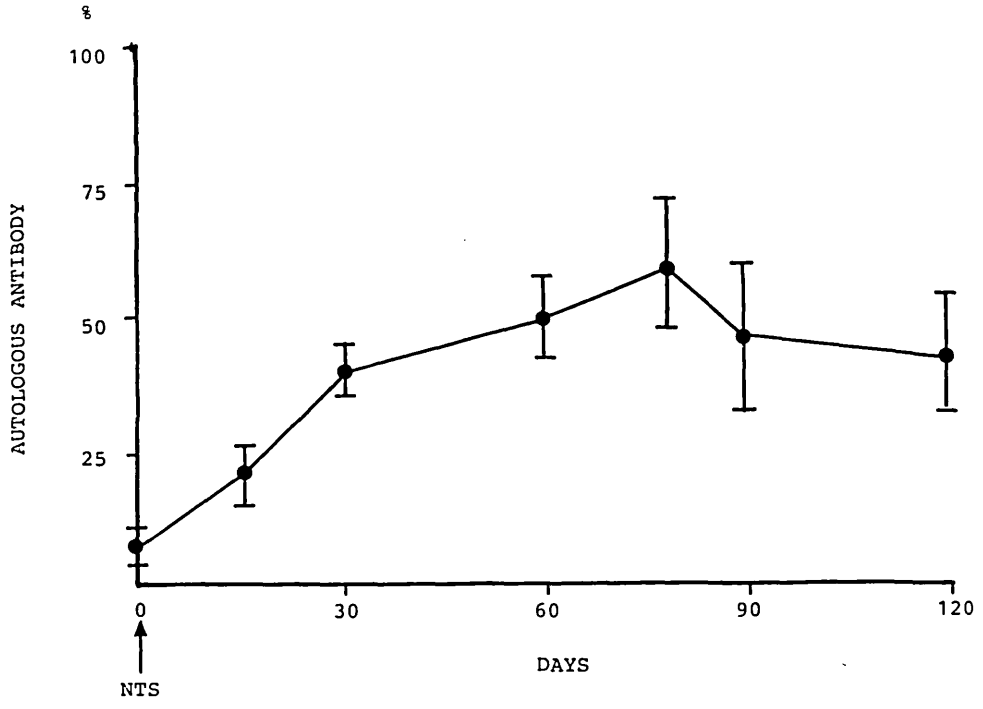


Figure 9 : The autologous antibody response in nephrotoxic nephritis

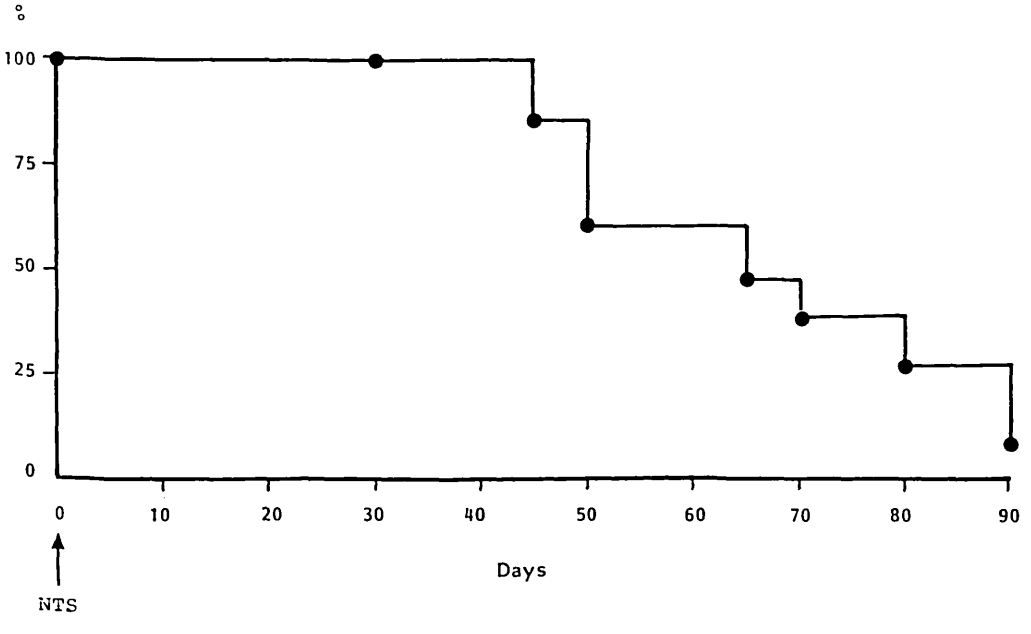


Figure 10 : Survival in nephrotoxic nephritis

On day 30, there was a mild focal and segmental sclerosing glomerulopathy. Less affected glomeruli showed thickening of the basement membranes and mesangial proliferation while the more severely affected ones showed different degrees of glomerular sclerosis. Tubular atrophy at this stage was variable, as well as interstitial cellular infiltrate, and reflected the severity of the adjacent glomerular damage. No renal calcification was detected.

At time of death, the renal morphological changes were invariably diffuse and severe. The majority of the glomeruli at this stage showed diffuse sclerosis with adjacent tubular atrophy and tubulo-interstitial calcifications (Figure 11) (Photos 1 to 6).

Immunofluorescence examination of renal sections on days 3, 30 and 90 showed the presence of both rabbit and rat gamma globulin (IgG) as well as rat C3 in a linear fashion. (Photos 7 & 8).

From these observations, I confirmed that nephrotoxic nephritis is a progressive renal disease similar to human immune mediated nephritis. I defined, on the basis of proteinuria and renal function, three different phases of the disease. I observed, like in some forms of human sub-acute nephritis (Schacht, 1976), that the progression from acute to chronic stages is characterised by the replacement of the early proliferative changes by late sclerotic ones.

For the first time in nephrotoxic nephritis the autologous immune response was quantitated; I noted an early and sustained increase in the level of circulating antibodies. It will be the aim of the

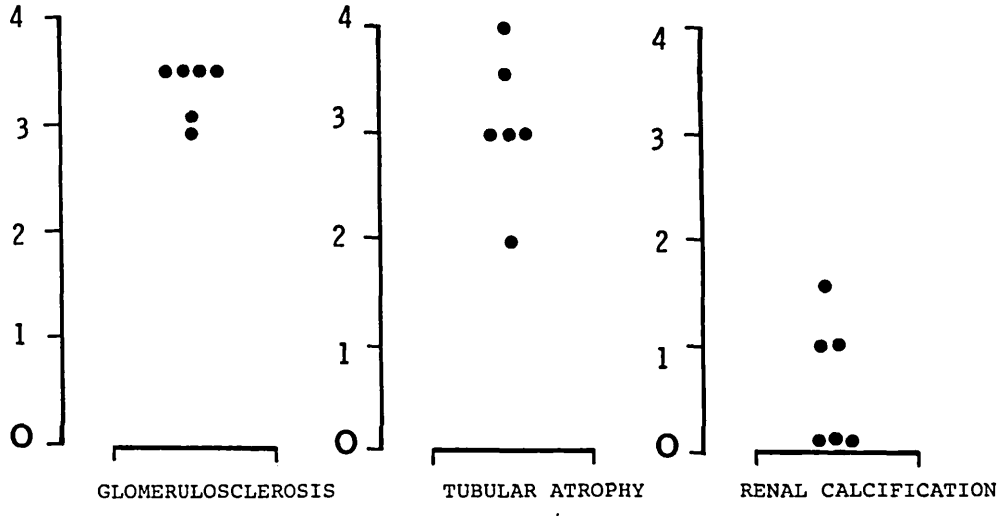


Figure 11 : Renal morphology changes at time of death from renal failure in nephrotoxic nephritis

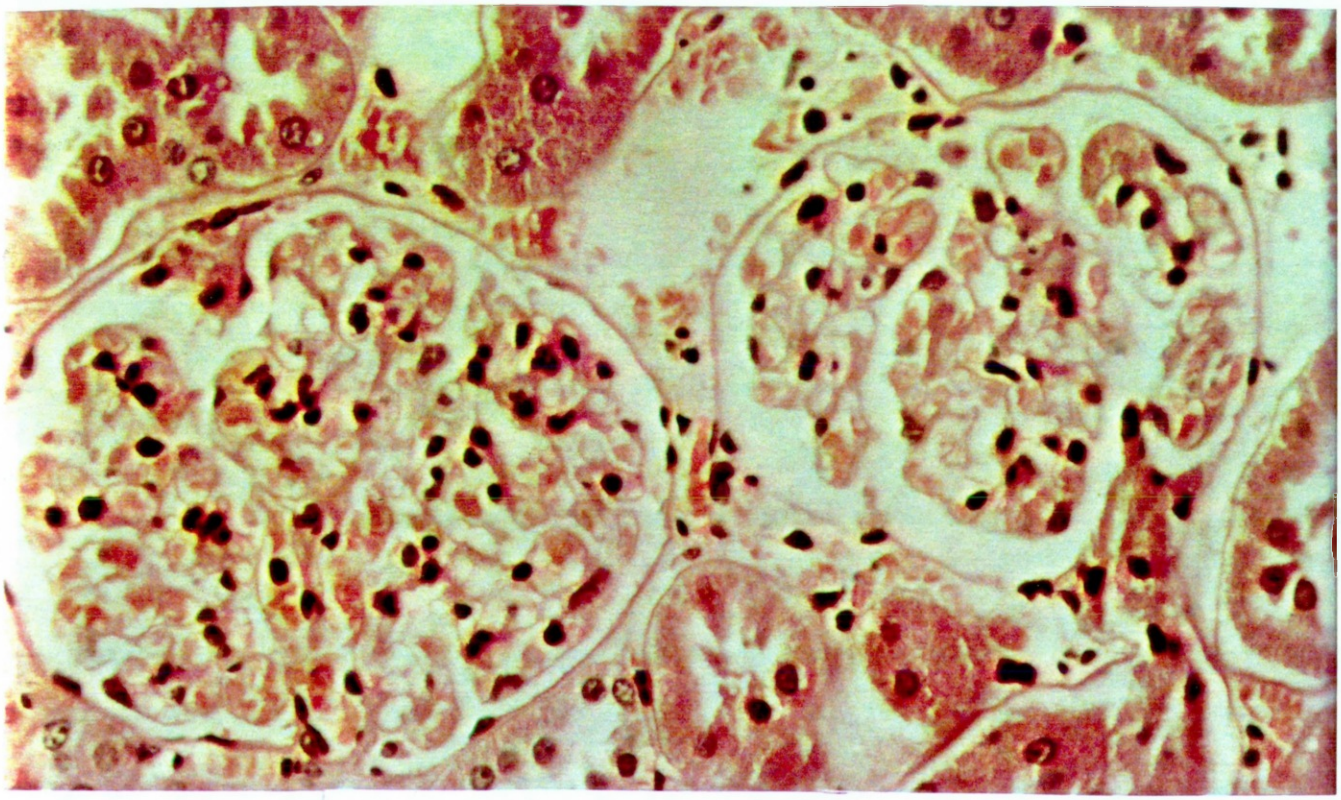


Photo 1 : Normal rat glomeruli (x25)

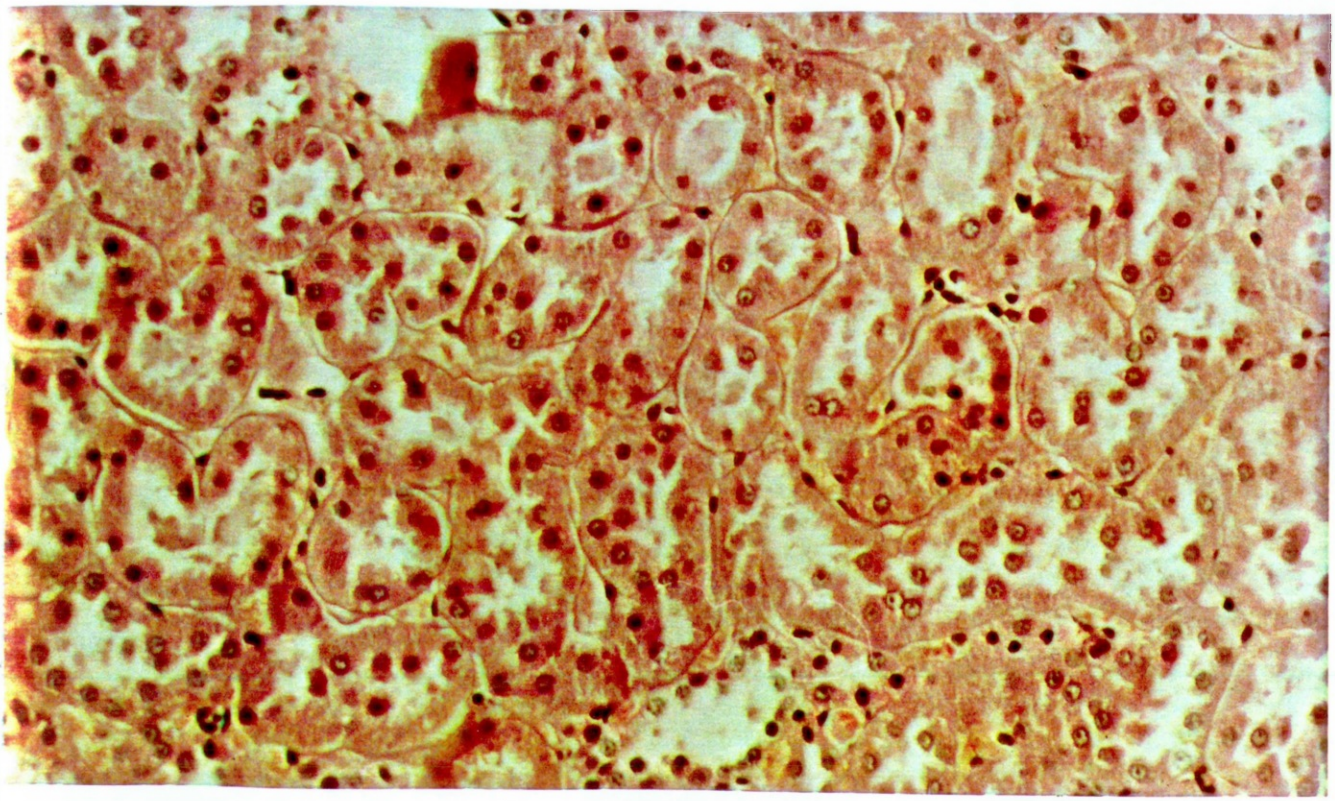


Photo 2 : Normal rat renal tubules (x10)

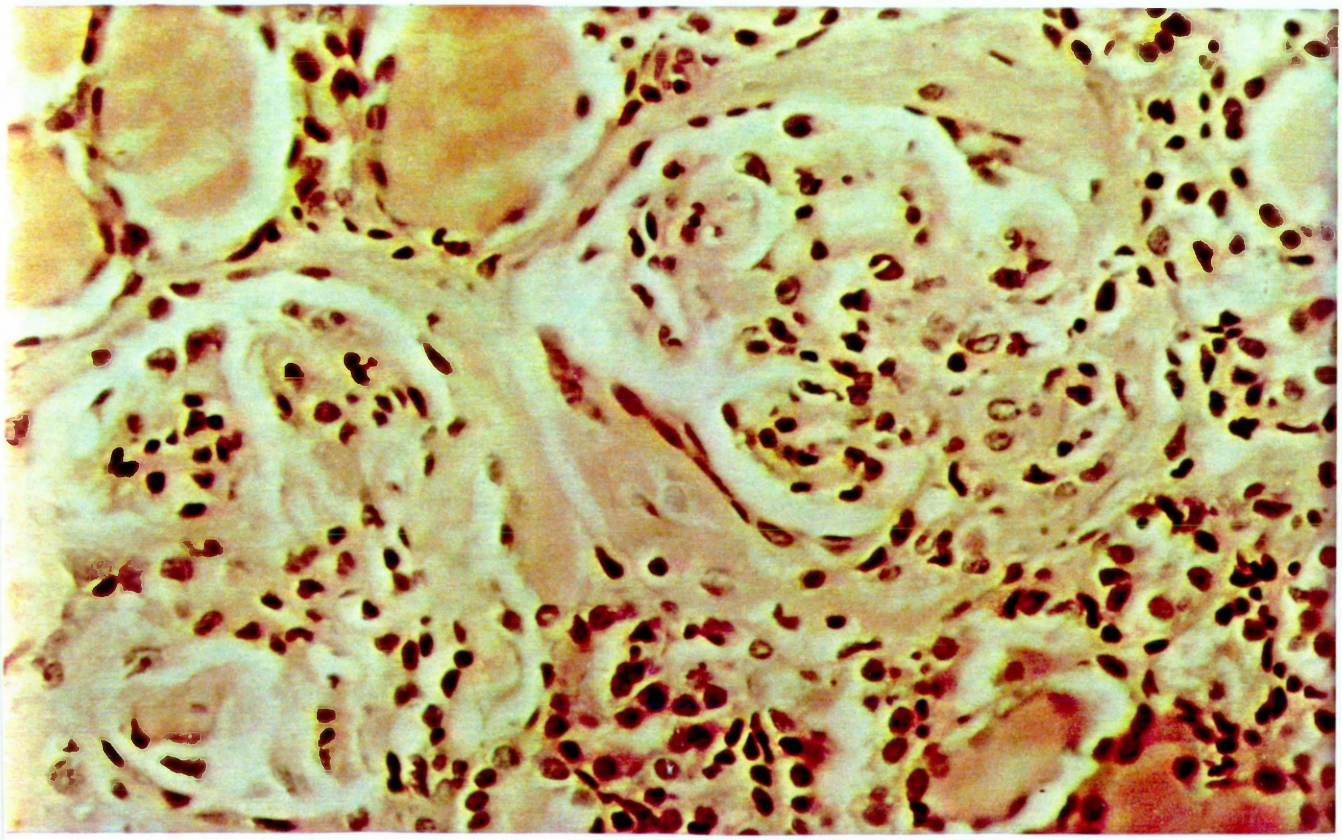


Photo 3 : Chronic nephrotoxic nephritis: Proliferative and sclerotic glomerular changes (x25)

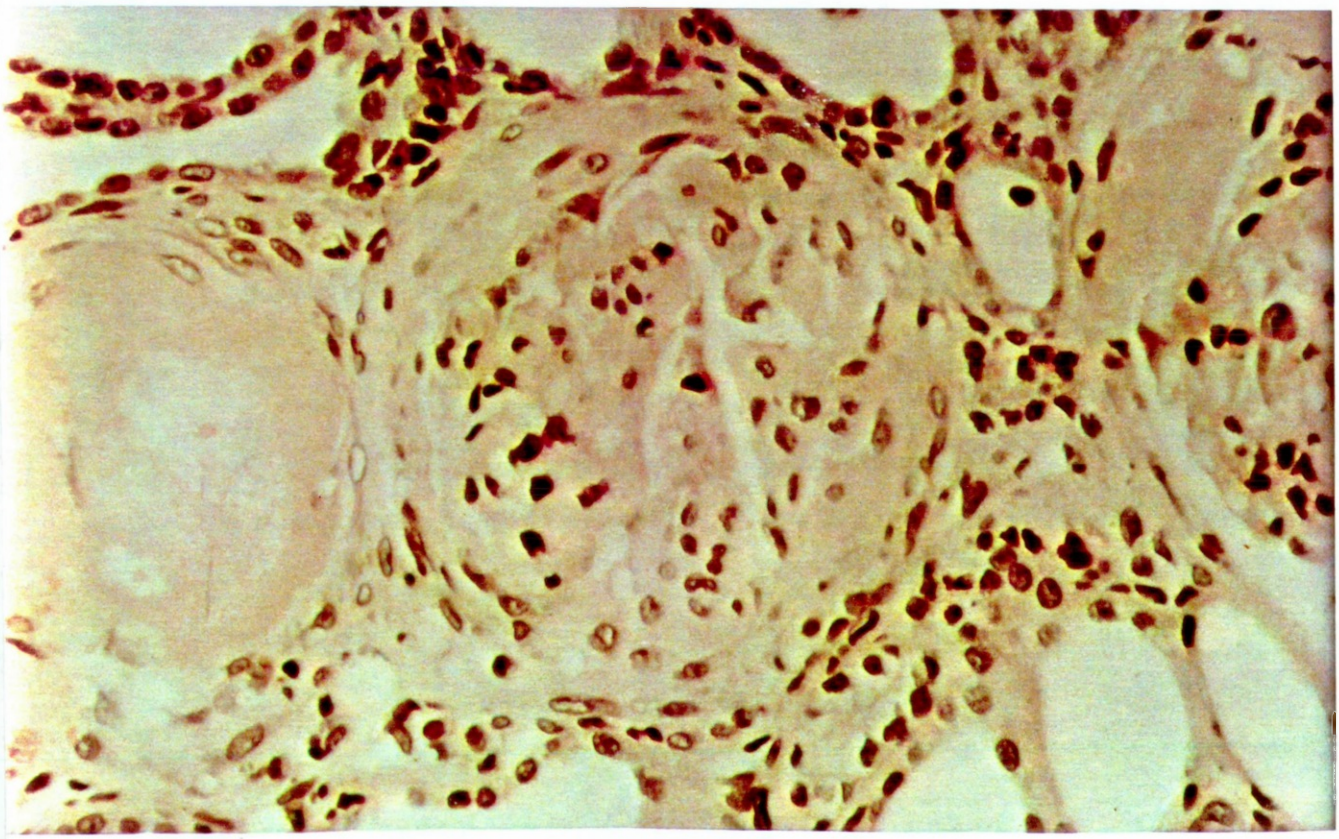


Photo 4 : Chronic nephrotoxic nephritis : Segmental and diffuse glomerulosclerosis (x25)

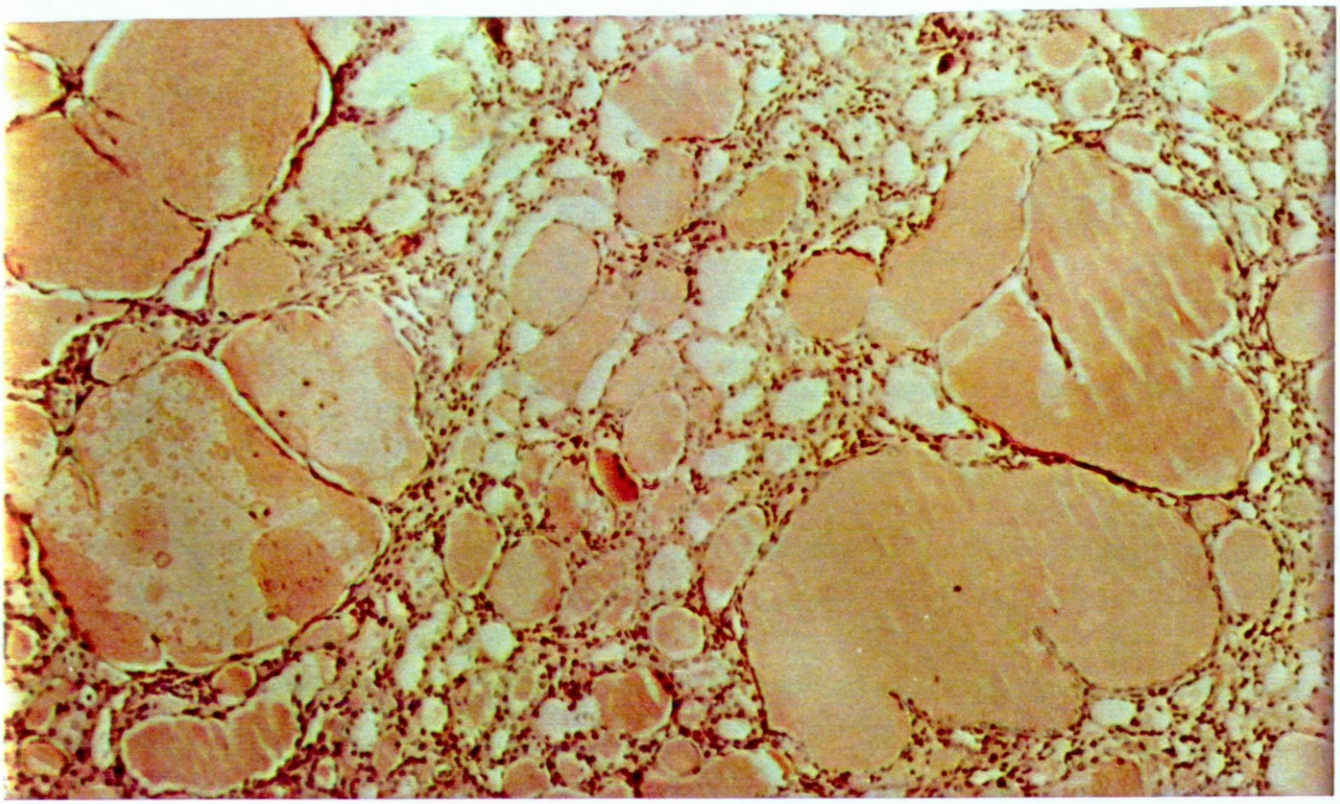


Photo 5 : Chronic nephrotoxic nephritis. Severe tubular dilatation and atrophy (x10)

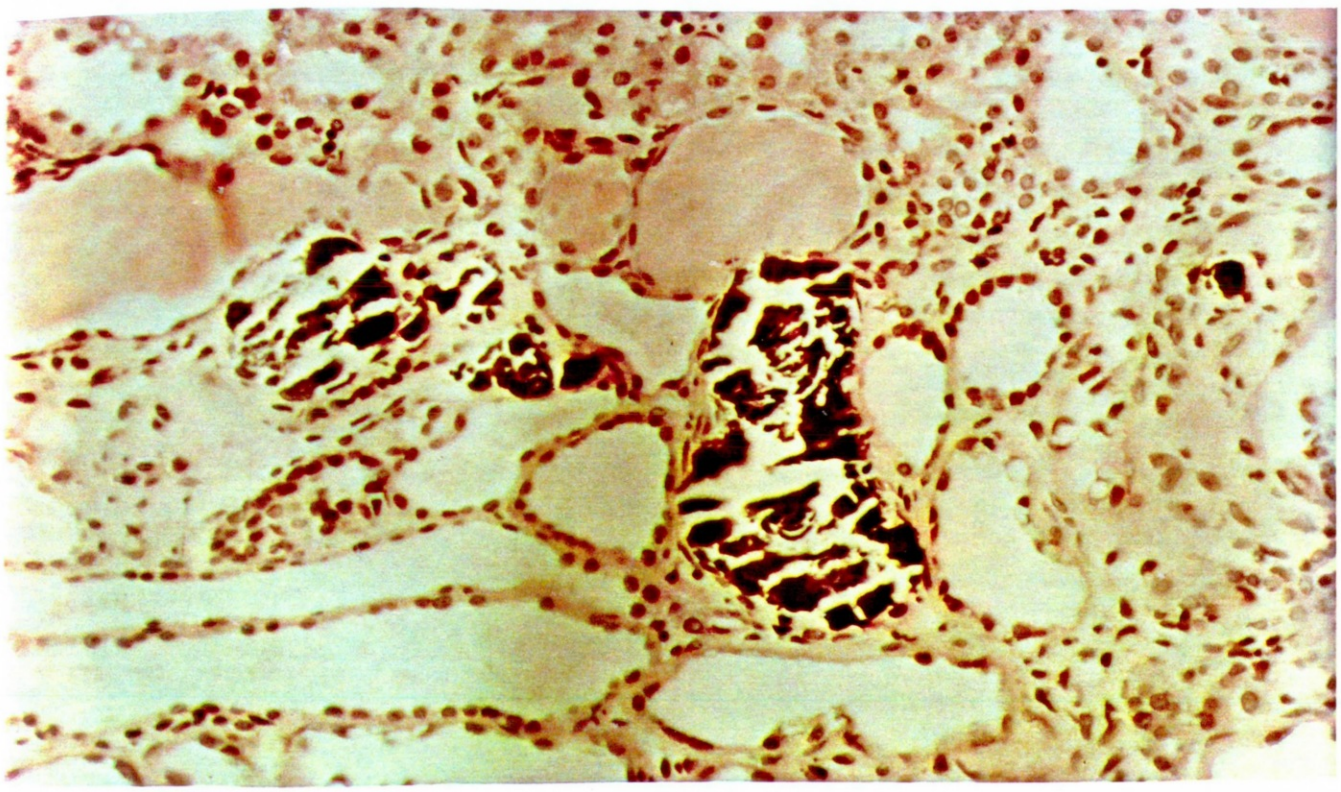


Photo 6 : Chronic nephrotoxic nephritis. Tubulo-interstitial infiltrates and calcifications (x10)

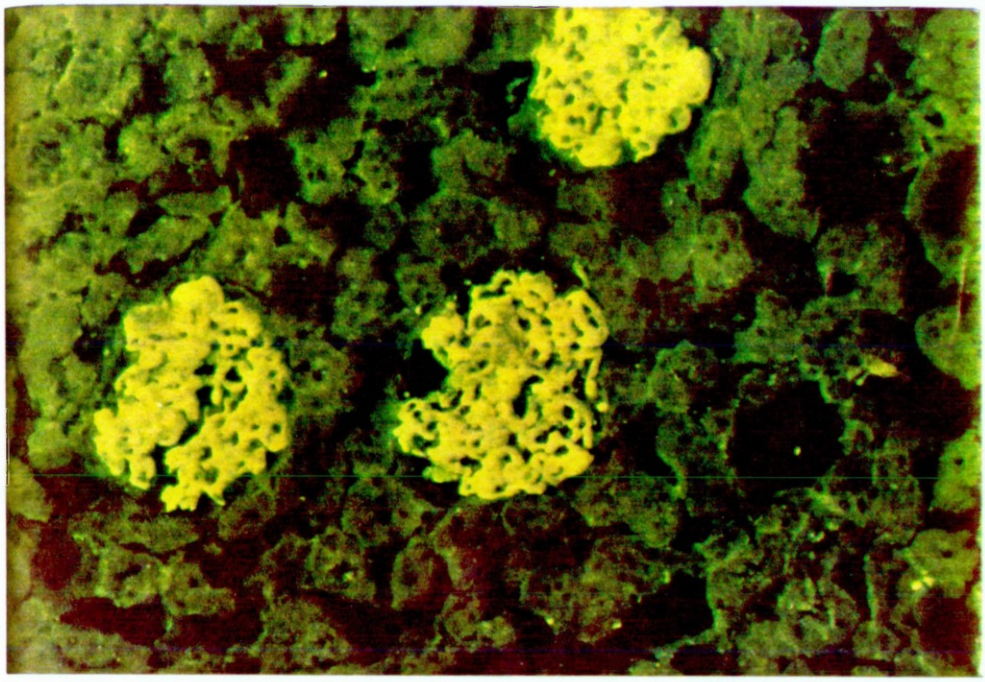


Photo 7 : Nephrotoxic nephritis - Deposition of rabbit heterologous IgG on the GBM of nephritic rats (x10)

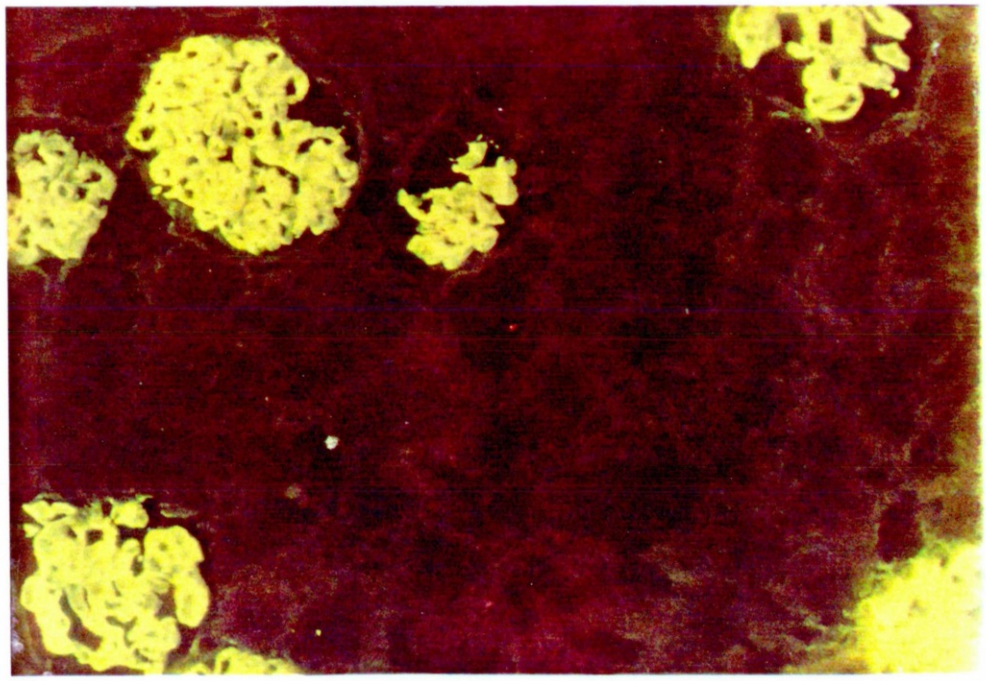


Photo 8 : Nephrotoxic nephritis - Deposition of rat's autologous IgG on the GBM of nephritic rats (x10)

following stage of my research to establish their role in the progression of the nephritis.

As previously stated, in order to establish the role of the immune autologous response in the progression of NTN, I designed a novel transplantation model whereby the course of single nephritic kidneys was compared. There it was essential to determine if single nephritic kidneys had a comparable course to the one I described above in the two kidney model.

(b) Effect of late unilateral nephrectomy on the course of NTN

Reduction in functional renal mass in man (Zuchelli, 1983) and in animals (Elema, 1971) is thought to accelerate the normal, ageing, process of decline in renal function.

These observations in man (Kiprov, 1982) and in animals (Shimamura & Morrison, 1975) were made when remnant kidneys were intact at the onset and when the host was young going through an active growth period (Kaufman, 1975). In man, Kiprov et al (1982) have recently reported a higher incidence of proteinuria, hypertension and renal functional impairment in patients with unilateral renal agenesis.

In rats, surgical reduction of renal mass accelerates the normally occurring glomerular sclerosis of ageing (Elema, 1971) as well as lesions of diabetic nephropathy (Steffes, 1978).

The course of nephrotoxic nephritis was not significantly affected by unilateral nephrectomy (UNx).

Rats weight

There was no significant difference in the rate of growth of nephritic rats with one and two kidneys. However growth was arrested in both groups in rats with severe chronic renal disease leading to end stage renal failure. The growth of nephritic rats was comparable to that of normal controls, until advanced renal failure developed.

Proteinuria

Normal non-nephritic rats had proteinuria levels not exceeding 20mg/24 hours. The rate of protein excretion did not vary significantly between normal rats with one and two kidneys.

First, in rats with NTN and two kidneys, the course of the nephritis was comparable to the one described in the previous experiment on the natural history of NTN. In these rats, acute proteinuria peaked between days 3 and 9 at 237 $\pm$ 50mg/24hrs. A comparable degree of proteinuria was observed in the nephritic experimental group at day 7, before uni-nephrectomy, 233 $\pm$ 55mg/24 hours. However in these rats following nephrectomy there was a significant increase in proteinuria, five days after the surgical procedure, 392 $\pm$ 66mg/24 hrs. In these animals, although the proteinuria remained higher than during the pre-nephrectomy stage, it decreased and by day 30 (232 $\pm$ 47mg/24 hours) it was no longer significantly different from the control nephritic group (298 $\pm$ 38mg/24 hrs).

It would seem therefore that unilateral nephrectomy after the acute phase of nephrotoxic nephritis, led to an increase in proteinuria; this increase although significant appeared short lived, lasting 2 to 3 weeks and subsided. (Figure 12)

The increase in proteinuria of nephritic rats after unilateral nephrectomy implied, to us, that nephritic kidneys were capable of responding to reduction in renal mass by hyperperfusion and hyperfiltration. Similarly, it meant that these kidneys might not have been hyperfiltrating or not maximally so at the time of nephrectomy during the sub-acute phase of the nephritis.

Renal function

Serum creatinine followed a comparable course in the two experimental groups; serum creatinine was increased by day 3 to 7 following the injection of the nephrotoxic serum, it remained elevated, although stable up to day 21 and started to increase in individual rats thereafter as renal function deteriorated and most animals died from renal failure by day 70. At day 45, the serum creatinine levels were 126 $\pm$ 16umol/l in two kidney nephritic rats compared to 167 $\pm$ 61umol/l in the nephrectomised nephritic ones; this difference was not statistically significant. (Figure 13)

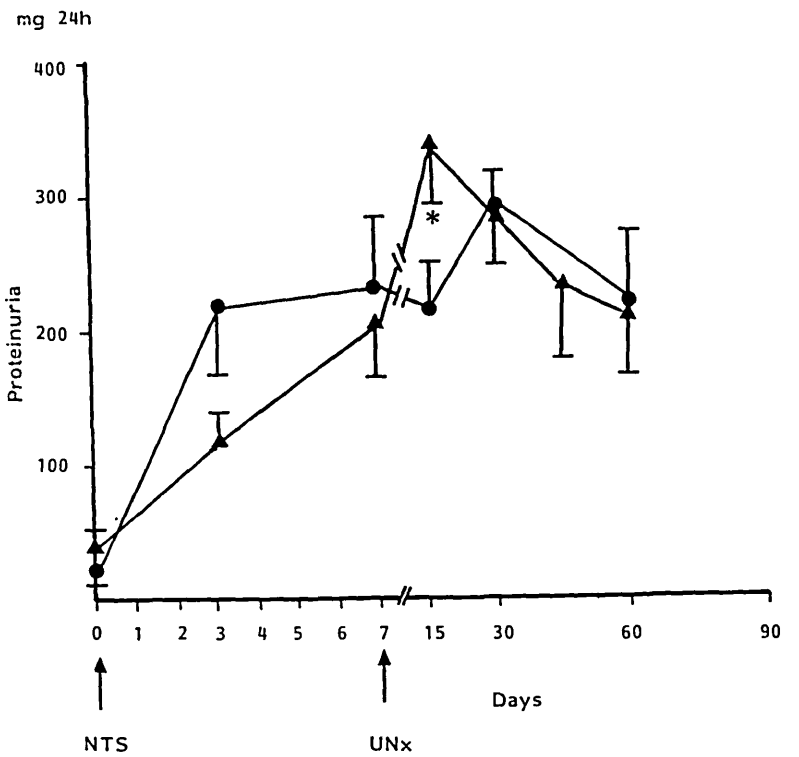


Figure 12 : Nephrotoxic nephritis - The effect of unilateral nephrectomy (UNx) on the course of proteinuria

(● — ●) 2 kidney NTN, (▲ — ▲) 1 kidney NTN*($p < 0,05$)

Survival

By day 60, 3 out of 5 rats with 2 kidneys and nephritis died from renal failure comparable to 3 out of 6 with unilateral nephrectomy and nephritis. No death was observed in normal, non-nephritic, controls. The experiment was ended on day 70, when the majority of rats died from renal failure. (Figure 14).

Renal morphology

Kidney weight was increased at time of death in the unilateral nephrectomised, nephritic, rats (3.20 $\pm$ .65 grams) compared to (2.02 $\pm$ .75 grams) in the nephritic controls. This difference was statistically significant, $p < 0.05$. Similarly, there was an increase in the kidney weight of normal uni-nephrectomised rats (3.6 $\pm$ .72 grams) compared to (1.35 $\pm$ .15 grams) in control animals, $p < 0.025$.

In nephritic animals, the renal histology showed no significant changes between the two nephritic groups; the glomerulosclerosis score, at time of death, was 3.35 $\pm$ .72 for rats with two nephritic kidneys and 3.33 $\pm$ .49 in the one kidney nephritic group. Changes in tubular atrophy and interstitial infiltrate were proportional to the degree of glomerulosclerosis.

From the data on the kidneys weight, it appears that there was an increase in the size of nephritic kidneys (Δ = 60%) after unilateral nephrectomy, although this increase was not as important as the one observed with normal, not diseased kidneys (Δ = 166%). Meanwhile, in nephritic rats the reduction in renal mass with the subsequent increase in kidney size did not affect adversely the underlying course of the nephritis.

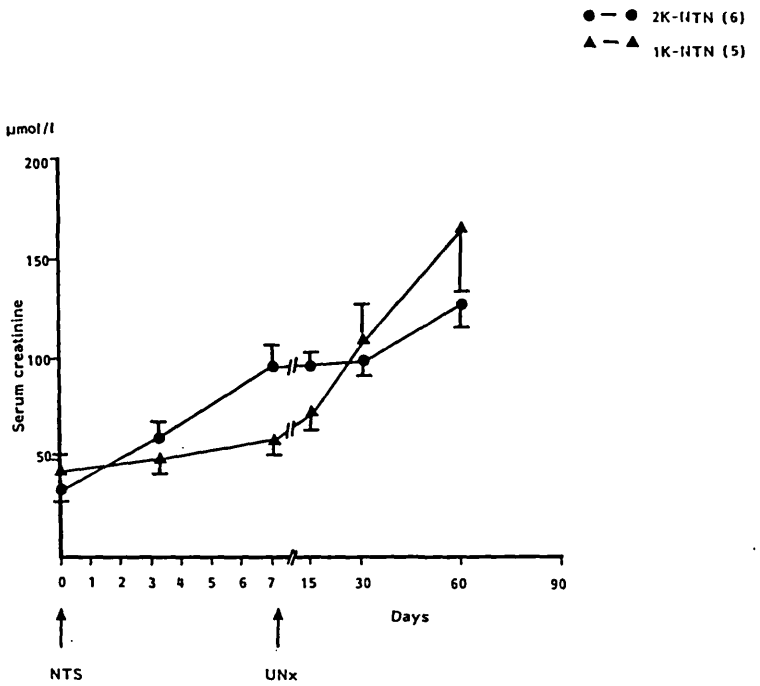


Figure 13 : Nephrotoxic nephritis - The effect of unilateral nephrectomy (UNx) on renal function

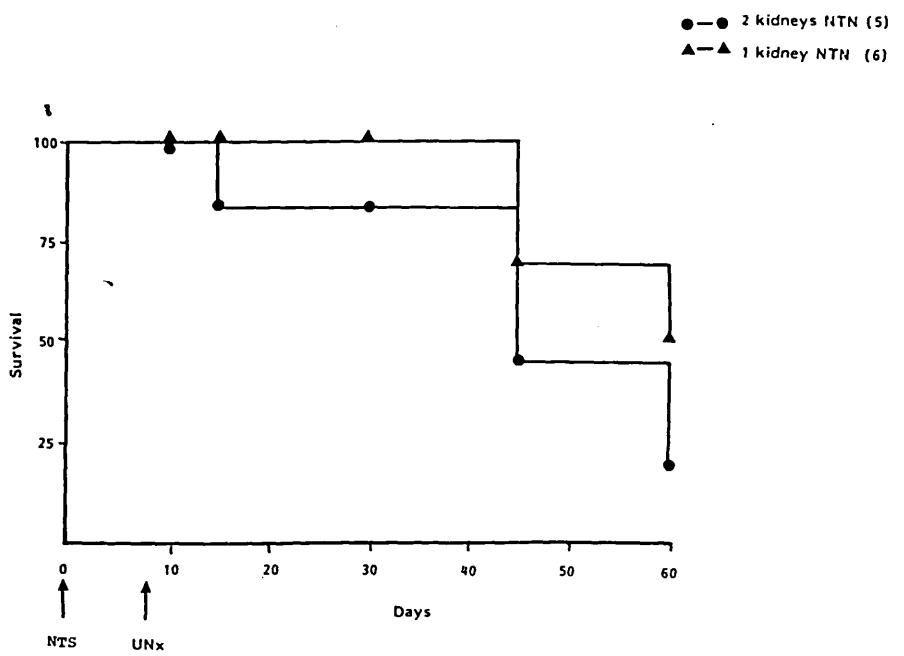


Figure 14 : Nephrotoxic nephritis - The effect of unilateral nephrectomy (UNx) on survival

From these data, I concluded that unilateral nephrectomy had no significant effect on the course of severe nephrotoxic nephritis in rats; the rapid decline in renal function following the injection of the nephrotoxic serum was not further accelerated by surgical reduction in renal mass. These results allowed me to proceed in my experimental process, transplanting nephritic kidneys and studying the progression of nephritis in individual kidneys, knowing that the course of the disease, in this model, was comparable in rats with one or two kidneys.

Equally important, the absence of harmful effects of nephrectomy in this model might imply that hyperperfusion and hyperfiltration was not a major feature of sub-acute NTN; in other models where these haemodynamic changes are known to occur further reduction in renal mass accelerated the rate of glomerular scarring (Steffes, 1978).

(c) Nephrotoxic nephritis - The role of immune environment in the progression of the disease

In preliminary studies I established that the course of NTN was not significantly affected by transplantation; The disease progressed in nephritic kidneys after transplantation into new hosts (Table 4).

proteinuria after transplantation decreased temporarily while the recipient's contralateral kidney was still present and reappeared unchanged, at its pretransplant level, when the contralateral kidney was removed and the rat left with a single nephritic kidney.

Renal function continued to decline after transplantation, providing the acute nephritis, in the original host, was severe enough.

In order to determine the role of the continuous autologous immune response in the progression of the nephritis, I designed the following experimental protocol:

Nephrotoxic nephritis was induced in 13 Lewis rats according to the telescoped model. The acute nephritis was allowed to progress beyond the acute and sub-acute stages. At day 30, the nephritic rats were bilaterally nephrectomised and both kidneys were transplanted into syngeneic recipients. The recipient rats were divided into two groups. The first pre-immunised against rabbit gamma globulin and recreating the donor, original, immune environment. The second, virgin with no circulating autologous antibodies at time of renal transplantation. (Figure 15)

	5 - 10 days		Day 30			Day 60		Day 90		Day 120	
	Creat.*	Protein *	Creat.*	Protein *		Creat.*	Protein *	Creat.*	Protein *	Creat.*	Protein *
Group I NTN	66 ± 5	112 ± 3.8	56 ± 4	153 ± 6		66 ± 2.3	148 ± 49	96 ± 13	166 ± 27	196 ± 76	183 ± 92
Survival	3/3		3/3			3/3		3/3		1/3	
Group II NTN + Tx	68 ± 10	105 ± 39	68 ± 50	119 ± 68	IIa	110 ± 67	168 ± 93	168 ± 104	161 ± 93	170 ± 91	203 ± 77
					IIb	121 ± 74	163 ± 80	174 ± 100	159 ± 100	179 ± 84	217 ± 108
Survival	13/13		13/13			8/8 8/8		6/8 6/8		3/8 3/8	
Group III Tx			46 ± 8	16 ± 8		57 ± 8	20 ± 14	61 ± 4	23 ± 4	60 ± 10	20 ± 6
Survival			4/4			4/4		4/4		4/4	

IIa = pre-immunized recipients, IIb = naive recipients

*Creat. = Serum creatinine $\mu mol/l^{-1}$, * Protein = proteinuria $mg/24h^{-1}$
(results expressed as Mean ± Standard deviation

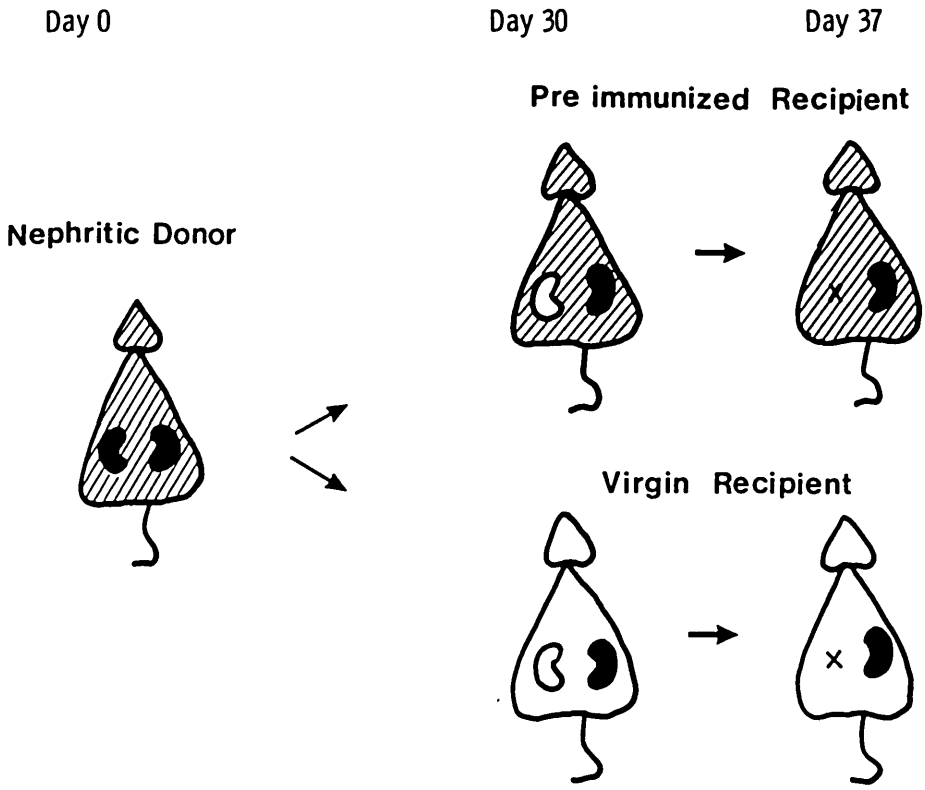


Figure 15 ; Experimental protocol,

Role of autologous antibody levels on the progression of chronic nephrotoxic nephritis

= Normal kidney

= Nephritic kidney

X = Nephrectomy

Right and left kidneys were transplanted from 13 nephritic rats on day 30. Right and left kidneys were equally distributed between the two recipient groups.

5 recipient rats died during the transplantation procedures; 4 from uncontrolled bleeding from transplant biopsies - a procedure then discontinued - and 1 from primary graft non function.

The course of 8 pairs of nephritic kidneys was studied in the two sets of recipients with one kidney transplanted into the pre-immunised host and the contralateral to the naive host. The recipient's contralateral, right, kidney was removed one week after transplantation of the nephritic kidney, leaving the recipient with one nephritic kidney only.

Six pairs of kidneys were followed until rats died from renal failure or were killed at the end of the experiment, 150 days after the induction of the nephritis. 2 rats died without renal failure; one on day 55 during an anaesthetic for blood sampling and the second from infection on day 60. At time of death the serum creatinine of these two rats were 80 and 70mol/l respectively.

Proteinuria

At time of transplantation, the donor rats proteinuria was 120 $\pm$ 12mg/24 hours. Following transplantation of the nephritic kidneys, there was a marked and significant fall in protein excretion (38 $\pm$ 4mg/24 hours). This decrease in proteinuria remained unchanged for 7 days up to the time of the nephrectomy

of recipient's own right kidney. At this stage, and following the recipient's right nephrectomy, the proteinuria rose sharply to a level comparable to the donor rats, pre-transplant, proteinuria (130 $\pm$ 16mg/24 hours). During the period following transplantation there was a progressive increase in proteinuria, concomittant with the simultaneous decline in renal function of the transplanted kidneys. (Figure 16).

The degree of proteinuria was remarkably comparable between right and left kidneys from individual donors in their new hosts ($r = 0,938$, $p < 0,001$). (Figure 17).

It seemed thatefore that the course of proteinuria in paired transplanted kidneys was identical regardless of the underlying humoral immune status of the host.

Renal function

In transplanted nephritic recipients, there was a considerable variation in the rate at which renal failure developed. 11 out of 14 rats died from renal failure within 90 days of transplantation (120 days from the induction of the NTN). Despite this heterogenous course of transplanted nephritic kidneys, the course of pairs of kidneys from the same donor was almost identical ($r = 0,962$, $p < 0,001$). (Figure 18).

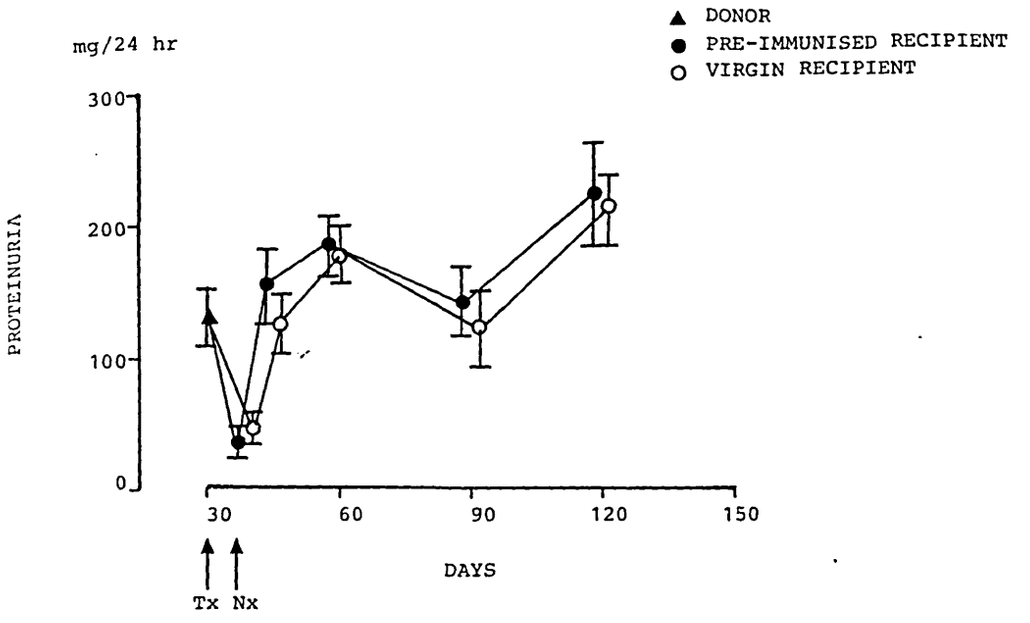


Figure 16 : Course of proteinuria after transplantation (Tx) of paired nephritic kidneys into separate recipients

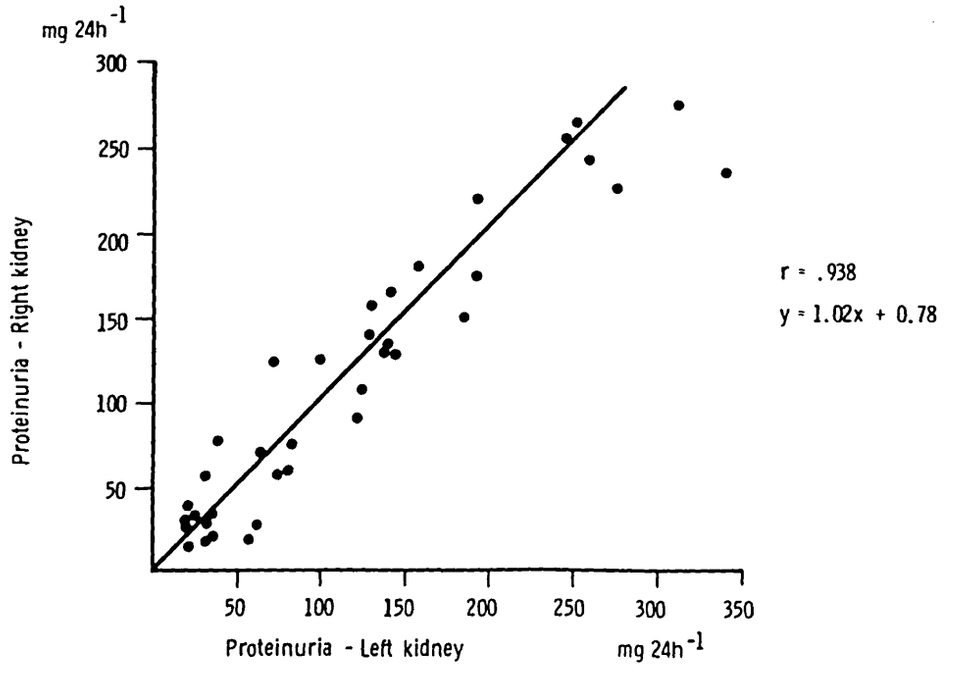


Figure 17 : Correlation between the proteinuria of transplanted paired right and left kidneys

Autologous antibody response

All donor rats had high titres of anti-rabbit globulin antibodies immediately before transplantation (66 ± 3%). The level of antibody response in these rats was similar to that of pre-immunised recipients at the same time (71 ± 6%). None of the naive recipients had detectable antibodies before transplantation (21 ± 4%) (normal, background range = up to 31%). After transplantation with nephritic kidneys, carrying rabbit gamma globulin, there was no appreciable change in the level of circulating antibodies in the pre-immunised group. However, low titres were detected (30 ± 5%) in naive recipients. Throughout the course of the nephritis the antibody titres in pre-immunised rats were considerably higher than in the naive recipients ($p < 0,025$). (Figure 19).

These data of highly significant difference in circulating autologous antibody titres confirm the success of this approach in creating different immune environments to study the effects of the autologous immune response on the progression of nephrotoxic nephritis.

Survival

11 out of 14 rats died during the course of the experiment from renal failure as judged by the level of serum creatinine and the severity of the renal morphological changes. A strong correlation was observed between the time of death for recipients of right and left kidneys from the same donor ($r = 0,968$). All paired recipients died within 7 to 10 days of each other. (Figure 20)

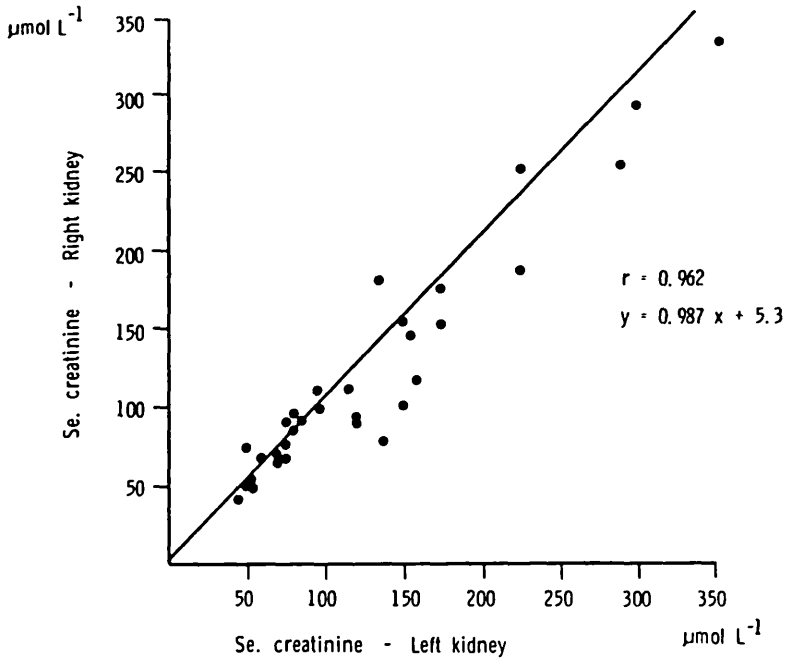


Figure 18 : Correlation between the renal function of transplanted paired right and left kidneys

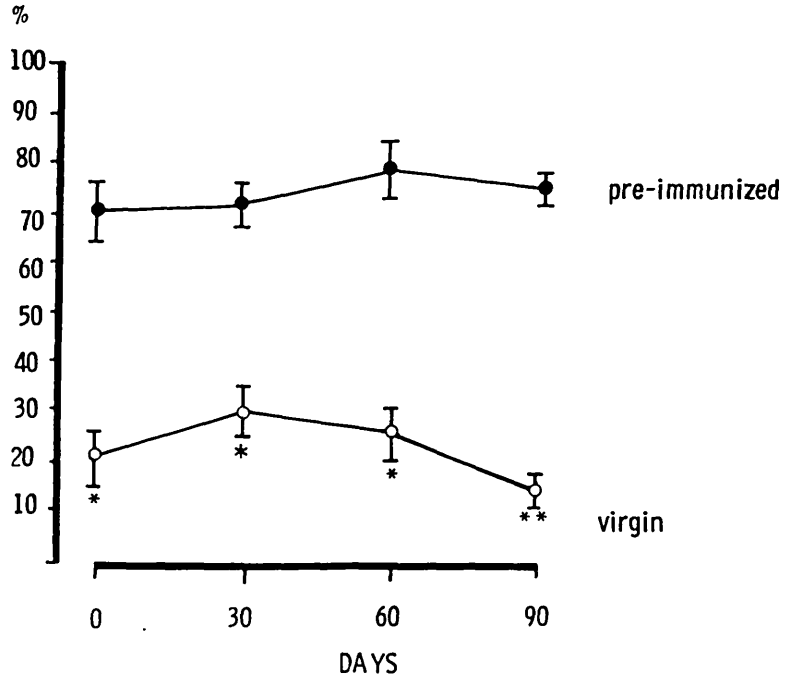


Figure 19 : Titre of circulating autologous antibody levels in recipients of nephritic kidneys

* $p < 0.05$, ** $p < 0.025$

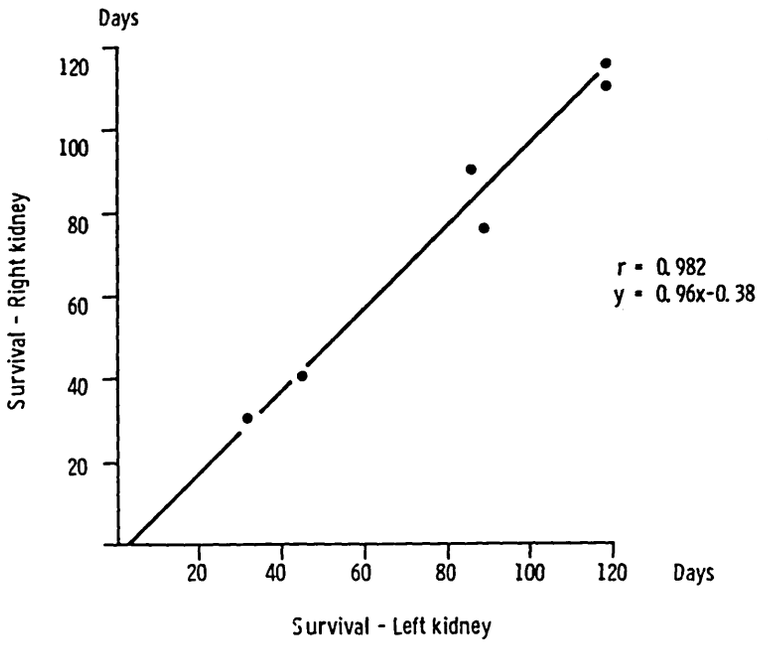


Figure 20 : Correlation between the survival of transplanted paired right and left kidneys. ($p < 0.001$)

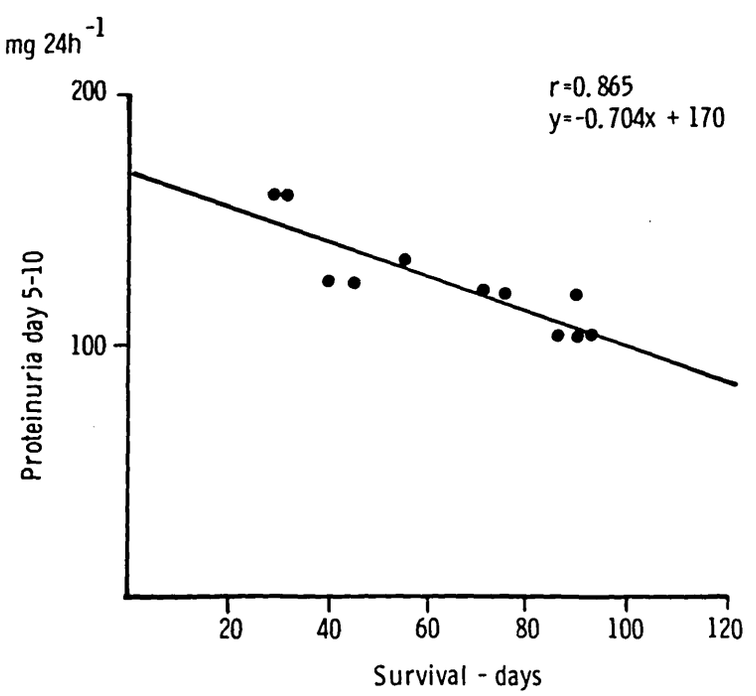


Figure 21 : Correlation between acute phase proteinuria of nephrotoxic nephritis and the survival of transplanted nephritic kidneys. ($p < 0.001$)

Renal morphology

At time of death, kidneys showed severe scarring with diffuse glomerulosclerosis, tubular atrophy and interstitial infiltrate as well as calcifications. Strong correlations were observed for glomerulosclerosis ($r = 0,91$) and tubular atrophy ($r = 0,83$) between pairs of nephritic kidney. No difference was observed at immunofluorescence in the amount of rat or rabbit gamma globulin deposited on the GBM between paired kidneys.

All these results imply that, in nephrotoxic nephritis, the progression of the disease occurs independently of the extent of the host's autologous immune response. Nephritis in paired kidneys progressed at the same rate regardless of the level of the circulating antibody titres.

In order to determine the factors influencing progression in this model, I studied the effect of severity of the acute injury and the residual sub-acute damage, at the time of transplantation, on the long course of the nephritis. There was no correlation between serum creatinine level during the acute injury phase of the nephritis and renal transplant survival. However, there was a strong correlation between the severity of early (days 5 to 10) proteinuria and transplant survival ($r = 0,88$, $p < 0.001$), but the shallow shape of this line (0,342) gives the measurement little predictive value (Figure 21). In contrast, there was an exceptionally strong correlation between transplant survival and late proteinuria (day 30) ($r = 0,966$, $p < 0,001$) as well as the graft survival and late (day 30) reciprocal of serum creatinine ($r = 0,952$, $p < 0,001$), (Figure 22,23).

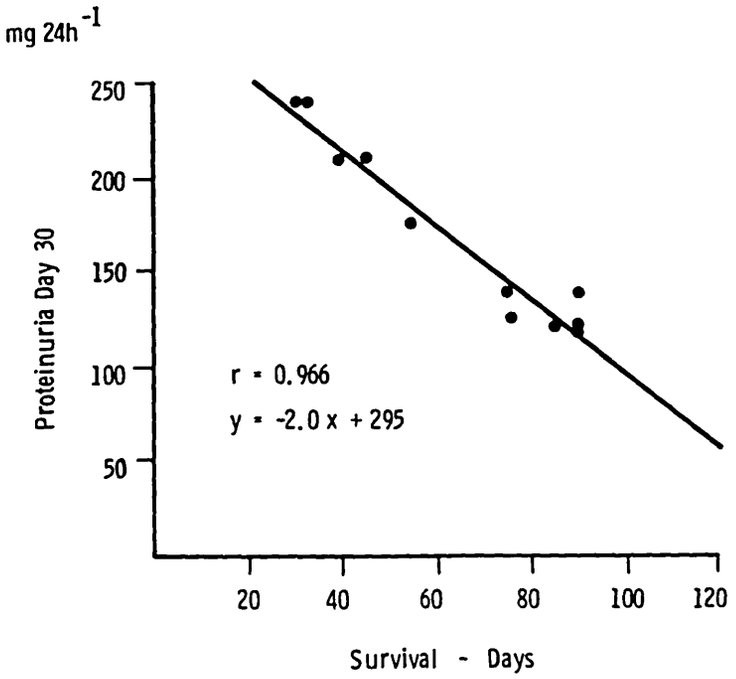


Figure 22 : Correlation between proteinuria on day 30 and the survival of transplanted nephritic kidneys ($p < 0.001$)

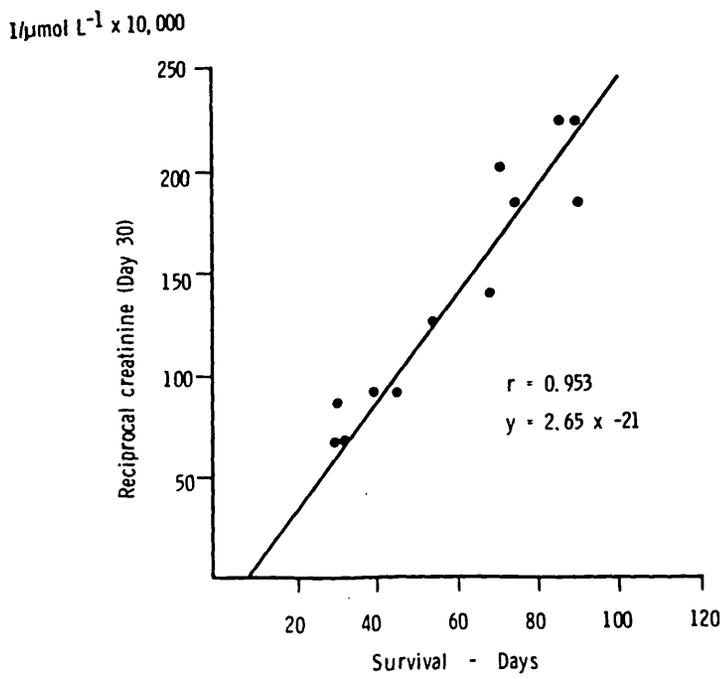


Figure 23 : Correlation between renal function on day 30 and the survival of transplanted nephritic kidneys ($p < 0.001$)

These results confirm that persistently high titres of autologous anti rabbit antibodies do not influence the development of chronic renal failure after nephrotoxic nephritis in rats; as 90% of the variation in nephritic kidneys survival was predetermined at time of the transplantation. Acute and sub-acute injury or compensatory changes determined to a major extent the prognosis of the nephritis in this model.

Discussion

As the course of nephrotoxic nephritis varies significantly between individual animals within the same experimental group, the study of factors affecting the long term course of the nephritis can be made extremely difficult to interpret. Transplanting pairs of nephritic kidneys from the same donor into separate syngeneic recipients potentially provides a method for controlled studies of glomerular scarring.

I have demonstrated the effectiveness of the double transplant approach and using it I showed that in contrast to the acute injury (Unanue & Dixon, 1965) the titre of autologous antibodies has little influence on progressive glomerular scarring in nephrotoxic nephritis.

These experiments have shown that the rate of renal scarring after NTN of single nephritic kidneys in syngeneic recipients followed a course comparable to that of intact nephritis animals. Furthermore, right and left kidneys from the same donors in different recipients behaved in an identical fashion. Each of eight pairs of rat's kidneys had the same course and those dying from renal failure did so within 10 days of each other. The close correlation between renal function

of paired kidneys demonstrate that this model provides an exceptionally good method of performing controlled studies on the development of glomerulosclerosis after nephritis.

This experiment also provides an ideal approach to assess the role of the immune environment in the progression of this immune mediated nephritis. In the telescoped model of NTN, acute injury is caused by the interaction on the GBM between the heterologous and circulating autologous antibodies (Unanue & Dixon, 1965). The acute proliferative inflammatory nephritis subsides within seven days of its onset and progressive sclerotic changes slowly develop (Fujimoto, 1980) leading to chronic renal failure within the ensuing 30 to 45 days. From these data I determined that progression of this chronic phase into end stage renal failure was not influenced by the titre of circulating autologous antibodies. Nevertheless I cannot rule out a permissive effect of these antibodies, as all naive recipients developed low titres of antibodies after transplantation. Nor can I exclude a contribution of the in situ immune interaction on the progressive renal scarring (Couser & Salant, 1980).

However, in this model, the major influence on the rate of glomerular scarring was the extent of injury to donor kidneys by the time of transplantation. This was indicated by close correlations between donor proteinuria and reciprocal of serum creatinine, with the long term functional survival of nephritic kidneys.

Presently, I can only speculate about the reasons why late proteinuria provides such a good guide to prognosis. It has been suggested that proteinuria per se is nephrotoxic in rats (Davis, et al 1978). More likely it would seem that the late proteinuria

could be the reflection of compensatory hyperperfusion and hyperfiltration of the remnant glomeruli (Brenner, 1982).

Irrespective of the mechanisms involved there are striking similarities between my results and the data on the development of chronic renal failure in patients treated for crescentic nephritis. In these patients, renal function during the acute phase injury provides a poor guide to prognosis. Whereas the late, sub-acute levels of renal function, immediately after treatment was found to be a much better indicator of the longterm outcome of the nephritis (Haworth, 1982).

Having established that humoral immunity is of secondary importance in the progression of nephrotoxic nephritis and that proteinuria is an important prognostic factor in this disease, I designed the next phase of my research programme.

In this phase I studied the influence of non-immune interventions on the course of the disease. I attempted to manipulate the extent of early and late proteinuria and observed the influence such changes in protein excretion had on the natural history of nephrotoxic nephritis.

In general, the second stage of the research concentrated on the effects of dietary interventions on the course of experimental chronic renal failure in rats, starting with the effects of a low protein diet on the course of the sub-total nephrectomy model of uraemia.

II. Effects of dietary manipulations on the progression of chronic renal disease

(a) Sub-total nephrectomy model

Dietary protein

Introduction :

It has been postulated that the progression of non-immune mediated nephropathies was the direct consequence of the haemodynamic changes that follow an acute functional reduction in renal mass (Hostetter, 1982b). Hostetter et al (1981b)have shown that the morphological and haemodynamic alterations following sub-total nephrectomy, in rats, could be prevented by dietary manipulations. A low protein diet known to decrease glomerular blood flow and glomerular filtration rate (Ichikawa, 1980) prevented the adaptive changes that characterised the remnant kidney (Brenner, 1982). It was assumed therefore that these compensatory changes were at the base of the glomerulosclerosis that underly the progressive chronic renal failure that occurs in this experimental model.

These observations were made over a short period and in an extreme model of eleven-twelths nephrectomy (Hostetter, 1981b).

Similarly, longer term observations were made by Kleinknecht et al (1979); these authors concentrated on young, weanling, and growing rats. In these animals a low protein diet improved the natural history of glomerulosclerosis following sub-total nephrectomy, although significantly hampered their rate of growth (Saluski et al, 1981).

However, the compensatory response to renal ablation has been shown by Kaufman and Hayslett to vary between growing and adult rats (Hayslett, 1983).

Finally, the majority of early data on the effects of low protein diet on experimental uraemia did not differentiate between the respective influences of protein and phosphate restrictions.

Therefore, I started this phase of my research programme exploring the effect of a low protein, normal phosphate, diet on the progression of chronic renal failure in adult rats following five-sixths nephrectomy.

Experimental protocol

I studied the course of chronic renal failure in two experimental groups of rats following sub-total nephrectomy on different diets. Group 1, consisted on 10 rats maintained on a normal (18%) protein diet, providing 13.5 grams/kg body weight/day. Group 2, consisted of 11 rats fed a low protein diet (8%) providing 6 grams/kg of body weight/day. The details of the diets are given in the Materials and Methods section. Animals were given a fixed amount of food (20 grams/animal per day and had free access to tap water). Two additional control groups were studied to determine the effects of experimental dietson normal rats. Five-sixths nephrectomies were performed in two stages; first the two-third of the left kidney was removed and one week later the right kidney was removed. Details of the procedure are given in the Materials and Methods section. The experimental diets were introduced two weeksafter the completion of the nephrectomies. (Figure 24).

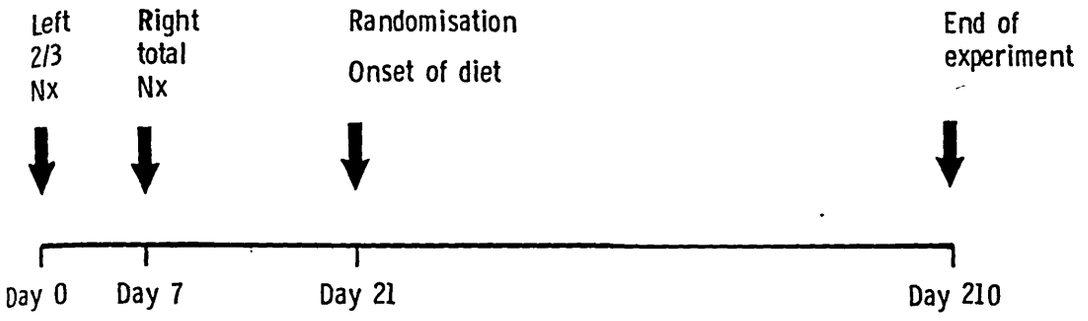


Figure 24 : Experimental protocol. Effect of low protein diet on the course of the sub-total nephrectomy model of chronic renal failure

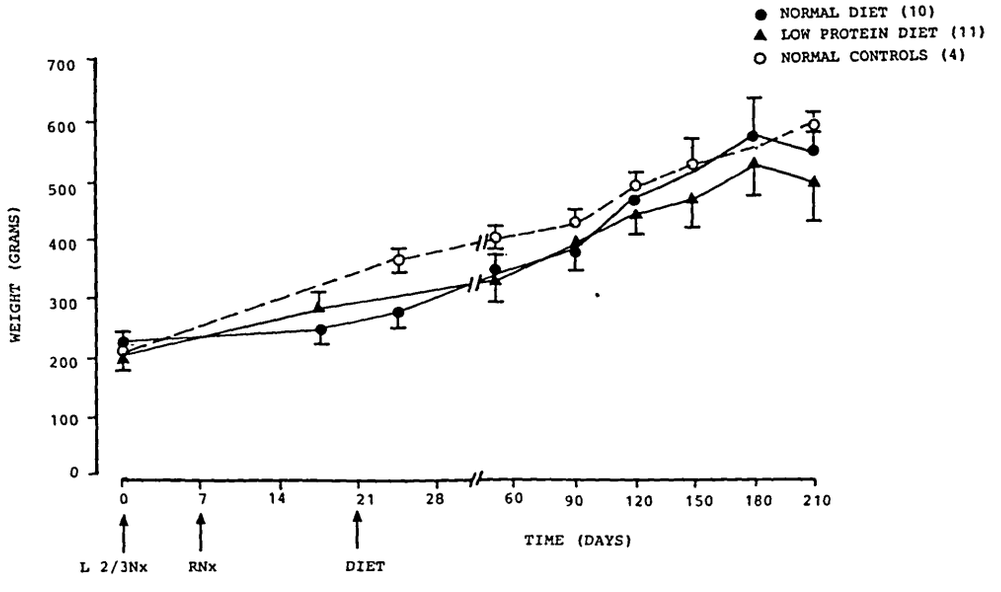


Figure 25 : Effect of low protein diet on the rat's growth rate following sub-total nephrectomy

Results :

General observations

Irrespective of the dietary content all rats recovered after nephrectomy, appeared healthy and grew normally during the course of the experiment. (Figure 25)

The normal growth emphasises that rats on the low protein diet were not malnourished.

After nephrectomy, the mean systolic arterial blood pressure rose from 110 ± 5mmHg to 130 ± 5mmHg. It was similar in both groups of rats and tended to increase further during the course of the study. (Figure 26)

Proteinuria

At the start of the experiment protein excretion was 25 ± 5mg/24 hours in normal rats on the experimental diets and did not increase immediately after nephrectomy in both groups of experimental rats; at day 60, rats in group 1, on a normal protein diet, had a mean protein excretion of 37 ± 10mg/24 hr, which increased to 271 ± 41mg/24hr by day 210. At day 60, proteinuria, in rats on a low protein diet, was comparable to group 1 and increased only slightly during the subsequent course to reach 83 ± 24mg/24h at day 210. This difference in proteinuria is significant, $p < 0.0125$. (Figure 27)

The extent of proteinuria in this model of chronic renal failure was decreased by the early introduction of a low protein diet.

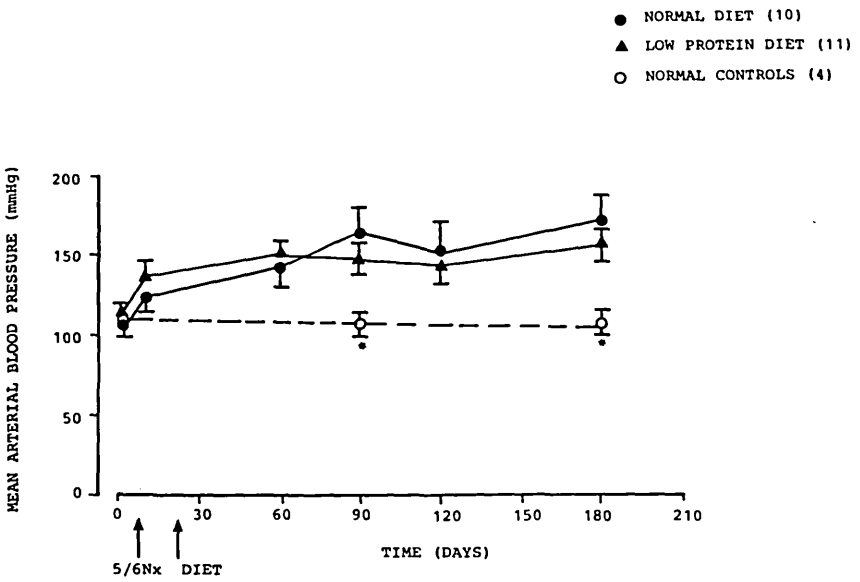


Figure 26 : Effect of low protein diet on blood pressure following sub-total nephrectomy. *$p < 0.005$

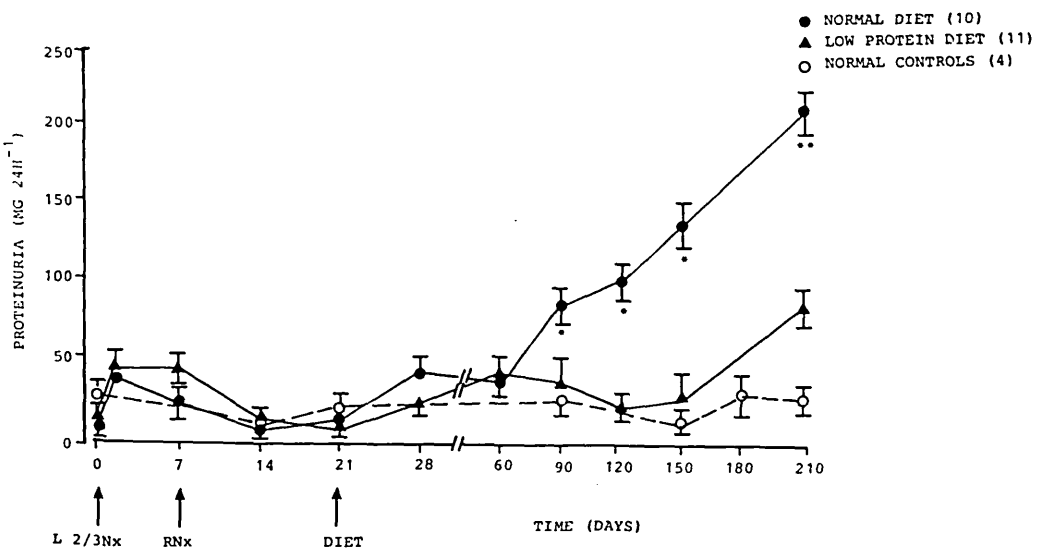


Figure 27 : Effect of low protein diet on the course of proteinuria following sub-total nephrectomy *$p < 0.005$, **$p < 0.0125$

Renal function

After the nephrectomies the mean serum creatinine rose from 45 to 100umol/l and was similar in the two groups when the diets were introduced on day 21. It remained so up to day 150, after which serum creatinine rose sharply in group 1 animals; there was no comparable rise in group 2 rats. At the end of the experiments the difference in plasma creatinine between the groups was statistically significant ($p < 0,0025$). These differences in plasma creatinine were attributable to changes in glomerular filtration rate assessed by 24hr creatinine clearance. (Figure 28, 29).

Animals on a normal diet, developed progressive renal failure while the ones on a low protein diet had to some extent a preservation of their renal function throughout the experiment.

Survival

Six of ten rats, on a normal diet, died from renal failure before the end of the experiment, compared with only two of eleven in group 2, on the low protein diet. This difference is highly significant, $p < 0,0028$. (Figure 30).

No differences in growth, blood pressure, proteinuria, renal function and histology were observed between the two control groups.

Renal morphology

At death from renal failure, or after killing at the end of the experiment, the kidneys from animals in both groups showed a wide range of glomerular and tubular lesions. Some glomeruli from

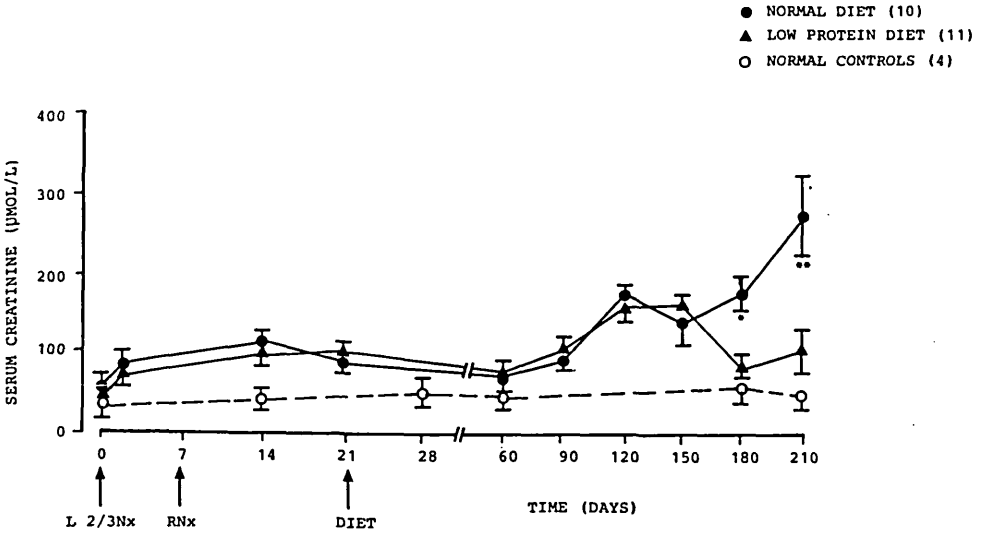


Figure 28 : Effect of low protein diet on renal function following sub-total nephrectomy. $^{*}p < 0.0125$, $^{**}p < 0.0025$

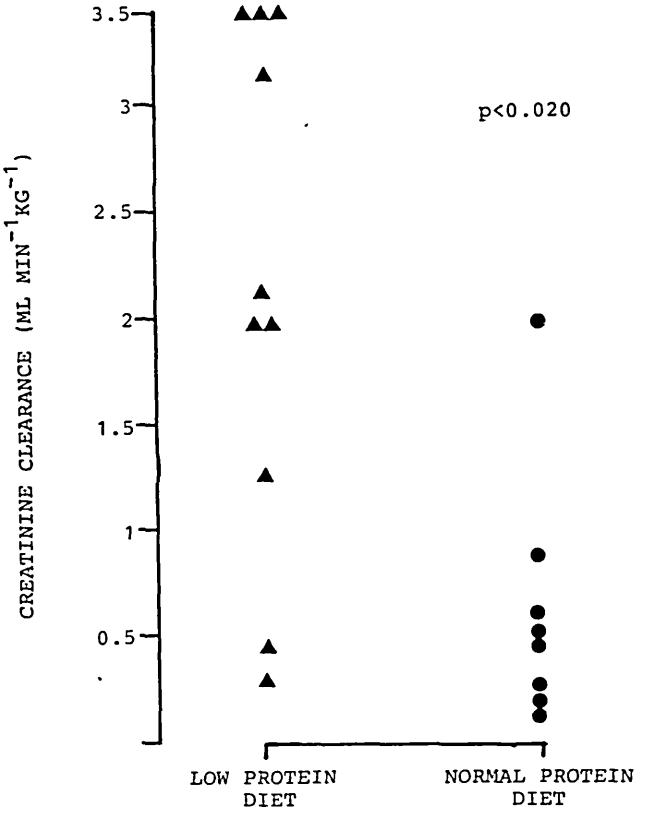


Figure 29 : Creatinine clearance at time of death for animals on a normal diet (●) and a low protein diet (▲) following sub-total nephrectomy

animals in both groups were totally sclerosed whilst others had segmental scars. The incidence of glomerulosclerosis was much higher and was more severe in Group 1. In this group at least 25% of glomeruli were totally sclerosed in all nine rats examined, whereas only three rats in Group 2 had this degree of sclerosis. This difference is significant, $p < 0.05$). There were comparable differences in the degree of tubular atrophy, which was present in seven of nine rats in Group 1 compared to three of ten rats in Group 2. In Group 1, four rats had a small proportion of glomerular tufts surrounded by crescents; none was noted in Group 2. No immunoglobulins were detected by immunofluorescence in glomeruli in rats from either group. (Figure 31) (Photos 9 & 10).

On electron microscopical examination, the predominant findings in Group 1 was hypertrophy of the epithelial and mesangial cells together with wrinkling and breaks in the glomerular basement membrane. Hypertrophic changes were less marked in group 2 animals.

Remnant kidneys weight was lower in animals fed a LPD 1.74 ± 0.48 when compared to those on a normal diet : 2.4 ± 0.67 - the difference reached statistical significance ($p < 0.0\ 5$) when kidney weight was adjusted to body weight; LPD: 0.31 ± 0.06 and normal diet : 0.51g/100g body weight.

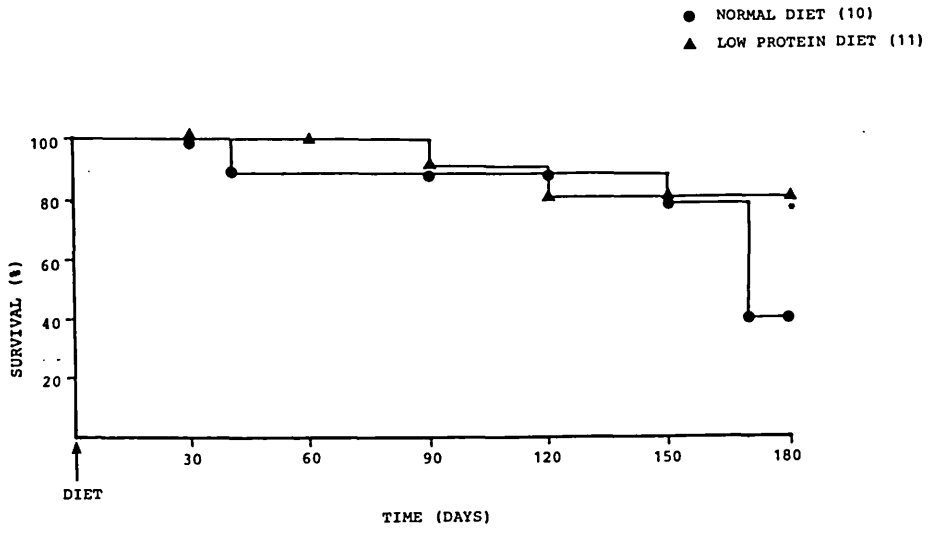


Figure 30 : Effect of low protein diet on survival following sub-total nephrectomy. *$p < 0.0028$

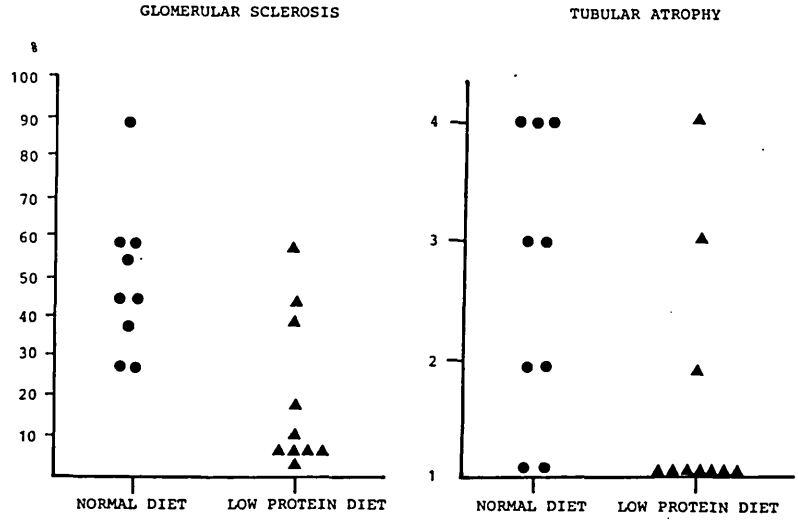
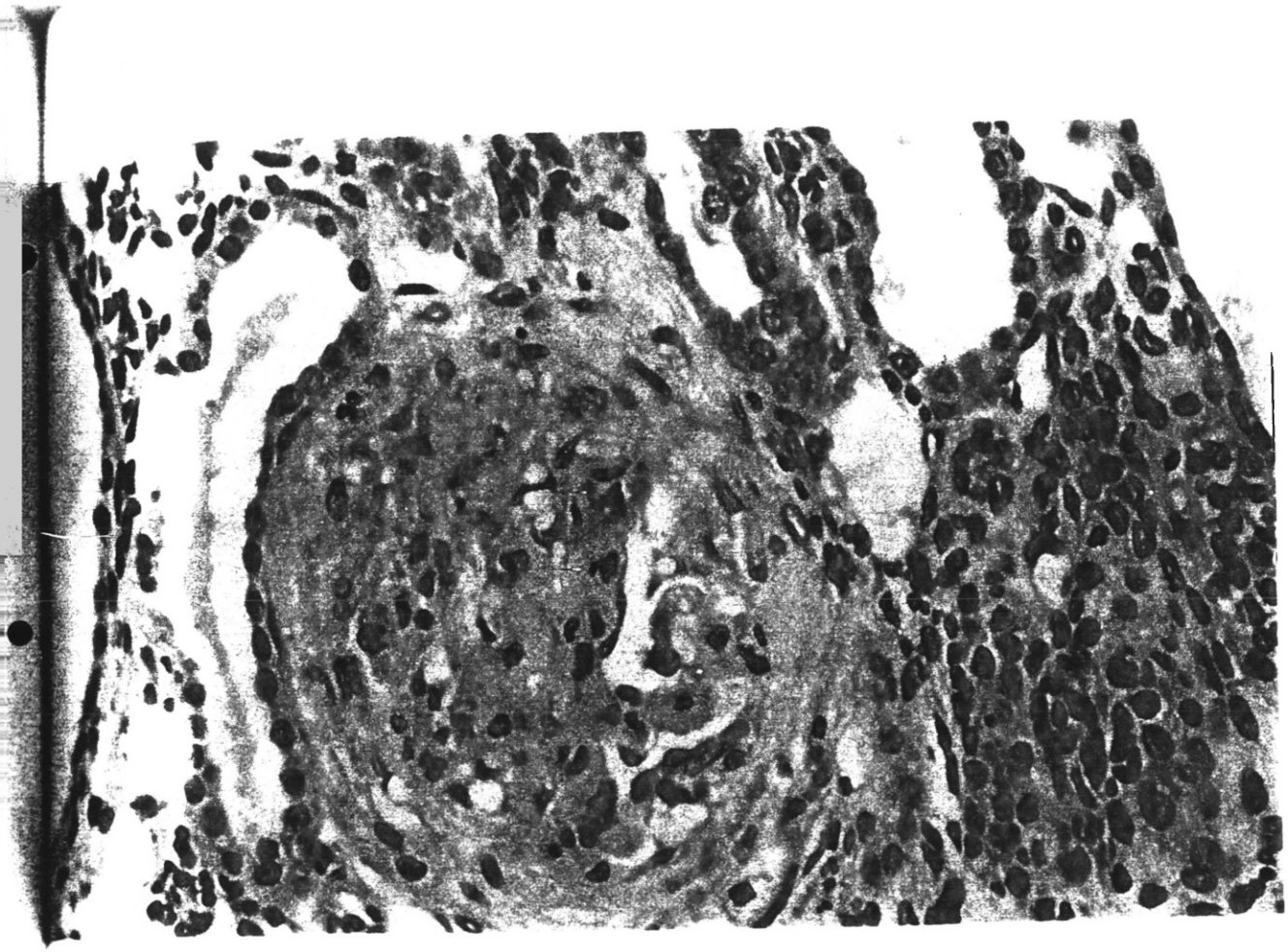
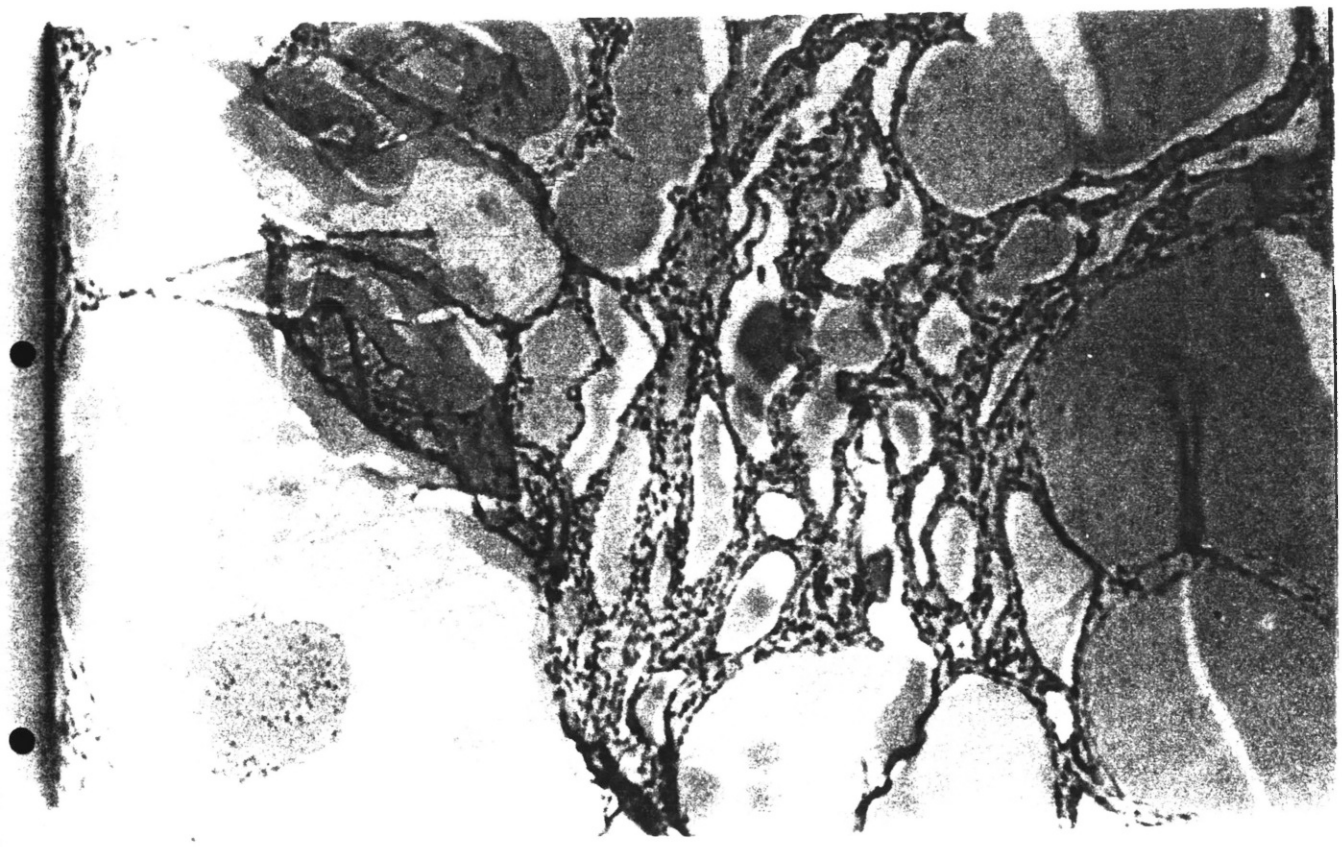


Figure 31 : Effect of low protein diet on renal morphology changes following sub-total nephrectomy. The significance of differences between the two experimental groups. $p < 0.05$





Discussion

Although the process of glomerular scarring is poorly understood it is well known that in both rats (Bolton & Sturgill, 1980) and human (Kaplan, 1975) there is a progressive increase in the proportion of sclerosed glomeruli with age. These sclerotic changes occur more rapidly after unilateral (Elema, 1971) or sub-total nephrectomy (Shimamura & Morrison, 1975). Lately evidence has been advanced to suggest that the rate of glomerulosclerosis might also be increased by reduction in renal mass in man; Patients with unilateral renal agenesis have an increased incidence of glomerulosclerosis (Kiprov, 1982) as well as patients submitted to unilateral nephrectomy (Zuchelli, 1983). These observations raise the question whether a similar mechanism of renal scarring might contribute to the development of renal failure after other types of renal injury. This process might therefore operate in glomerulonephritis or in vesico-ureteral reflux after the acute insult has significantly reduced the amount of viable renal tissue. If so, such sclerosis might proceed independently of the primary disease well after it had resolved. After sub-total nephrectomy of rats, massive adaptive changes take place immediately (Hayslett, 1979); these consist of hypertrophy of the kidney which is largely complete after two weeks (Malt, 1969) and which is accompanied by a significant increase in renal blood flow (Hayslett, 1979) and single nephron glomerular filtration rate (Deen,1974).

In the ensuing three months, nephrectomised rats develop extensive glomerulosclerosis of the remnant kidney (Shimamura & Morrison, 1975). It is uncertain, however, to which extent the sclerosis is predetermined by the degree of early adaptive changes and to what extent it depends on the persistence of the increased blood flow or other compensatory

alterations. In the later case, the rate of progressive scarring might be reduced by appropriate, although delayed, therapeutic interventions.

I chose to study the effect of protein restriction on glomerular sclerosis because it has previously been shown to delay spontaneous scarring and the development of renal failure in ageing rats (Berg & Simms, 1960). My results clearly show that moderate protein restriction also delays the onset of proteinuria and decreases the incidence of glomerulosclerosis and renal failure after five-sixths nephrectomy. It is probable that the protection I observed is directly attributable to the protein restriction. Previously other dietary changes, and in particular phosphate restriction have been shown to limit the development of renal failure in rats after partial nephrectomy (Ibels, 1978). The experimental diets I used contained identical amounts of calcium and phosphorus and differed only in their protein and starch content.

Similarly, the results cannot be explained by the general effect of malnutrition on the inflammatory response, as the rats in both groups were not malnourished and grew normally during the course of the experiments.

Hypertension could not explain the results; although blood pressure increased after the nephrectomies, it did not differ significantly between nephrectomised rats fed with either diet. It has recently been demonstrated that a diet supplemented with tryptophan improved the hypertension after sub-total nephrectomy without affecting the development of chronic glomerulosclerosis (Kaysen & Kropp, 1983).

I can only speculate as to the mechanisms of protection by a low protein diet on the course of this nephropathy. Severe protein restriction has been shown to reduce renal hypertrophy as well as the increments in renal blood flow and single nephron glomerular filtration rate (Hostetter, 1981) after sub-total nephrectomy

As I was principally interested in the late development of scarring I therefore delayed starting the diets until 14 days after the completion of the nephrectomy, by which time most of the adaptive changes would have occurred. This, together with the fact that serum creatinine in both groups remained the same for a further 20 weeks, suggests that the protection from the glomerular sclerosis that was observed was not predetermined by the extent of the acute adaptive response. However, the protective effect of the diet could be mediated by long term changes of renal blood flow, on single nephron glomerular filtration rate as well as by changes in fluid and solute excretions.

Finally, in the remnant kidney model proteinuria develops and might in itself participate in a vicious circle that leads to further glomerular damage. In this model, like in other experimental ones in the rat, persistent proteinuria could irreversibly damage the glomerular basement membrane (Davies, 1978). Whether early proteinuria in the remnant kidney model reflects structural glomerular damage (Shea, 1980) or whether it is the mere reflection of the compensatory high pressure within glomeruli in unknown. However, the low protein diet by decreasing or delaying the onset of significant proteinuria might protect the glomeruli from further scarring.

It is important to remember that protein restriction also has several effects on the metabolism (Waterlow & Jackson, 1981) and causes a reduction in plasma triglyceride concentration (Liepa, 1980) and of thyroid function (Edozien, 1978); theoretically, either or both these could protect from the development of glomerular sclerosis.

Having established that moderate protein restriction affected favorably the cause of this, non immune, model of chronic renal failure, I directed my attention back to the immune mediated nephrotoxic nephritis model.

(b) Nephrotoxic nephritis

1. Acute effects of dietary manipulation

Introduction

During the early stages of my research I showed that proteinuria is an important factor in the progression of chronic nephrotoxic nephritis. The subsequent experiment on the sub-total nephrectomy model showed a beneficial effect of low protein diet, with improved renal function possibly secondary to the prevention of severe proteinuria by such a diet.

In this part of my research, I decided to explore the effects of dietary protein and solute depletion and overload on the proteinuria of nephrotoxic nephritis.

There is some experimental evidence in animals to suggest that proteinuria in other models of nephritis could be harmful and lead to irreversible renal damage. Davies et al (1978) showed that the

injection of bovine serum albumin (BSA) to rabbits led to the development of intrinsic proteinuria with subsequent glomerular morphological changes. Similarly, in rats with amino nucleoside induced nephropathy, the degree of glomerular scarring is proportional to the degree of proteinuria (Glasser, 1977).

Previously, solute loading with high protein diet (Rumsfeld, 1956). hypertonic saline (Strauss, 1975), urea and manitol (Finlayson & Baumann, 1956) have all been shown to increase urine protein excretion in normal rats.

I attempted through this study to extend these observations to nephrotoxic nephritis in rats, as the first step in altering the longterm course by manipulating the proteinuria in this disease.

Experimental protocol

A mild, slowly progressive, nephrotoxic nephritis was induced in a cohort of six adult rats.

Experiments were started 60 days after the induction of the nephritis. At this stage, the rats had a stable but significant proteinuria (125 $\pm$ 8mg/24h).

Animals were fed a standard medium protein diet (18%) supplemented in succession with protein, urea and sodium chloride followed by injections of frusemide and acetazolamide.

Each experimental subset lasted for 5 days with the first 2 for baseline assessment followed by 3 days for the dietary or pharmacological interventions.

One week was allowed between each experimental subset for reequilibration.

Diets

Animals were fed a standard,medium protein diet (18%) providing 13,5 grams/day/kg of body weight of protein (casein).

The low protein diet provided 6gr/day/kg, of protein (8%). The high protein diet provided 53,5gr/day/kg of protein (78%). Rats ate their daily food allowance and had free access to water - these diets are detailed in the Materials and Methods section.

Urea was supplemented at a rate of 30 grams/kg/day. Sodium chloride was supplemented at 90mmol/kg/day. The urea, and salt supplementations were administered by gavage in divided daily doses.

Diuretics

Rats on a standard diet were injected with :

1. Frusemide (im) : 40mg/three times daily
2. Acetazolamide (im) : 125mg/twice daily

Measurements

During the dietary supplementations daily measurements were made of rat's serum albumin and creatinine as well as 24 hour urine for : volume, protein, albumin, light chains, urea, creatinine and sodium.

During the pharmacological interventions only serum albumin and creatinine as well as urine protein, albumin and light chains were measured.

Results

1. Low and high protein diets

Immediate, reversible, changes were observed on the low and high protein diets; urine volume, protein, sodium and urea content all varied proportionally to the protein intake.

On the high protein diet significant changes in urine protein excretion of these nephritic rats occurred. The 24 hour proteinuria increased by 212%, while albumin increased by 248%, fractional excretion (FE) of albumin by 372% and light chains by 214%. (Figure 32)

Concomittantly to these changes increments in urine volume (246%), sodium (242%) and urea (342%) occurred. (Figure 33)

Since a high protein diet induces an osmotic diuresis and increases the urine excretion of free water, sodium and urea, I proceeded to attempt to dissect the individual effects of these variables on the proteinuria of nephritic rats.

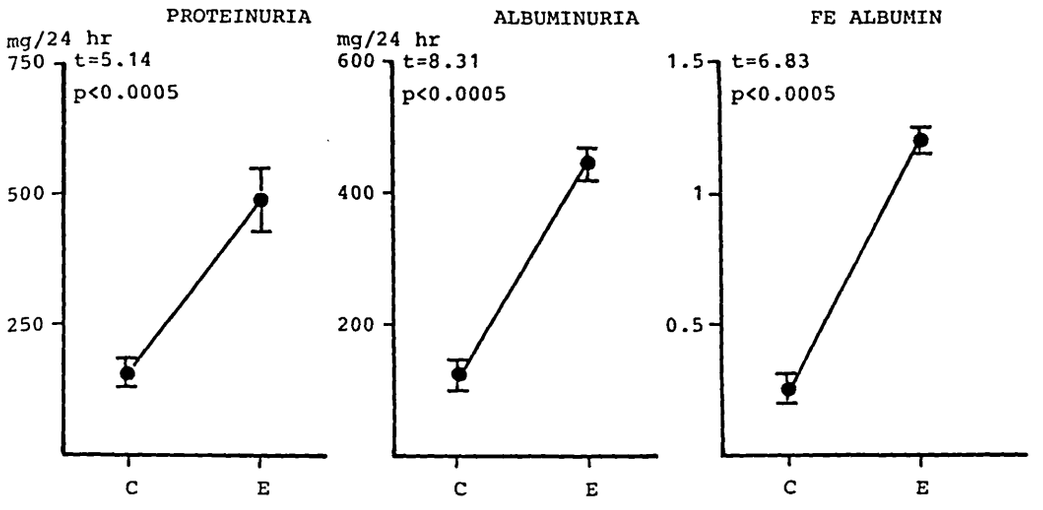


Figure 32 : Effect of acute dietary protein supplementation on urinary protein excretion in nephrotoxic nephritis

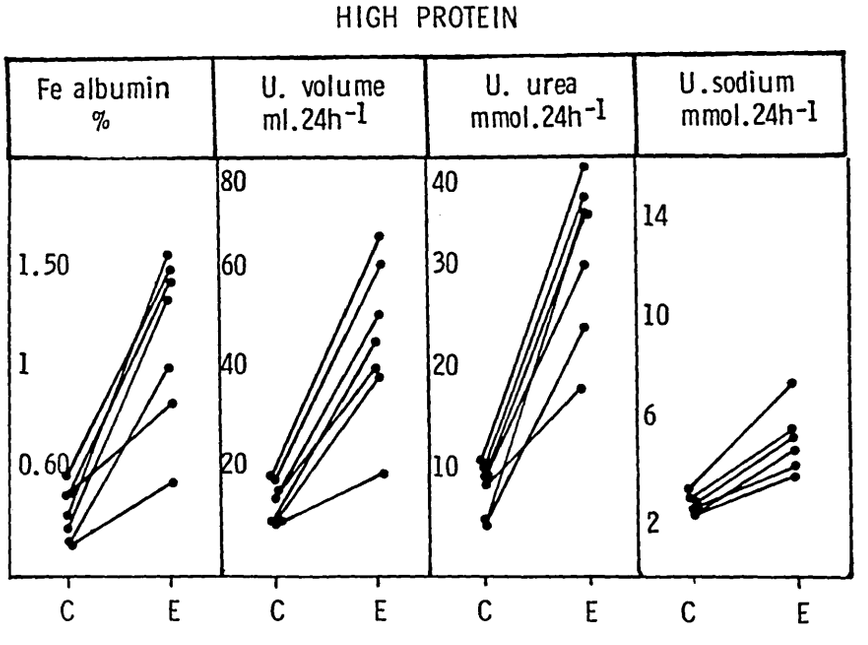


Figure 33 : Effect of acute dietary protein supplementation on urine albumin and solutes excretions in nephrotoxic nephritis

2. Urea loading

Increasing the urea intake was aimed at reproducing a similar osmotic diuresis to that observed with the high protein diet; this was achieved with daily increments in urea of 30gr/kg of body weight/day.

The observed urine volume increment of 270% was comparable to that obtained with the high protein diet, however the sodium excretion increased by only 50% while the urea increment reached 385%.

Similarly, urine protein increased after urea loading; protein 154%, albumin 154%, FE albumin 270% and light chains 416%. (Figure 34,35). The increase in FE albumin with urea loading was significantly less than with a high protein diet, $p < 0.05$.

3. Sodium chloride loading

Salt loading with 90mmol/kg/day of sodium chloride led to a substantial increase in proteinuria. However, the increments in proteinuria were significantly less than with the high protein diet; protein (171%), albumin (116%), FE albumin (200%) and light chains (285%). (Figure 36).

Simultaneously urine volume increased by 384%, sodium by 225% and urea decreased by 39%. (Figure 37).

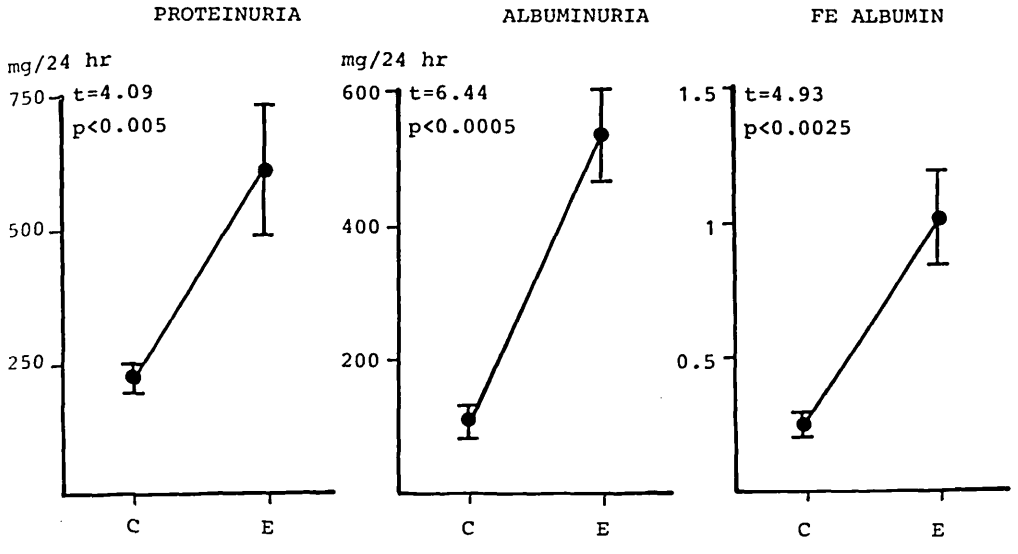


Figure 34 : Effects of acute dietary urea supplementation on urinary protein excretion in nephrotoxic nephritis

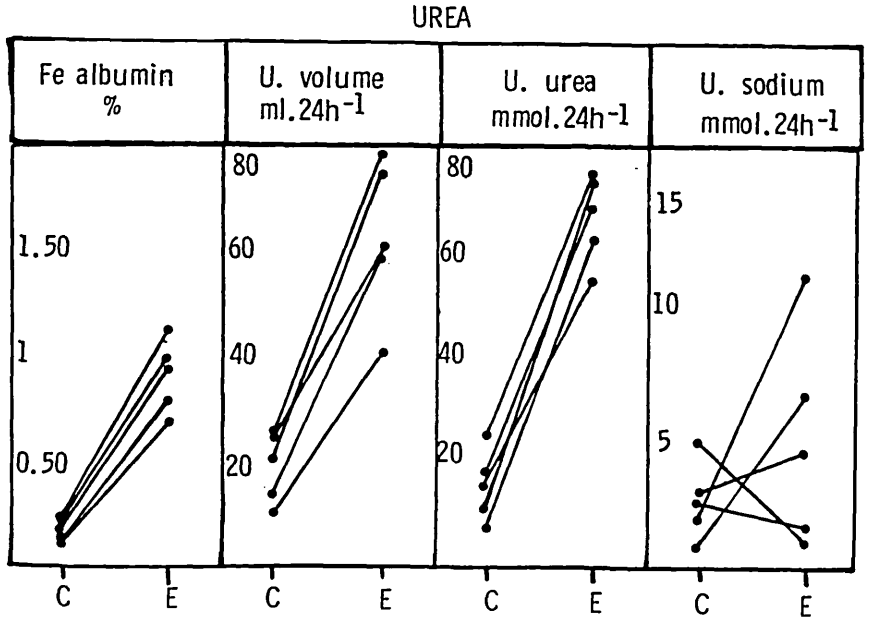


Figure 35 : Effect of acute dietary urea supplementation on urine albumin and solutes excretions in nephrotoxic nephritis

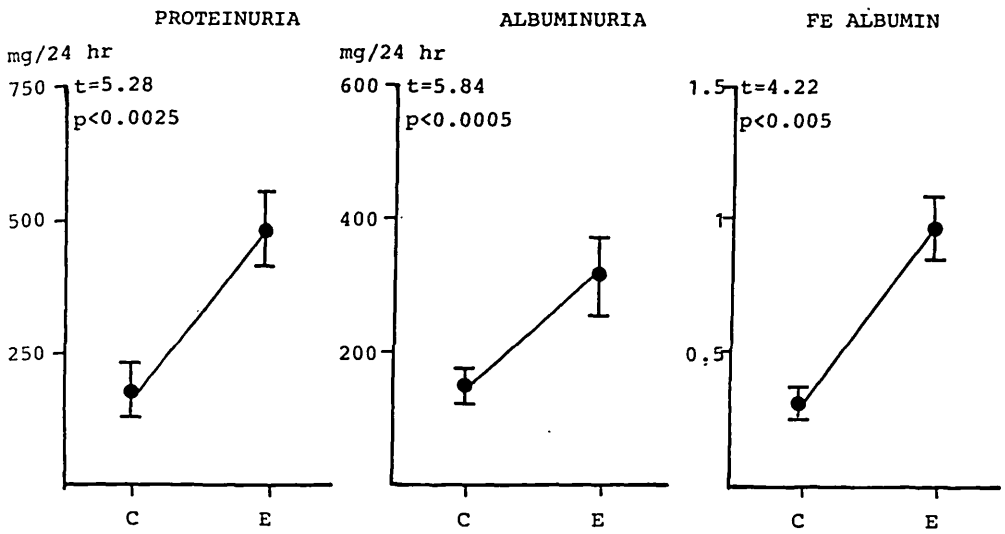


Figure 36 : Effect of acute dietary salt supplementation on urinary protein excretion in nephrotoxic nephritis

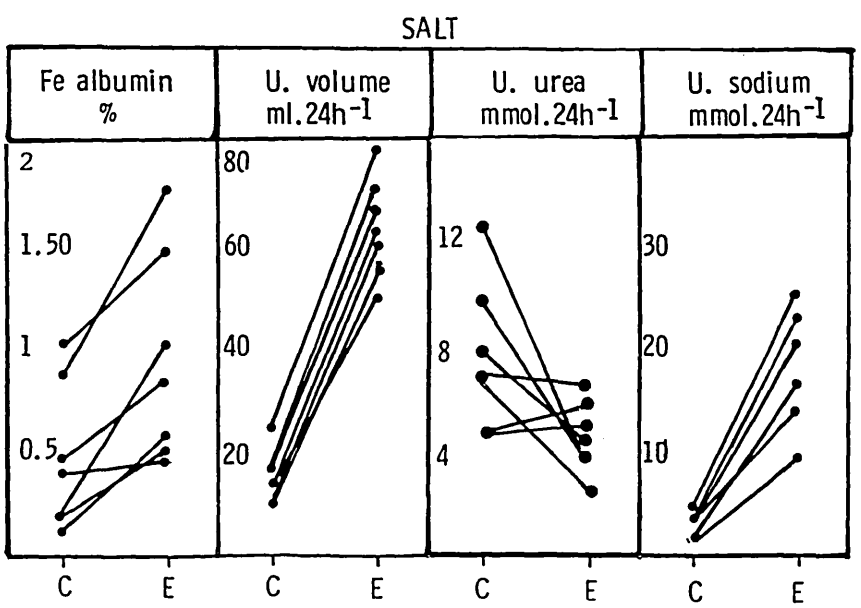


Figure 37 : Effect of acute dietary salt supplementation on urine albumin and solutes excretions in nephrotoxic nephritis

The salt loading experiment seemed to dissociate urine protein excretion from urea; as increased dietary salt intake led to an increase in proteinuria with no concomittant rise in urine urea excretion.

From these short term experiments on the proteinuria of nephrotoxic nephritis it became apparent that urine protein excretion in this condition is not solely the reflection of underlying organic damage. The acute, reversible, changes induced in protein excretion by different dietary manoeuvres imply a functional contribution to the proteinuria of nephrotoxic nephritis. During these experiments, and as the different dietary interventions induced an osmotic diuresis with increased proteinuria, it appeared that urine protein excretion correlates with urine volume ($r = 0.73$, $t = 6.1$, $p < 0.001$) as well as with the sodium excretion ($r = 0.84$, $t = 7.5$, $P < 0.001$). (Figures 38 & 39).

All through the different experiments a close correlation was observed between albumin and light chain excretion ($r = 0.78$, $t = 6.3$, $p < 0.001$).

One of the mechanisms through which an osmotic diuresis could interfere with urine protein excretion is by affecting the proximal tubule protein reabsorption capacity. In order to assess the contribution of the proximal tubule to the proteinuria of nephrotoxic nephritis, we attempted to inhibit its reabsorption capacity with acetazolamide a known proximal tubule carbonic anhydrase inhibitor. We compared the effect of acetazolamide on proteinuria with that of a loop diuretic, frusemide.

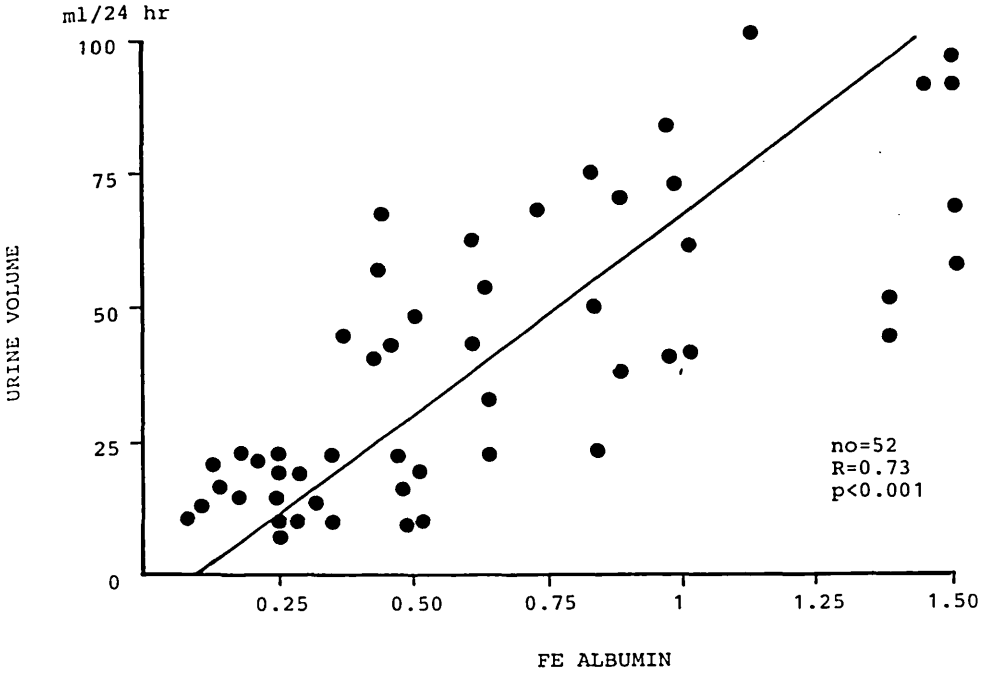


Figure 38 : Correlation between fractional excretion (FE) of albumin and urine volume in nephrotoxic nephritis

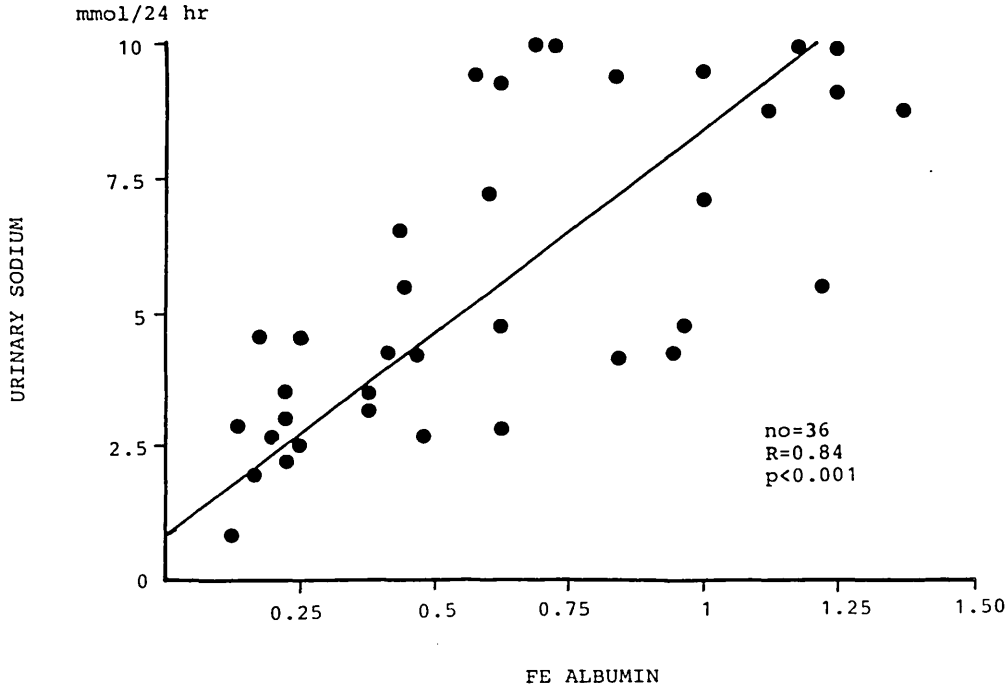


Figure 39 : Correlation between the fractional excretion (FE) of albumin and urine sodium in nephrotoxic nephritis

4. Acetazolamide

Acetazolamide at a dose of 125mg/twice daily increased proteinuria in NTN; proteinuria increased by 38%, albuminuria : 63%, FE albumin 100% and light chain excretion 71% (Figure 40).

This effect was acute, starting on the day of the first injection, and lasted throughout the three experimental days.

5. Frusemide

Frusemide at a dose of 40mg/three times daily, increased urine volume in nephritic rats by 129%. However, frusemide had an opposite effect on proteinuria to acetazolamide; proteinuria and albuminuria decreased by 81% and 78% respectively. Meanwhile the FE albumin increased marginally by 67 %. (Figure 41).

From this second set of experiments with two different diuretics, I concluded that frusemide and acetazolamide had opposite effects on proteinuria in nephritic rats.

It is tempting to speculate that the effect of acetazolamide on proteinuria is mediated by its inhibitory action on the proximal tubule reabsorption processes; the observed increment in light chain excretion would support this concept.

Discussion

Proteinuria is often considered the reflection of the severity of the underlying nephropathy and structural renal damage (Gang & Kalant, 1970). In animals with experimental nephritis it is

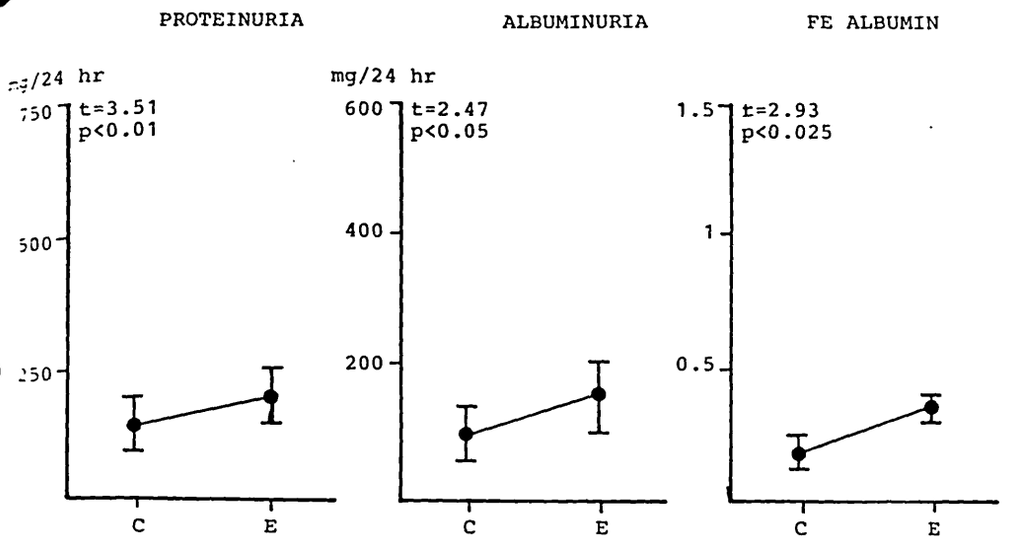


Figure 40 : Acute effects of acetazolamide on urine protein excretion in nephrotoxic nephritis

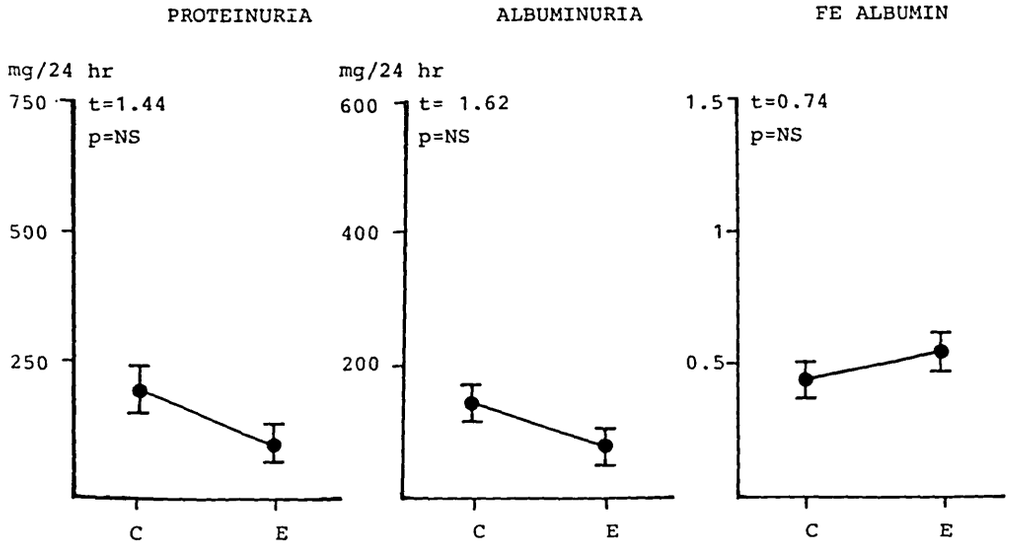


Figure 41 : Acute effects of frusemide on urine protein excretion in nephrotoxic nephritis

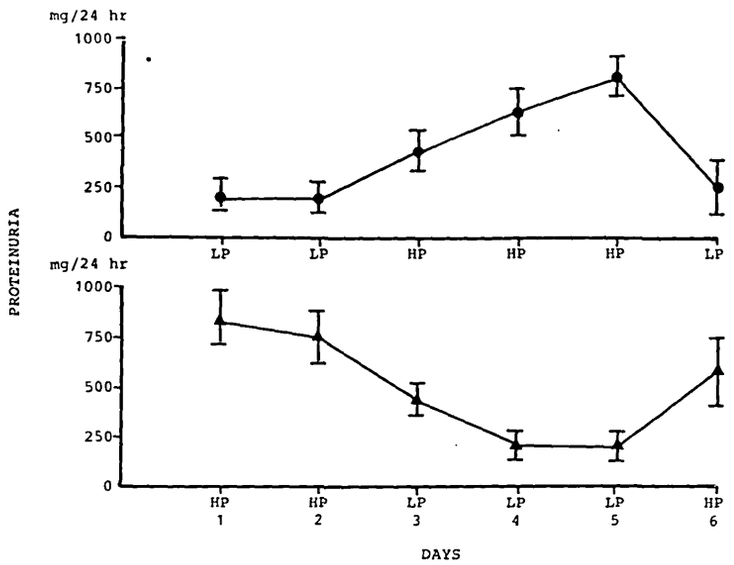


Figure 42 : The acute effects of high (●) and low (▲) protein diets on the proteinuria of nephrotoxic nephritis

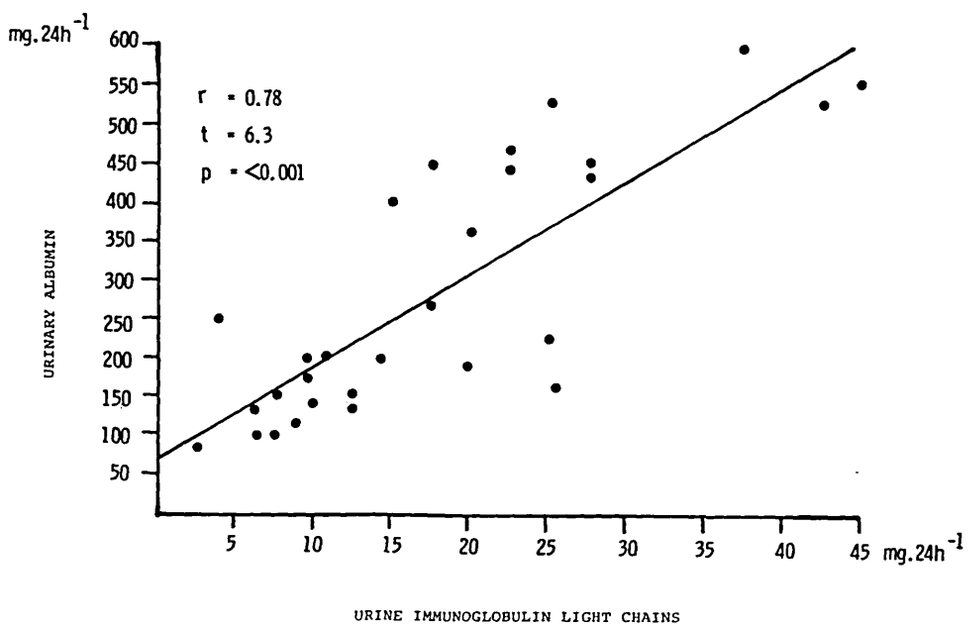


Figure 43 : Correlation between the urine excretions of albumin and immunoglobulin light chains in nephrotoxic nephritis

currently believed that proteinuria, early in the course of the disease results from the structural damage to the glomerular basement membrane with the subsequent loss of its negative charge coating (Kreisberg, 1979). However, it has been recently advanced that proteinuria could in itself perpetuate the glomerular injury and lead to progressive renal scarring (Davies, 1978). In man, proteinuria, not only reflects the severity of the nephropathy but is thought to be an important prognostic factor (Cameron, 1979a). The progression of some minimal change nephropathies to focal glomerulosclerosis is believed by some to be the direct consequence of long standing proteinuria (Cameron, 1978). In other forms of nephrotic syndrome, the persistence of proteinuria and the absence of remissions are considered poor prognostic signs, as these patients invariably develop chronic renal failure (Idelson,1977). Finally, persistent proteinuria might lead in some patients to mesangial overload and subsequent dysfunction with resulting glomerular sclerosis (Couser & Stilmant, 1975). In the early experiments on the role of immune response in the progression of NTN I confirmed that the severity of sub-acute proteinuria correlates closely with the functional life of nephritic kidneys.

It would seem, therefore, possible that therapeutic modifications of proteinuria after nephrotoxic nephritis might affect its long term course.

Previous workers have demonstrated that alteration in dietary protein (Rumsfeld, 1956), amino acids (Mogensen & Solling, 1977), urea (Finlayson & Baumann, 1956) and salt (Strauss, 1975) content altered the proteinuria of normal rats.

My results show a similar effect of changes of urine solutes excretions on proteinuria of nephritic rats.

These changes were acute and reversible affecting protein, albumin, and light chain excretion to a comparable extent. (Figure 42 & 43).

Many mechanisms could account for the observed changes in protein excretion. Increased glomerular or tubular damage is unlikely since the changes induced are reversible, with proteinuria returning to baseline after discontinuation of the dietary or pharmacological interventions.

An increase in the glomerular filtration rate (GFR) secondary to intravascular volume expansion is likely to have contributed to the observed changes in proteinuria after protein, urea and salt supplementations. However, as the fractional excretion of albumin also increased it is likely that other factors came into action. Similarly, changes in GFR cannot explain the changes occurring after acetazolamide treatment.

Similarly, modifications of renal and glomerular blood flow can account for some of the observed modifications of urine protein excretion; a low protein diet is known to decrease the glomerular plasma flow in rats (Ichikawa, 1980) through the increase in glomerular pre and post capillary resistances. Equally, a decrease in glomerular perfusion and filtration could significantly increase the amount of protein reabsorbed (Van Liew, 1970) leading to a decrease in proteinuria.

An opposite effect on proteinuria could be attributed to a high protein, high solute, diet; such diet increases plasma volume, decreases peripheral vascular resistances and increases glomerular filtration rate (O'Connor & Summerill, 1976).

The effects of plasma volume expansion on proteinuria have been the subject of many studies (Parving, 1974). Shirley et al (1957) attributed the increased permeability of capillaries to protein after plasma volume expansion to stretching of the capillary pores due to raised intraluminal pressure. Meanwhile, Studer et al (1973) suggested an increase in protein diffusion across capillary walls following plasma volume expansion.

It is therefore conceivable that increased circulating blood volume accounts for some of the changes in proteinuria observed in our experiments.

A tubular contribution to the changes in proteinuria cannot be ignored. Since the bulk of protein reabsorption, in rats, occur in the proximal tubule (Galaske,1979), an inhibition of this process would lead to an increase in protein, albumin and light chain excretion (Mogensen & Solling, 1977).

The proximal tubule handling of sodium, water and amino acids is likely to be interdependent (Ullrich, 1976). Inhibition of sodium and water absorption at this level by the osmotic diuresis induced by high protein diet, urea, and hypertonic saline could interfere with protein reabsorption at this site. Similarly, acetazolamide inhibition of proximal sodium reabsorption led to

increments in the excretion of albumin and light chains. Meanwhile, a similar diuretic effect induced through frusemide acting at the loop of henle would not be expected to alter the overall protein excretion.

From these studies I clearly determined that proteinuria in nephrotoxic nephritis is not solely the reflection of the underlying structural damage. Dietary manipulations have major effects on the magnitude of protein excretion following an acute nephritis. These effects are likely to be multifactorial with involvement of preglomerular, glomerular and post glomerular, tubular, mechanisms.

These observations in nephritic rats could have important implications if proteinuria, in itself, was nephrotoxic, as diets and manoeuvres increasing it would accelerate the course of the underlying nephropathy. Equally, the long term prognosis of experimental nephritis might be favorably influenced by diets and interventions aiming at reducing the magnitude of the urine protein leak.

Therefore, during the next phase of my research I studied the long term influences of dietary protein, urea and salt on the natural history of nephrotoxic nephritis.

2. Chronic effects of dietary manipulations

i. Dietary protein

Introduction

As early as 1939 Farr and Smadel have reported that the course of experimental nephrotoxic serum nephritis (NTN) could be altered favorably by dietary protein restrictions. In their experiments, a low protein diet prevented the occurrence of the severe glomerulosclerotic lesions observed when rats were fed a normal diet. However, in these early experiments the mechanism of protection was not understood and more so the diet used was likely to be equally restricted in phosphorus.

Recently, Haut et al (1980) have showed that progression to chronic renal failure in NTN could be slowed by dietary phosphate restrictions. Animals fed a low phosphate diet had a significant decrease in the degree of renal calcium deposition and were protected against severe tubular and glomerular scarring.

Therefore, the respective roles of dietary protein and phosphorus restrictions on the course of nephrotoxic nephritis remains unclear, since diets deficient in phosphorus are known to decrease the animal's food consumption and might lead to an overall reduction in dietary and protein intake. In the remnant kidney model of chronic renal failure Laouari and Kleinknecht (1982) have shed some doubt as to the beneficial effect of dietary phosphate restriction.

I undertook this phase of my research to determine the specific influences of low and high protein diets on the progression of nephrotoxic nephritis.

My experimental diets were isocaloric and carefully matched in their phosphate content. I hoped therefore to be able to dissociate the effects of dietary protein manipulations from changes in phosphate intake.

This study was aimed at studying the influence of dietary protein on the functional and morphological characteristics of chronic experimental glomerulonephritis. I hoped in this study to extend my observations on the effect of low protein diets to an immune mediated model of nephritis.

On the other hand, I was interested in evaluating the effect of a very high protein intake on the course of such nephritis. A high protein diet has been shown to be detrimental on the course of chronic renal failure in rats with reduced renal mass (Lalich, 1975), however an equally raised protein intake failed to alter renal function in the same model in dogs. In these animals Bovee et al (1979) showed an increased incidence of glomerulosclerosis without functional repercussions, implying a poor correlation between the effects of the high protein intake on glomerular morphology and renal function.

Finally, I was interested to determine through this experiment the long term effects on renal function and morphology of the increased proteinuria resulting from the increased protein and solute load.

Experimental protocols

This experiment was divided into three experimental sub-sets :

1. The effects of early introduction of low and high protein diets on the natural history of NTN

2. The effect of early dietary introduction on the renal morphology during the early stage of the nephritis

3. The effect of late dietary introduction on the course of NTN

Experiment (1) :

Seven days after induction of the nephritis by injection of NTN, the rats were randomised into three experimental groups matched for the severity of proteinuria. Group I : 9 rats, kept on a normal protein diet, Group II : 8 rats, started on a low protein diet and Group III : 8 rats, started on a high protein diet.

Three control groups of 4 rats each on the different diets were simultaneously studied.

Experimental rats were biopsied at day 30 and when the experiment was terminated on day 90.

Experiment (2) :

This second experiment was performed to assess the influence of diet on the renal morphology during the first 30 days after the induction of the glomerulonephritis. On day 7, rats were segregated into three groups matched for the severity of glomerular injury. The three

experimental groups consisted of 8 rats each. The renal morphology was assessed on days 3 and 30 after the induction of the nephrotoxic nephritis.

Experiment (3) :

A third experiment was designed to see if late introduction of the diets influenced the progression of NTN. The rats were allocated to one of 3 matched groups for proteinuria on day 30 and then followed until day 120, Renal morphology was assessed on days 30 and 90. Each experimental group consisted of 8 rats.

Diets

The experimental diets are detailed in the Materials and Methods section.

Low protein diet : provided 6 grams of protein/day/kg of body weight

Medium protein diet : provided 13.5 grams " " " "

High protein diet : provided 45 to 50 grams " " "

The three diets were isocaloric and had a similar sodium, calcium, and phosphate composition differing only in their protein and starch content.

Rats were provided with 20 grams of food/rat/day, however, the eating habits of the three experimental diets varied; both the animals on low and normal protein intake consumed all their food allowance, while rats on the high protein diet consumed approximately 16 to 18 grams/day. All rats had free access to tap water.

Assessment of disease progression

Serial weekly measurements were made of rats weight, blood pressure, serum and urine creatinine, serum autologous antibody titres and 24hr urine protein excretion. Rats kidneys were biopsied according to the experimental protocol and renal tissue was processed for light microscopy and immunofluorescence.

Results

Experiment 1 : The effect of early dietary protein introduction.

Proteinuria

Within 24 hours from the introduction of the experimental diets, proteinuria changed proportionally to the dietary protein intake; a low protein diet decreased the proteinuria significantly, while the high protein diet had an opposite effect on urine volume and protein excretion. Proteinuria remained proportional to the amount of protein ingested up to day 30 when it further increased in individual animals as renal failure developed. (Figure 44)

Renal function

Serum creatinine in the three experimental groups followed a similar course up to day 30. Afterwards, rats fed a normal diet developed renal failure as reflected by the increase in serum creatinine to 125mg $\pm$ 15umol/l on day 60. Animals on the low protein diet showed no significant changes in their renal function, while rats fed a high protein diet followed an intermediate course. (Figure 45)

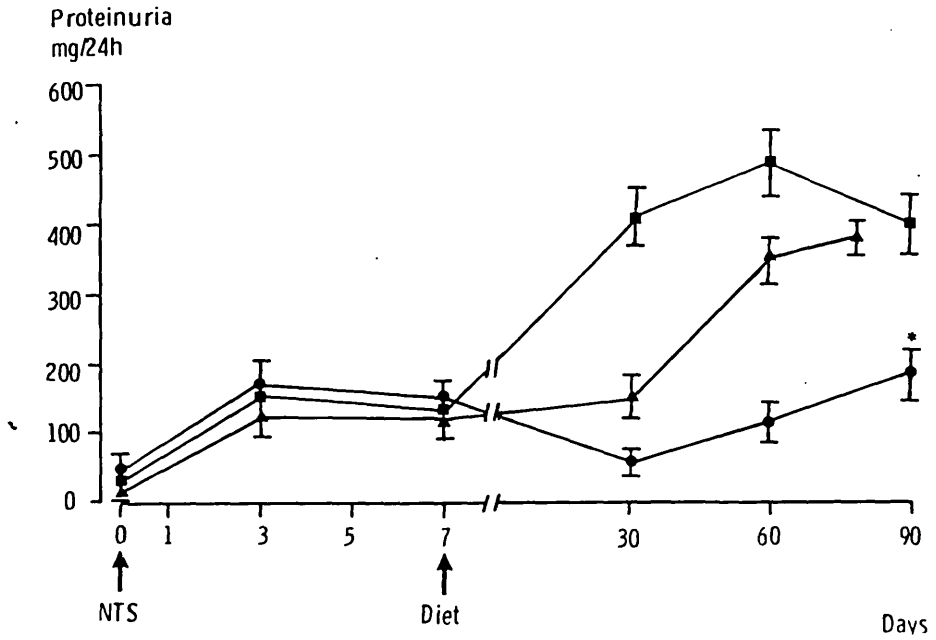


Figure 44 : Nephrotoxic nephritis - Effect of dietary protein manipulations on the course of the proteinuria (▲—▲) normal diet, (●—●) low protein, (■—■) high protein diet. *p < 0.025

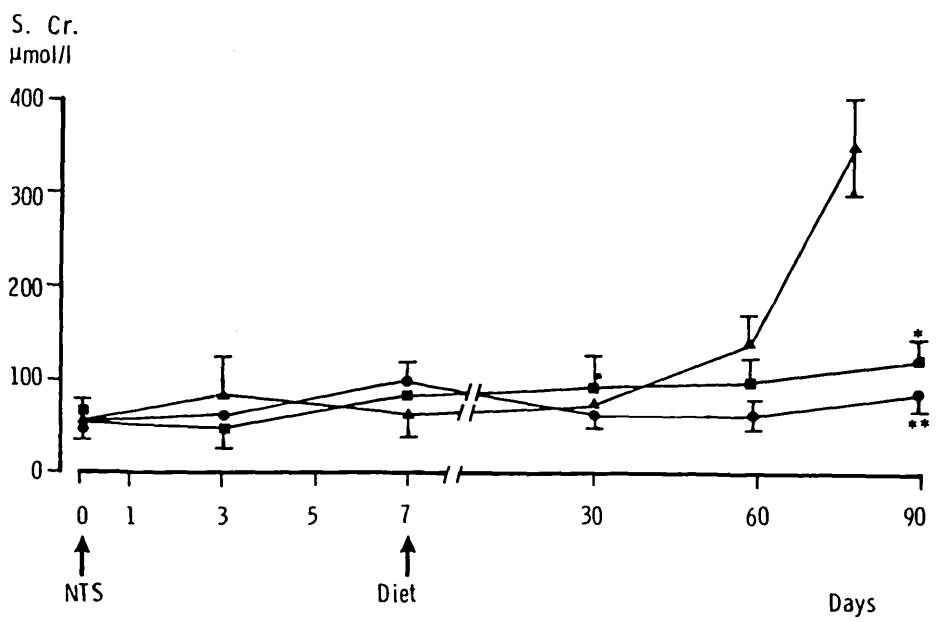


Figure 45 : Nephrotoxic nephritis - Effect of dietary protein manipulations on the course of renal function (▲—▲) normal diet, (●—●) low protein and (■—■) high protein diet. *p < 0.031, **p < 0.025

The low protein ($p < 0,025$) and to a lesser extent the high protein ($p < 0,031$) diets protected rats with nephrotoxic nephritis from severe renal failure.

Survival

While 8 of 9 rats on the normal diet died from renal failure, none died in the low protein diet group ($p < 0,0025$). Only 2 of 8 rats died from renal failure in the high protein diet group ($p < 0,0035$). (Figure 46).

Renal morphology

In this experiment, renal morphology was not assessed before the rats were randomised into the three experimental groups on day 7. However, when first sampled on day 30, although the mean glomerulosclerosis score was greater in group I (normal diet) than in the other two groups, the difference was not statistically significant. Similarly, there were no significant differences in the degrees of tubular atrophy and tubular calcifications were equally absent.

In contrast, by day 90 or at time of death, there were marked differences between the groups in each of the morphological indices. The extent of glomerulosclerosis ($3,4 \pm 0,9$) was greater in group I rats when compared to animals fed a high protein diet ($2,5 \pm 0,1$) and those on a low protein intake ($1,8 \pm 0,5$). Results from the assessment of tubular atrophy were different; group I rats were again the most severely affected ($3 \pm 0,2$) but results from the other groups were much more similar; group II ($1,1 \pm 0,2$) and group III : ($1 \pm 0,5$).

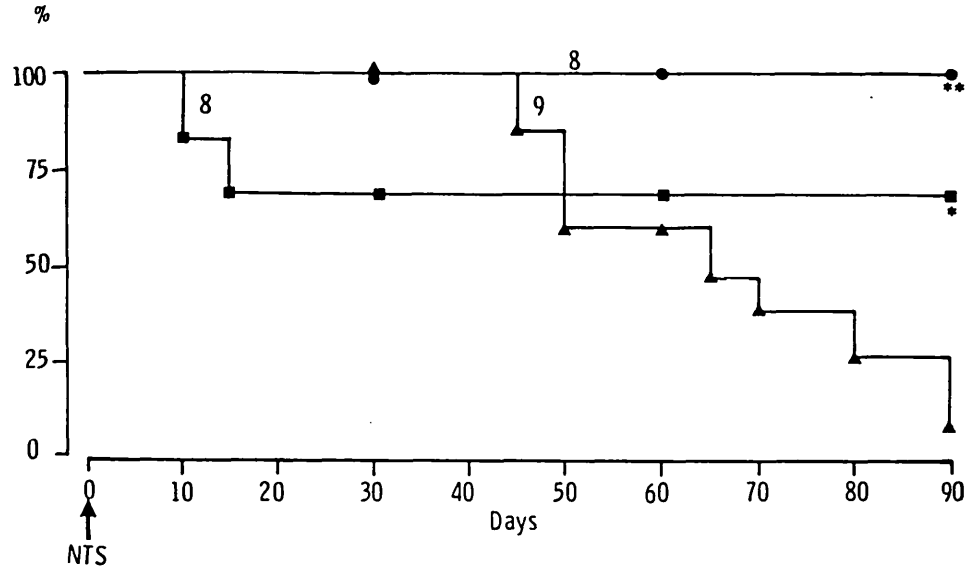


Figure 46 : Nephrotoxic nephritis - Effect of dietary protein manipulations on survival. The differences between the low protein ($^{**}p < 0.0025$), the high protein ($^{*}p < 0.0035$) and the normal diet were highly significant

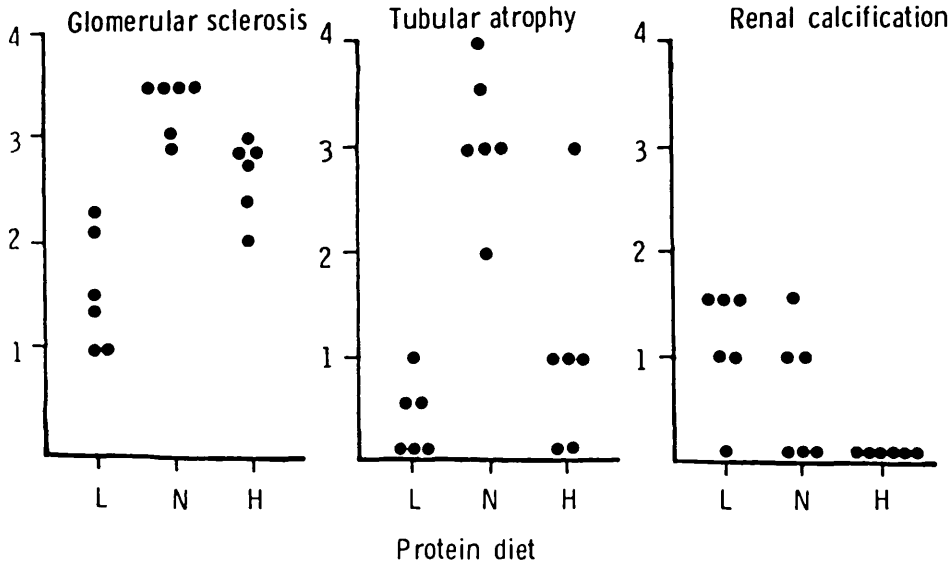


Figure 47 : Nephrotoxic nephritis - Effect of dietary protein manipulations on renal morphology

None of the rats fed the high protein diet developed tubular calcifications, compared to 3 of 6 rats on a normal diet and 5 of 6 rats on the low protein diet. (Figure 47,48).

Animals weight, blood pressure as well as the autologous antibody response were comparable in the three experimental groups.

The experimental diets had little effect on the proteinuria, renal function and histology of the three control groups.

Results from this experiment demonstrate the protective effects from renal failure of low and high protein diets when introduced in the early stages after the induction of nephrotoxic nephritis.

To confirm these results and to ascertain the influence on renal morphology in the first three weeks on the diets, experiment II was performed. Similarly, the following experiment was designed to exclude failure of matching as the cause for the differences in renal morphology observed on day 30 in experiment I.

Experiment (2) :

All rats had severe autologous phase injury with proteinuria of 128 $\pm$ 15mg/24 hours, with a concomittant increase in serum creatinine from 45 $\pm$ 2.5umol/l to 75 $\pm$ 4umol/l. Autologous phase injury in this experiment was similar to that observed in experiment (1). The results of renal biopsies taken on day 3 are shown in Figure 49). On day 30, rats on the normal diet had the most severe degree of glomerulosclerosis, with protection provided by low and high protein diets. (Figure 49)

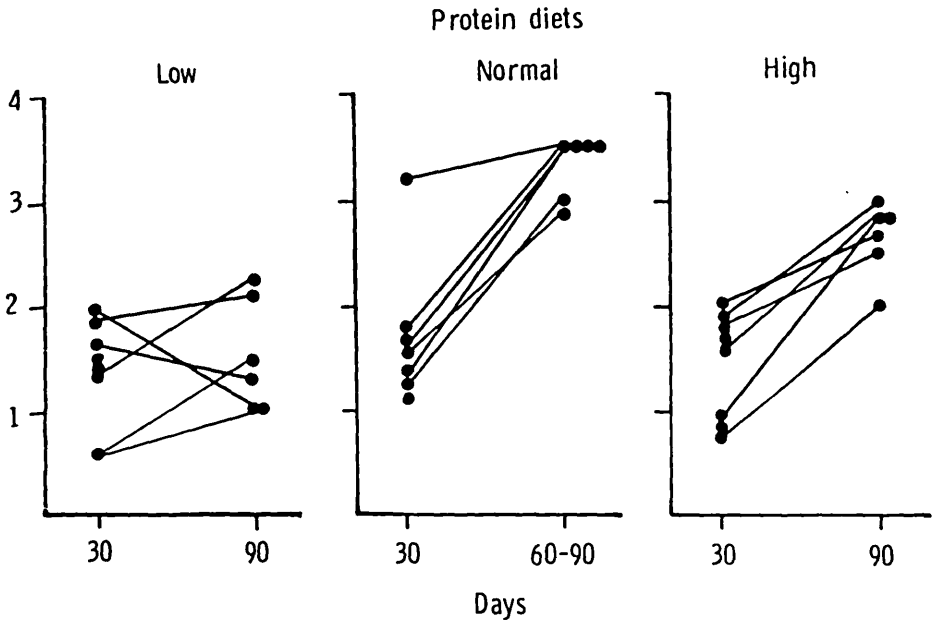


Figure 48 : Nephrotoxic nephritis - Effect of early dietary protein manipulations on glomerulosclerosis

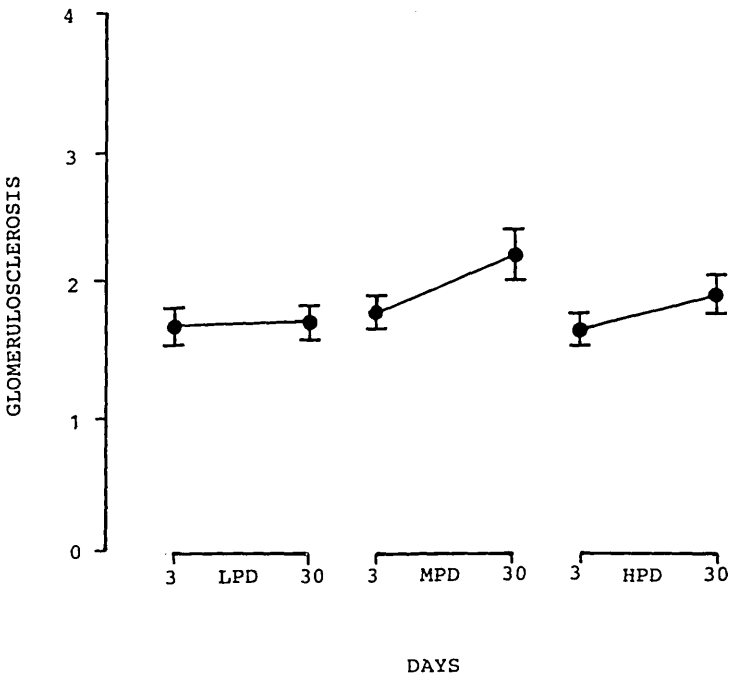


Figure 49 : Nephrotoxic nephritis - The effect of early dietary protein manipulations on glomerulosclerosis

Experiment 3 : The effect of late dietary protein introduction.

In the first two experiments, the diets were introduced 7 days after induction of glomerulonephritis at a time the acute glomerular inflammation, though subsiding, was still present. The third experiment was designed to see whether introduction of the diets on day 30, influenced the subsequent progression.

Twenty four rats were studied after all had a telescoped autologous injury; the proteinuria : 95 ± 10mg/24hr and serum creatinine 68 ± 7umol/l on day 3 reflected a less severe acute phase injury than in the two previous experiments.

Proteinuria

The course of proteinuria and the severity of the nephritis were comparable in the three experimental groups up to day 30, when the diets were introduced.

After the introduction of the experimental diets, proteinuria varied proportionally to the amount of protein ingested. (Figure 50)

Renal function

On a normal diet, the renal function remained preserved up to day 45 (serum creatinine : 47 ± 6,5umol/l). Afterwards the incidence of renal failure increased with serum creatinine reaching 148 ± 40umol/l on day 120. Rats on a low protein diet developed mild renal functional impairment with serum creatinine reaching 67 ± 4umol/l on day 120 ($p < 0,010$). Animals fed the high protein diet, although similarly protected, developed moderate renal failure; serum creatinine 105 ± 35umol/l on day 120 ($p < 0,025$).

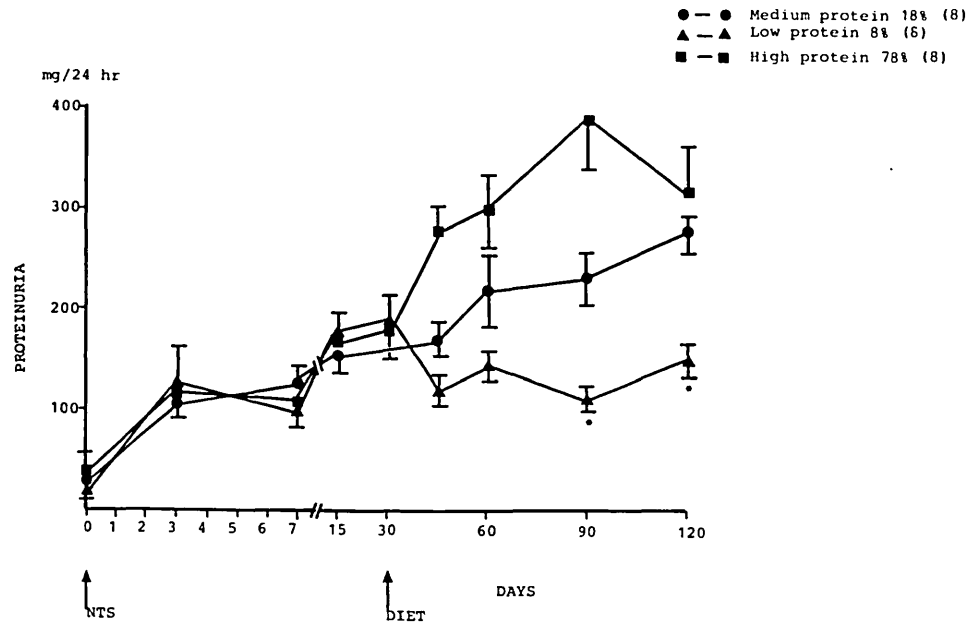


Figure 50 : Nephrotoxic nephritis - Effect of late dietary protein manipulations on the course of proteinuria
*p < 0.025

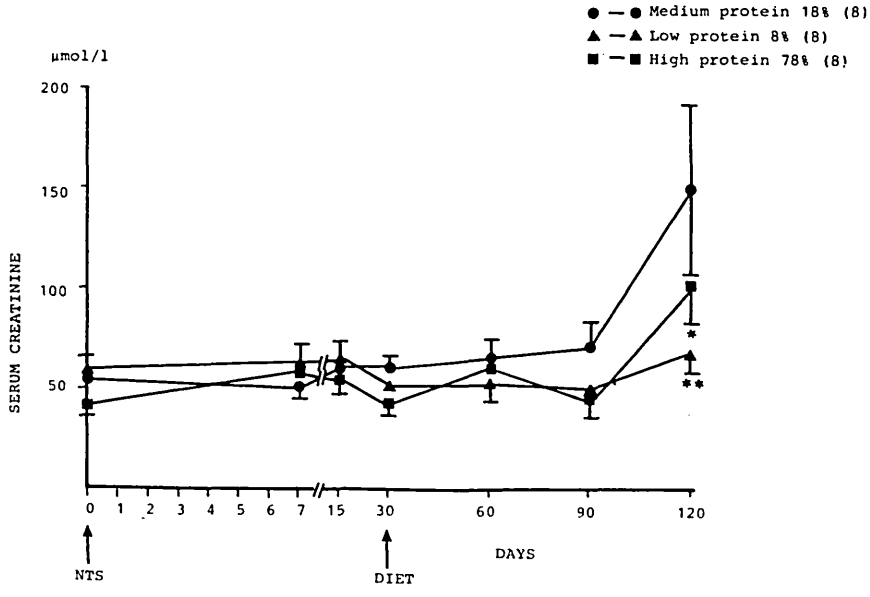


Figure 51 : Nephrotoxic nephritis - Effect of late dietary protein manipulations on the course of renal function. *p < 0.025, **p < 0.010

In the high protein diet group 3 of 8 rats developed renal failure, compared to 2 of 8 on the low protein diet and 6 of 8 on the normal protein intake. (Figure 51)

It seemed therefore that the late introduction of low and high protein diets protected rats with nephrotoxic nephritis from the development of severe renal failure. These results confirm the ones observed when the diet was introduced on day 7.

Survival

On a normal diet 6 of 8 rats died from renal failure by day 90, compared to 1 of 8 on the low protein diet ($p < 0.0025$) and 2 of 8 on the high protein diet ($p < 0.0035$).

Renal morphology

At day 90 the renal morphological lesions were severe in rats on a normal diet; glomerulosclerosis at this stage was 3.20 ± 0.40 and tubular atrophy was 2.62 ± 0.80. Both rats on low and high protein diets were protected from severe renal scarring; on a low protein diet, glomerulosclerosis was minimal at 1.68 ± 0.40 and tubular atrophy was 1.30 ± 0.20. On a high protein diet glomerular scarring was more severe than on the protein restricted diet 1.85 ± 0.40 while tubular atrophy was milder 1.07 ± 0.15.

The influence of diet on the relation between glomerular sclerosis and tubular atrophy after NTN :

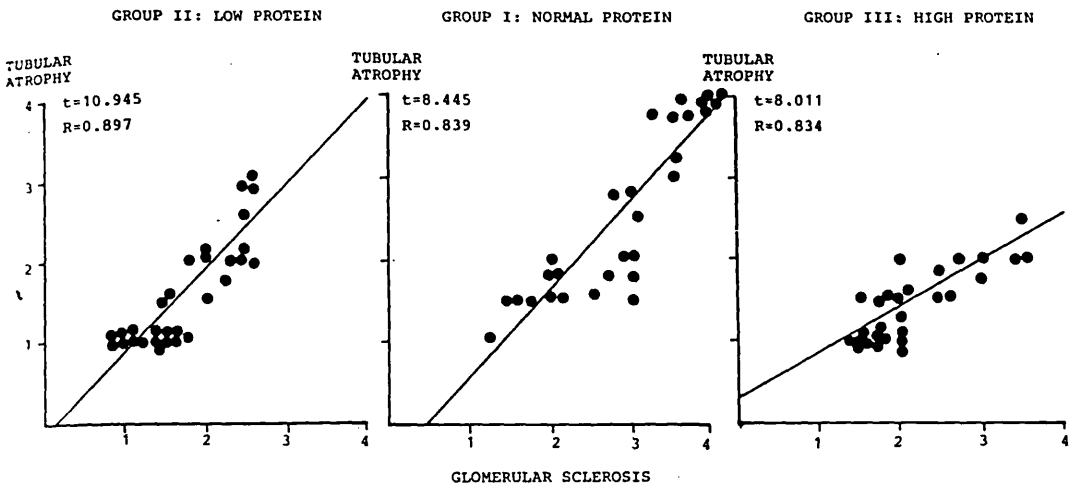


Figure 52 : Nephrotoxic nephritis - Correlations between tubular atrophy and glomerulosclerosis on the three experimental diets.

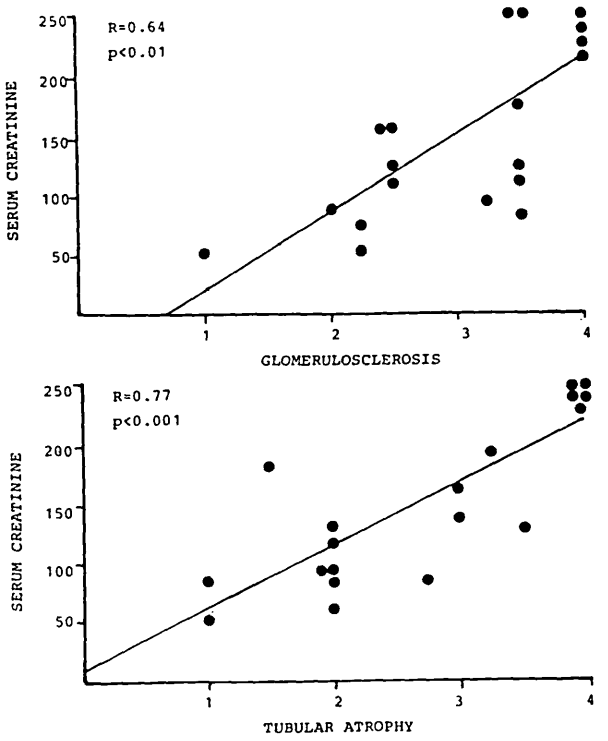


Figure 53 : Nephrotoxic nephritis - Correlation between glomerulosclerosis, tubular atrophy and renal function in rats on a normal diet

In all three experiments the rats fed on a high protein diet had glomerulosclerosis scores that were close to those found on the normal diet and had tubular atrophy scores that tended towards those found on the low protein diet; this disparity between the severity of glomerulosclerosis and tubular atrophy was most pronounced in animals followed for 90 days. This observation together with that in experiment I of a progressive decrease in the severity of tubular calcifications with increasing dietary protein intake, suggested that the relationship between the severity of tubular interstitial scarring and that of glomerular scarring might be influenced by the diet. The correlation between glomerular sclerosis and tubular atrophy was a strong one in the three experimental groups; group I $r = 0,792$, group 2 : $r = 0,896$, group 3 : $r = 0.766$ (Figure 52). The slope of the regression equation in group 3, on the high protein diet, is significantly different from the other two groups. Thus on a high protein diet the degree of tubular atrophy is significantly less for any degree of glomerulosclerosis. These findings became more relevant when we examined the correlations between renal function and glomerular and tubular morphological changes; a stronger correlation ($r = 0,77$ $p < 0,001$) was observed between serum creatinine and the extent of tubular atrophy compared to glomerulosclerosis ($r = 0,64$, $p < 0,01$). (Figure 53) while on a normal diet.

Discussion

The understanding of the pathophysiology of chronic renal failure is fundamental in designing successful therapeutic approaches to slow the rate of decline in renal function of chronic nephritides.

Brenner et al (1982) have recently postulated that progression of renal diseases to chronicity might be mediated by the haemodynamic compensatory changes that follow an acute reduction in renal functional mass. These authors suggested that compensatory glomerular hyperperfusion and hyperfiltration are the common pathway to glomerulosclerosis once a critical reduction in renal tissue was reached (Hostetter, 1982a). These observations were made in the remnant kidney model of chronic renal failure.

Whether, these sub-acute adaptive changes of glomerular haemodynamics occur in other experimental models of chronic renal disease is unknown. Indeed, in severe nephrotoxic nephritis, where all the glomeruli are diffusely involved by the acute injury process, Blantz and Wilson (1976) showed that these glomeruli tend to vasoconstrict and therefore be underperfused with a decrease in their filtration rate.

Experimental progressive sub-acute and chronic immune mediated glomerulonephritis might therefore respond to acute injury in a different manner to the sub-total nephrectomised kidney.

We attempted through this series of experiments to explore the mechanisms involved in the protective effects of changes in dietary protein, and to speculate as to the pathophysiology of experimental chronic glomerular diseases.

In these experiments, we observed that both low and very high protein intakes had some beneficial effects on the course of nephrotoxic serum nephritis.

A low protein diet prevented renal failure through its protection from severe glomerulosclerosis and sub sequent tubular atrophy. On the other hand, a high protein intake seemed to have slowed the progression of the nephropathy through decreasing the severity of the tubular scarring and calcifications.

The mechanisms underlying the effects of these diets on renal function and morphology are likely to be multifactorial. A low protein diet has intrarenal as well as systemic effects that could affect the rate of progression of nephrotoxic nephritis. Such a diet is known to reduce renal, glomerular, blood flow and increase glomerular arteriolar resistances (Ichikawa, 1980). It is precisely this mechanism that is thought to be operational in the protection against progressive renal scarring in the remnant kidney model (Hostetter, 1981a). Whether immune mediated chronic glomerulonephritis have hyperperfused glomeruli or segments of glomeruli remains to be determined. In this case the low protein intake might be protective in the nephrotoxic nephritis model through a similar mechanism to the one thought to prevent scarring in the sub-total nephrectomy model.

Similarly, a low protein diet is known to decrease the overall protein synthesis (Waterlow & Jackson, 1981) and might therefore prevent or reduce the amount of collagen deposited in the interstitium of chronically affected kidneys; Striker et al (1978) showed extensive deposition in the renal interstitium of collagen, and postulated that such deposits lead to interstitial fibrosis and eventually end stage renal failure.

A low protein diet has major metabolic and endocrine effects; it is known to reduce serum lipids (Liepa, 1980) and affects thyroid hormones (Rabolli, 1977), as well as insulin, glucagon and corticosterone secretions (Edozien, 1978). The role of the thyroid in the progression of nephrotoxic nephritis in rats has recently been emphasised by Tomford et al (1981) who showed an improvement in the course of the disease when rats were thyroidectomised. The role of other hormones in the progression of renal diseases is entirely speculative at this stage, but might prove of major importance.

Finally, a low protein diet significantly reduced the extent of the proteinuria during the sub-acute and chronic phases of nephrotoxic nephritis. This effect could conceivably contribute to the beneficial action of the low protein diet providing excessive proteinuria is nephrotoxic in itself (Davies, 1978). It is doubtful, however, that excess proteinuria is harmful to nephrotoxic nephritic kidneys as rats on a high protein diet had a fourfold increase in their protein excretion with no ill effects on the renal function.

On the other hand, the high protein diet with its increased solute load is likely to affect the kidneys through its local, intrarenal effects as well as its systemic actions. It is of interest that such diet, in these experiments, protected the renal tubules and interstitium from severe scarring and calcifications. These calcifications are thought to accelerate the decline in renal function and increase the severity of the tubulo-interstitial lesions in nephrotoxic nephritis; their prevention is known to protect nephritic kidneys from further renal function deterioration (Karlinsky, 1980).

The mechanism through which a high protein diet prevents nephrocalcinosis could be mediated by its known hypercalciuric (Bell, 1975) and urine acidifying effects (Whiting & Draper, 1980). These dietary influences could alter the calcium x phosphate product and prevent calcium phosphate deposition in the scarred kidneys.

Similarly, a high protein diet with its increased urinary solute load with subsequent osmotic diuresis could prevent renal tubules ischaemia and collapse, in a manner comparable to the protective effect of mannitol on ischaemic renal tubules; An increased urine flow induced by mannitol was found to prevent tubular collapse after an acute renal injury.

Equally important could be the effect of high protein intake on renal haemodynamics. Maddox et al (1975) observed a decrease in glomerular blood flow following severe nephrotoxic nephritis. While Blantz and Wilson (1981) emphasised the role of α-adrenergic vasoconstriction in the reduction of blood flow to nephritic glomeruli. A high protein diet, with its plasma volume expanding and peripheral vasodilatory, effects could counteract the haemodynamic change of nephritic glomeruli and prevent severe renal failure.

A high protein diet has systemic metabolic and hormonal effects (Edozien, 1978) that could contribute to its overall action in the kidneys.

This study is likely to pioneer new avenues of research into the therapy of progressive chronic renal diseases; as it clearly demonstrated more than one pathway to chronic renal failure in chronic glomerular diseases.

Both glomerulosclerosis and tubulo-interstitial scarring can ultimately lead to end stage renal failure in glomerular disorders.

In man, as early as 1968, Risdon and de Wardener stressed the importance of tubular atrophy in the progression of renal diseases and impairment of renal function. These authors observed a closer correlation between creatinine clearance and tubular atrophy compared to glomerulosclerosis.

More recently, Riemenschneider and Bohle (1980) emphasised the predominant role of increased interstitial volume in the impairment of renal function in chronic glomerulonephritis. These authors implied that enlarged renal interstitial volume due to the accumulation of collagenous fibres influenced glomerular filtration by narrowing the postglomerular vessel network.

From these and other studies (Mackensen et al, 1979) it would seem that tubular scarring is a major pathway to end stage renal failure in chronic glomerulonephritis. Its prevention by a high protein diet in this study had important, beneficial, repercussions on the progression of chronic, experimental, glomerulonephritis.

Therefore, it is conceivable that in the future prevention of progressive glomerular disease will be achieved through both the prevention of glomerulosclerosis and tubulo-interstitial scarring.

ii. Dietary urea

Introduction

In the previous sections it was observed that a high protein diet increased urine output and proteinuria in short as well as longterm experiments, in nephrotoxic nephritic rats.

Moreover, it seemed that a high protein diet had an overall beneficial effect on the course of the nephropathy; renal function was preserved and the severity of the morphological changes were reduced.

The action of high protein intake seems to have selectively affected and protected the renal tubular and interstitium while preventing renal calcifications. Meanwhile the glomerulosclerosis development and progression was less affected.

The mechanism of action of high protein diet on the renal tubulo-interstitium remains hypothetical; it could be due to a specific effect of the diet on the kidney or be the result of a non specific action. Indeed, a protein rich diet is known to expand the intra-vascular volume, increase renal blood flow, increase glomerular filtration rate (Ichikawa, 1980), increase tubular perfusion and flow beside its osmotic diuresis action.

In the next phase of my research I attempted to determine the respective contributions of osmotic diuresis and volume expansion on the course of nephrotoxic nephritis.

In order to evaluate the contribution of osmotic diuresis on the course of the disease, I used a high urea diet. This diet was aimed to reproduce the urea loading effect of the high protein intake, while rats were fed a normal protein diet

Osmotic diuresis, induced by mannitol, was shown to have a protective effect on the tubular damage induced by noroadrenaline; In this model of acute ischaemic renal failure an increased osmolar excretion had a potentially salutory effect when the agents inducing an osmotic diuresis had renal vasodilating capacities (Patak, 1979). Whether an osmotic diuresis, induced by urea could reproduce such beneficial effects in the nephrotoxic nephritis model of chronic renal failure was to be explored in this experiment.

Experimental protocol

Accelerated nephrotoxic nephritis was induced in 13 rats. The acute phase of the disease was left unperturbed with rats fed a normal diet.

On day 7, as the acute injury was subsiding I randomised the rats into two experimental groups.

Group 1, with 6 rats left on the normal diet

Group 2, with 7 rats on a normal protein diet supplemented by urea at 30gr/day/kg of body weight.

Similarly, I studied two control groups of 4 rats each on the experimental diets.

Assessment of nephritis progression was done at weekly intervals with renal function and proteinuria estimations. Renal morphology was evaluated at days 30 and 90, when the experiment was terminated.

Results

Urea supplementation markedly accelerated the course of the nephritis.

Proteinuria

In group 1, on the normal diet, proteinuria was high on day 3 (98 ± 15mg/24hr), this was followed by further increase, with proteinuria peaking on day 7 at (127 ± 24mg/24hr). The level of protein excretion slowly decreased and stabilised over the following 2 weeks. From day 30 on, proteinuria increased and reached (218 ± 46mg/24hr) on day 90.

Animals on a high urea diet had comparable levels of proteinuria days 3 and 7. However, once they were started on the urea supplementation, proteinuria increased significantly, and remained high on days 15 (340 ± 47mg/24hr), 30 (360 ± 86mg/24h), and 60 (322 ± 48mg/24h). The difference in protein excretion between the two groups was statistically significant, $p < 0.05$. (Figure 54).

It seems therefore that a high urea intake maintained a significant osmotic diuresis throughout the 90 experimental days; urine volume and subsequently proteinuria were considerably increased.

Renal function

Animals fed a normal diet had a significant increase in serum creatinine during the first week following the injection of nephrotoxic serum. During the sub-acute stage of the nephritis

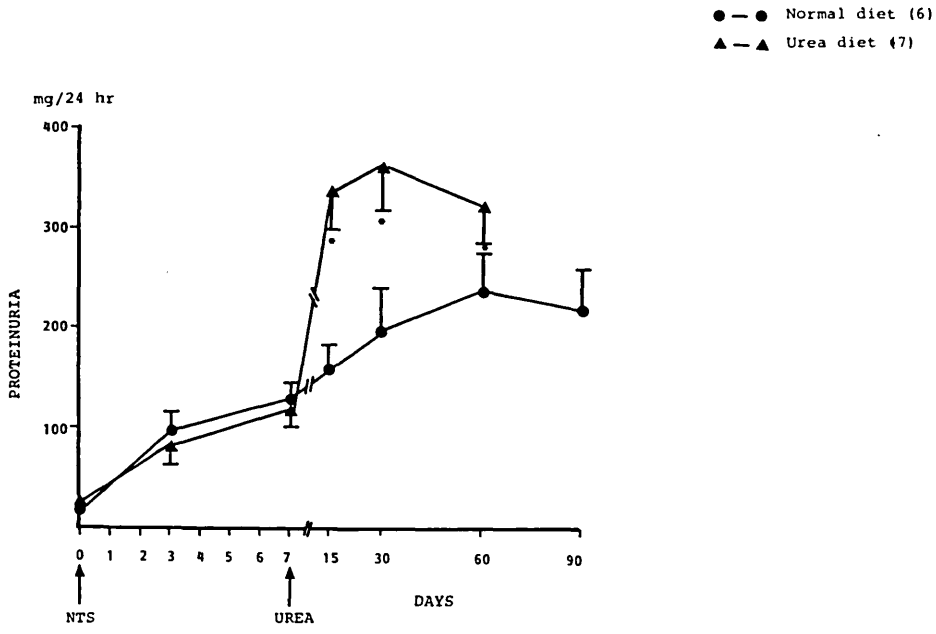


Figure 54 : Nephrotoxic nephritis - Effect of dietary urea supplementation on the course of proteinuria - $^{*}p < 0.05$

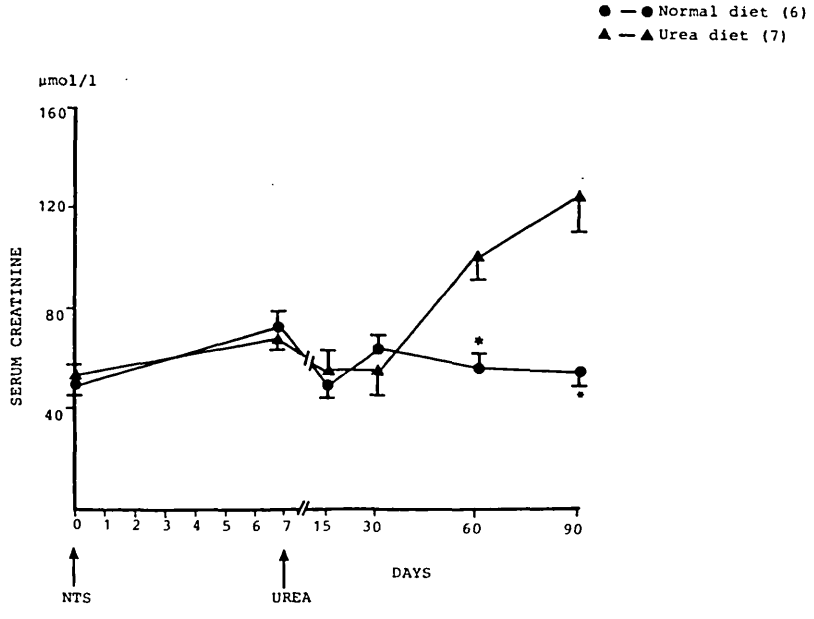


Figure 55 : Nephrotoxic nephritis - Effect of dietary urea supplementation on renal function - $^{*}p < 0.025$

(days 15 to 21) the serum creatinine returned towards normal and stabilised at this level up to day 90 (60 ± 8umol/l). In this group, the nephritis was milder than expected, with no significant (1/6) death from renal failure up to day 90.

In comparison, rats on the urea supplemented diet, although having a similar increment in their serum creatinine during the acute phase, had an overall more severe course; during the sub-acute phase their renal functions were still comparable to animals in group 1, however from day 30 onward, renal failure proceeded at an accelerated rate with the serum creatinine reaching 106 ± 23umol/l on day 60 and 125 ± 24umol/l on day 90 ($p < 0,025$). (Figure 55)

Survival

On a normal diet, 1 of 6 rats died from renal failure in comparison to 5 of 7 in group 2. This difference was significant ($p < 0,0035$). (Figure 56).

Renal morphology

Glomerulosclerosis was comparable in the two experimental groups on day 30, although slightly more severe in the urea fed rats. The glomerular and tubular scarring processes were accelerated in the urea group after day 30. Glomerulosclerosis remained unchanged between days 30 and 90 in normally fed rats, compared to a sharp increase in its severity in the urea fed rats; day 30 : 1,4 ± 0,3, day 90 : 2.5 ± 0,4. (Figure 57).

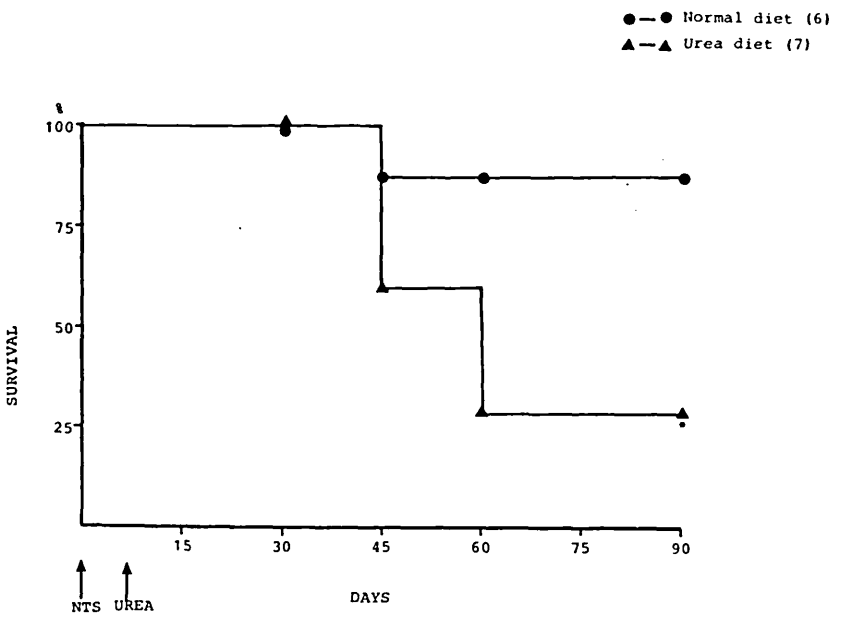


Figure 56 : Nephrotoxic nephritis - Effect of dietary urea supplementation on survival. * $p < 0.0035$

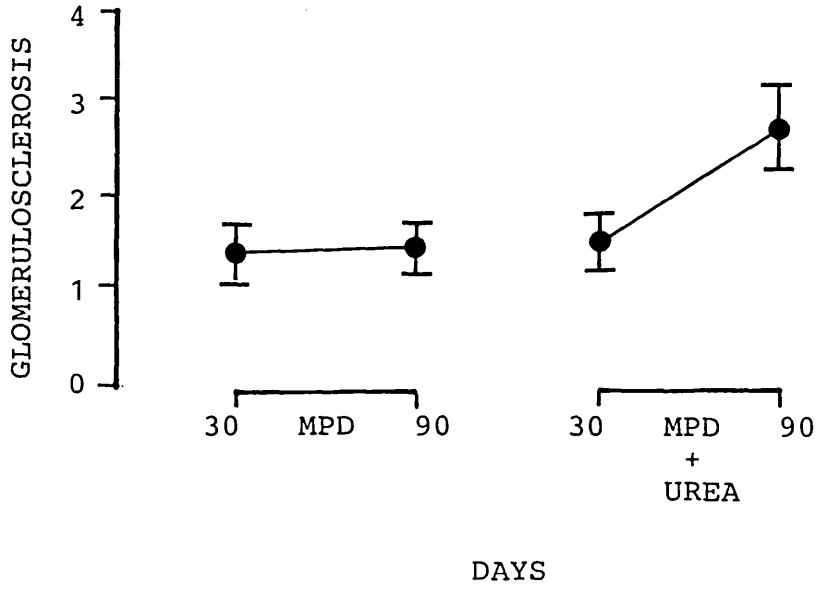


Figure 57 : Nephrotoxic nephritis - Effect of dietary urea supplementation on renal morphology

The tubulointerstitial changes paralleled the ones in glomerulosclerosis, in the two groups.

No significant changes in proteinuria, renal function (serum creatinine) or renal morphology (light microscopy) were observed between normal rats on the experimental diets.

Discussion

Although a high protein diet seemed beneficial to the course of renal failure in nephritic rats, a diet high in urea and with a normal protein content seems detrimental to the progression of renal scarring in nephrotoxic nephritis.

The urea supplemented diet induced an osmotic diuresis and significantly increased proteinuria, in a manner comparable to the high protein diet. However, it would seem that a high urine flow secondary to an osmotic diuresis is not in itself sufficient to prevent renal tubule collapse and subsequent glomerular scarring.

In other models of renal failure where glomerular vasoconstriction is predominant (Teschan & Lawson, 1966), osmotic diuresis has a protective, beneficial, effect. However protection was often obtained when the osmotic diuresis was concomittant to, or even preceeded, the onset of ARF. Moreso, the osmotic diuresis protective effect was blunted when renal resistances were increased prior to the ischaemic injury (Patak, 1979).

It is conceivable, therefore, that a urea induced osmotic diuresis failed to provide the same renal vasodilatory effect as a high protein diet. A high protein diet, has an alpha-adrenergic blocking action, and could provide further vasodilatory influences by supplying precursors to a variety of vasoactive amines (Bender, 1975).

Schnermann showed that an osmotic diuresis, by increasing the nephron, distal, fluid delivery and flow, could lead to reflux glomerular vasoconstriction (1981).

Finally, many authors have repeatedly observed a drop in glomerular filtration during sustained osmotic diuresis with increased proximal intratubular pressure (Navar, 1982).

Recently, Sadowski et al (1978) have described a new experimental model in the dog. In this model, the injection of urea into the renal artery induced proteinuria, reduced renal function and led to glomerular morphological changes. The authors postulated a direct nephrotoxic action of urea on the glomerular basement membrane. Here again, urea infusion led to a decrease in renal blood flow.

If the sub-acute and chronic phases of nephrotoxic nephritis are characterised by ischaemic changes secondary to glomerular vasoconstriction, an osmotic diuresis failing to reverse these changes might not be expected to improve the long term course of the nephritis.

Proteinuria was significantly increased by urea ingestion, it is conceivable that increased proteinuria with additional glomerular ischaemia combined to cause the observed deleterious effect.

I concluded from these experiments, that the high protein diet's beneficial effect on the course of the nephrotoxic nephritis is not entirely dependent on the changes in urine volume and solute excretion induced by such diet.

iii. Dietary salt and DOCA

Introduction

During this experiment I attempted to study the early effects of intravascular volume expansion and the late effects of hypertension on the course of nephrotoxic nephritis.

I have previously shown that intravascular volume expansion with a high protein, high urea and high salt diet increased the proteinuria, acutely, in nephrotoxic nephritis.

Longterm treatment of nephritic rats with a high protein diet proved beneficial while treatment with a high urea diet although inducing a similar osmotic diuresis was found to be harmful.

Following deoxycorticosterone acetate (DOCA) injections and increased dietary salt intake (1%) rats become immediately volume expanded, however hypertension is delayed (Selye, 1943). The onset of hypertension usually occurs 2 to 3 weeks from the institution of the DOCA and salt treatment.

Therefore, I was able to study the early phase (30 days) after DOCA and salt administration when volume expansion was predominant and rats were normotensive. This was followed by the late phase (days 30 to 90) when rats became hypertensive.

Previous workers have shown a detrimental effect of hypertension on the course of immune mediated experimental nephritis in rats. In both nephrotoxic nephritis (Motoki, 1978) and the autologous immune complex nephritis (Iversen, 1981) hypertension increased proteinuria and increased the renal scarring

process. Renal function was variably affected by hypertension in these two experimental models of chronic renal disease. Similarly, in both these models hypertension was exacerbated by the underlying nephritic process (Tikkanen, 1980).

Experimental protocol

Nephrotoxic nephritis (NTN) was induced according to the telescoped model.

Once the acute phase subsided by day 7, rats were randomised into two experimental groups.

Group 1, 8 rats were left untreated as the control, nephritic group

Group 2, 7 rats were treated with DOCA and salt.

These rats were injected twice weekly with DOCA : 0,25mg (im). The rat's drinking water was supplemented with sodium chloride to a final concentration of 1% and with potassium chloride to a final concentration of 0,2%. Concomittantly we studied two groups of normal rats: 7 treated and 5 untreated with DOCA and salt.

Assessment of disease progression

- The experiment was conducted over 90 days
- Serial weekly measurements of rats weight, blood pressure, renal function and proteinuria were assessed
- Renal morphology was examined at the end of the volume expansion phase (day 37) and at the end of the hypertension phase (day 90)

Results

The overall course of the nephritis was of moderate severity in the control group, with one death from renal failure during the observation period. The course of the nephritis was not significantly affected by concomittant volume expansion with and without hypertension.

Weights

The rate of rats growth varied considerably between the two nephritic experimental groups. Animals on DOCA and salt had a slow growth rate compared to near normal growth in untreated nephritic rats ($p < 0,05$). (Figure 58)

Hypertension

Rats with untreated nephrotoxic nephritis did not develop significant hypertension. Rats with NTN and DOCA-salt remained normotensive up to day 30 from the onset of the steroid and salt supplementation. Afterwards, hypertension developed, in group 2, and progressively increased. At days 45 , 60 and 90, the difference between the two experimental groups was significant; on day 45, group 1 : 121 $\pm$ 3mmHg and group 2 : 155 $\pm$ 6mmHg ($p < 0.05$), and day 90 : group 1 : 123 $\pm$ 2mmHg, group 2 : 175 $\pm$ 5mmHg ($p < 0,025$). (Figure 59)

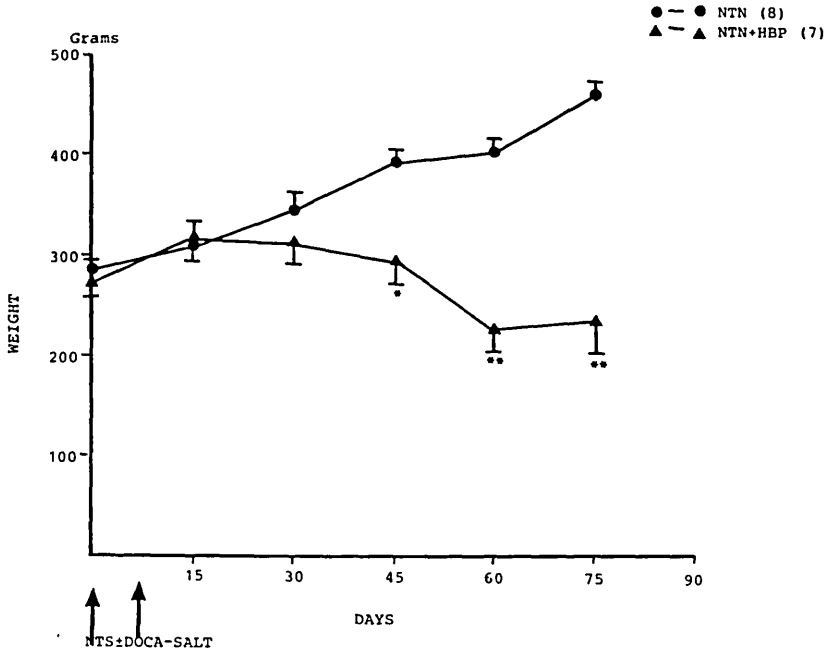


Figure 58 : Nephrotoxic nephritis - The effect of DOCA and salt supplementation on rat's growth rate
*p $<$0.05, **p $<$0.025

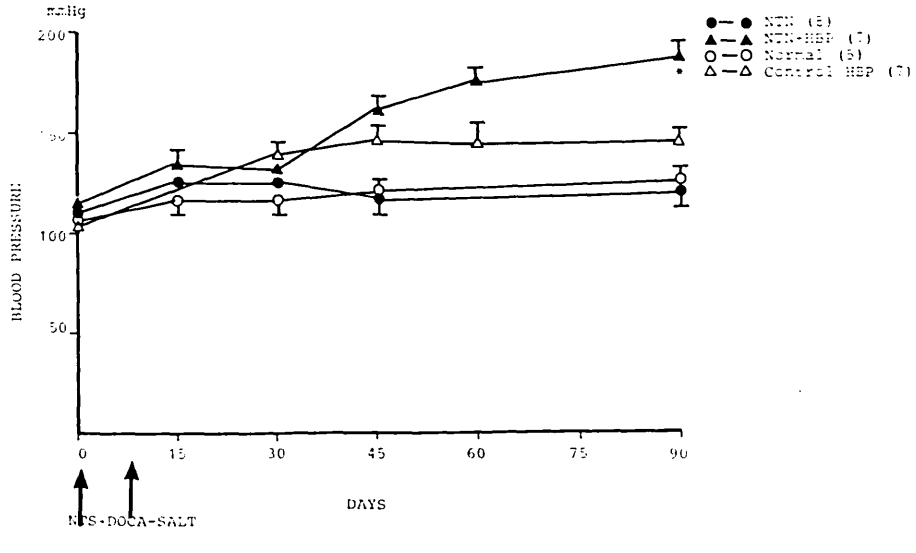


Figure 59 : Nephrotoxic nephritis - The effect of DOCA and salt supplementation on blood pressure - The significance of differences between nephritic rats with and without hypertension : *p $<$ 0.025

Equally interesting was the observed acceleration of hypertension in rats with nephritis compared to the control hypertensive ones; hypertension in nephritic rats occurred earlier (28 to 35 days) compared to (35 to 45 days) in controls, and was more severe ($p < 0.05$). (Figure 59).

Proteinuria

During the early phase of volume expansion, proteinuria varied proportionally to urine volume. Animals, treated with DOCA and salt, had a marked increase in their urine volume and proteinuria; this effect was short-lived and reversible, maximum within 24 hours of DOCA injection and decreasing following 48 to 72 hours.

After DOCA injections, nephritic rats increased their urine output by 275 $\pm$ 21%, with comparable increments in proteinuria (315 $\pm$ 40%). A close correlation was observed between urine volume and proteinuria in this experimental group.

During the hypertension phase of the experiment, proteinuria became fixed in rats of group 2. (Figure 60)

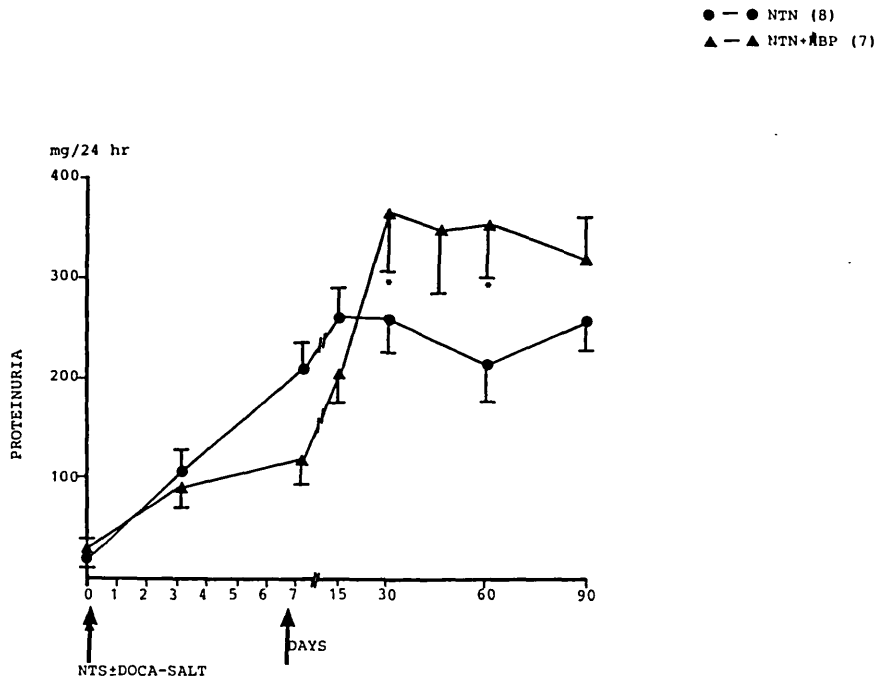


Figure 60 : Nephrotoxic nephritis - Effect of DOCA and salt supplementation on the course of proteinuria.
* $p < 0.05$

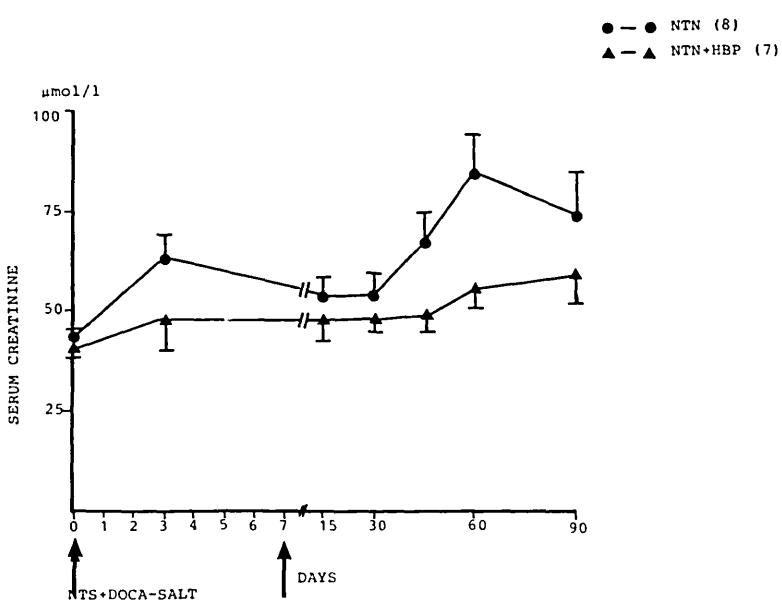


Figure 61 : Nephrotoxic nephritis - Effect of DOCA and salt supplementation on renal function

Renal function

Serum creatinine followed the usual pattern of nephrotoxic nephritis in both experimental groups; it increased during the first week following the induction of the nephritis, with subsequent normalisation during the sub-acute stage (days 15 to 30) and late increase between days 30 and 60. However, in view of the moderate course of the nephritis in these rats, at day 60 serum creatinine was 80 ± 17umol/l. The renal function, in animals with nephritis and DOCA-salt was not different at any stage, from the nephritic control group. (Figure 61)

Survival

1 of 8 rats died in the nephritic control group from renal failure on day 54. No death was observed in the hypertensive rats.

Renal morphology

In both groups, glomerular, tubular and vascular changes were of proportional severity.

At day 30, the glomerulosclerosis score was 1,57 ± 0.73 in group 1 with nephritis and 2 ± 0,65 (mean ± SD) in the nephritis-DOCA-salt group. These changes were more severe by day 90 : group 1 : 2.34 ± 0.90 compared to group 2 : 2.63 ± 1,19.

Although the glomerular lesions were slightly more severe in the hypertensive rats, the difference did not reach statistical significance. The severity of tubular lesions was comparable between group I : 2.06±0.65 and group II : 2.07±0.91.

Control, non nephritic, rats had normal renal function and histology throughout the experiment (days 30 and 90). The growth rate in the two control groups was comparable. Hypertension developed slowly in the non nephritic rats up to days 35-45 and was significantly less severe than in nephritic hypertensive rats.

Discussion

From these data, we failed to show a significant detrimental effect of volume expansion and hypertension on the course of nephrotoxic nephritis.

Although renal morphological changes were slightly more severe in the hypertensive group, the renal functions were comparable in the two experimental groups over the 90 days of the study.

It is likely that the observed changes in proteinuria following mineralocorticoid and salt administration represent the result of the intravascular volume expansion; stretching of the glomerular capillary wall (Shirley, 1957) and alterations of the glomerular basement membrane pore size (Myers, 1982) could explain the increase in protein leak of nephritic rats. These changes in urine volume and proteinuria over a long observation period confirm similar ones noted in the previous experiments over a much shorter (3 days) experimental span.

Here again, as after a high protein diet, although proteinuria increased there was no deleterious effects of such increase on the course of the nephropathy.

Increased salt intake and conceivably volume expansion was shown by Lalich et al (1975) to accelerate the rate of renal scarring after unilateral nephrectomy. A comparable result was not observed, in this experiment, in rats with nephrotoxic nephritis.

It is likely that the pathophysiology of glomerulosclerosis following unilateral nephrectomy of normal kidneys is not comparable to that following a diffuse acute nephritis as in nephrotoxic serum nephritis. In the first, where glomeruli are increased in size and with increments of their blood flow and filtration, additional increase in either could lead to further glomerular damage (Hostetter, 1982a).

By comparison, in nephrotoxic nephritis where glomeruli might be ischaemic and hypoperfused, volume expansion with the subsequent increase in renal blood flow might be beneficial. On the other hand, prolonged salt induced hypertension has been shown by Azar et al (1974) to induce a renal vasoconstrictive response.

Conclusions as to the role of hypertension on the progression of nephrotoxic nephritis cannot be made on the basis of this observation, as 90 days of hypertension are probably unlikely to be sufficient for significant changes to take place (Matthiesen & Tuck, 1978).

Summary of animal experimentation

From the series of experiments in rats with chronic renal diseases I determined some of the factors that influence the course of the underlying nephropathies.

Firstly, I showed in the nephrotoxic nephritis model of chronic renal failure, that the progression to chronic renal failure with severe glomerular scarring was not significantly affected by the magnitude of the host's systemic immune response. Nephritis progressed to end stage renal failure in animals with and without high titres of circulating autologous antibodies. The determinant of disease progression in this model seemed to be the extent of the renal injury during the sub-acute stage of the disease; with proteinuria at this late stage a very reliable indicator of the longterm, course of the nephritis. Secondly, we demonstrated a beneficial effect to both low and high protein intake on the course of experimental renal failure. These experiments showed that the course of a non immune mediated model of uremia, the remnant kidney model, could be substantially improved by a dietary protein restriction. In this model glomeruli are normal at the onset of the nephropathy and therefore could readily respond to the hemodynamic alterations induced by the diet.

Finally, I extended these observations to the immune mediated nephrotoxic nephritis model. In these rats I showed that a low protein diet protected from the development of renal failure and death from uremia. Glomerulosclerosis was minimal in animals fed the low protein diet. Similarly, in the same model I demonstrated that a high protein intake was equally protective against progressive

renal failure. It seemed, however, that the increased protein intake protected the kidneys mainly against tubulo-interstitial scarring and calcifications.

From these data, in chronic nephrotoxic nephritis I established that progression to end stage renal failure requires severe combined glomerular and tubular scarring. Protection from either glomerulosclerosis or tubulo-interstitial scarring can prevent death from uremia.

Fourthly, I showed that the beneficial effect of a high protein diet is unlikely to be solely mediated by its osmotic diuresis effect as a high urea, normal protein, diet failed to reproduce the same protection in nephrotoxic nephritis.

And finally, I cast some doubts on the nephrotoxicity, in experimental models of renal disease, of proteinuria as diets rich in protein, urea and salt all increased the amount of urinary protein with variable influences on the underlying nephropathies. In these models, I established however, that proteinuria was not the sole result of the organic renal damage as pre-glomerular, glomerular and post-glomerular contributions all seemed to participate in its expression.

The next phase of my research was addressed at applying some of these findings in exploring the pathophysiology of chronic renal diseases in man.

This phase was divided into two experimental subsets; the first looking into the effects of low protein diets on the progression of chronic renal failure of different etiologies. The second attempting to determine the acute effects of low and high protein intake on renal physiology and correlate these findings with the ones observed during the long term experiment.

B. Results of clinical investigations in man

I. Role of dietary protein in the progression of chronic renal disease

Introduction

In man, chronic renal diseases often progress relentlessly, with end stage renal failure a fairly predictable outcome. The rate of progression of nephropathies, although variable between individuals does not seem to be influenced by the nature of the underlying disease (Mitch, 1975).

The mechanisms involved in the progression of nephropathies from the acute to the chronic stages are largely unknown. However, recent attempts at understanding the pathophysiology of chronic renal failure in experimental animals have yielded interesting results and opened new avenues of research (Brenner, 1982).

In experimental animals, it would seem that progressive renal scarring is a multifactorial process with genetic, immune and haemodynamic influences all contributing to the final destruction of the renal parenchyma. The relevance of some of these factors in the progression of renal diseases in man is still subject to research and controversy.

In rats, for instance, haemodynamic factors are believed by some to play a major role in the progressive sclerotic changes that affect the glomeruli during the chronic stages of nephritides (Hostetter, 1982b). Two glomerular haemodynamic patterns have emerged as likely contributors to the changes in glomerular morphology following an acute renal injury.

The first, glomerular overperfusion and hyperfiltration and the second consisting of glomerular ischaemia and hypofiltration.

Glomerular hyperperfusion and hyperfiltration occurs when remnant glomeruli compensate for the loss of adjacent functional renal mass by reducing their arteriolar resistances and therefore increasing their blood flow and filtration rate (Deen, 1974). These changes although beneficial in the short term, in preserving renal function are likely to be harmful in the longterm by leading to progressive glomerular attrition.

The concept of remnant glomerular hyperperfusion and hyperfiltration has been advocated by Brenner et al. These authors observed these haemodynamic changes in the remnant kidney model of chronic renal failure (Hostetter, 1981b). Similar observations were made in the diabetic kidney and in experimental chronic pyelonephritis (Lubowitz, 1969).

In these conditions glomeruli are morphologically normal at the onset of the experiment. One would therefore expect them to maintain their vascular reactivity and rapidly adjust their perfusion and filtration to accomodate for the loss of adjacent nephrons.

On the other hand, in conditions where glomeruli are severely affected at the onset of the nephropathy, as in experimental glomerulonephritis a different haemodynamic pathway was observed. In this case, glomeruli responded to injury by a severe vasoconstrictive reaction leading to glomerular ischaemia and underfiltration (Blantz & Wilson, 1976).

Therefore, it would seem that in experimental models of chronic renal failure, a spectrum of glomerular haemodynamic changes could be observed.

Whether, similar changes occur in the renal microvasculature of patients with chronic renal diseases is unknown.

In this experimental section I attempted to determine, through dietary interventions, if comparable renal haemodynamic pathways could be envisaged in man.

In animals, the reduction of dietary protein intake has proved beneficial in preventing the compensatory changes of the remnant glomeruli (Hostetter, 1981b). Moreso, we have shown that such a diet slowed the rate of decline in renal function, delayed the onset of proteinuria, decreased the incidence of glomerulosclerosis and eventually significantly reduced the mortality from renal failure in rats after sub-total nephrectomy. In this model, where glomerular hyperperfusion and hyperfiltration predominate during the sub-acute and chronic stages of the nephropathy a diet low in protein, known to reduce these haemodynamic changes had prevented glomerulosclerosis.

Similarly, in man dietary management of patients with chronic renal failure has been potentially extremely beneficial; a low protein diet, not only, alleviates the symptoms of terminal uraemia but equally it was shown to slow the rate of progression of chronic nephropathies. This effect on renal failure was

recently shown by Maschio et al (1982) to be optimal when the dietary restrictions were introduced early in the course of the nephropathies. However the response of patients to a low protein diet, although generally favorable, is extremely variable and erratic with some patients showing a dramatic improvement while others continue to follow their course to end stage renal failure unaffected.

I undertook this three part study, in man, in order to delineate the influence of dietary protein variations in chronic renal diseases and to determine the factors that could predict the response to the diet as well as those that might affect the response to dietary protein restriction.

Results

Experiment I

The progression of renal failure in most patients occurred in a linear, predictable, fashion. However, the rate of progression of chronic renal failure varied according to the underlying nephropathy; patients with CGN had an overall faster rate of 1/serum creatinine decline (- 0,916) when compared to patients with CTIN (- 0,506) ($p < 0,025$) as well as patients with HNS (- 0,751) and PKD (- 0,572). The differences between CGN and HNS, PKD did not reach statistical significance.

Similarly, proteinuria in the glomerulonephritis group (3,57 $\pm$ 0,65g/24 hours) was significantly higher than in the CTIN group (1,46 $\pm$ 0,47g/24 hours), ($p < 0,05$) as well as in the HNS patients

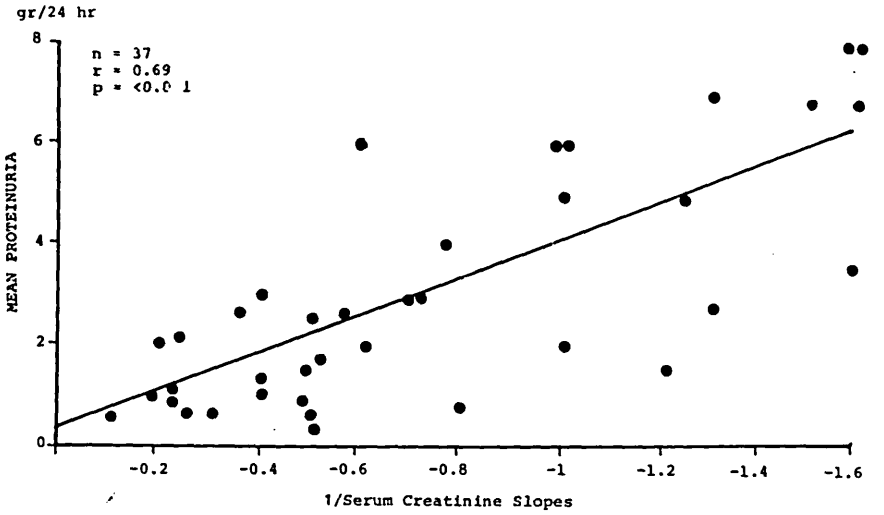


Figure 62 : Correlation between proteinuria and the rate of decline in renal function in patients with different nephropathies

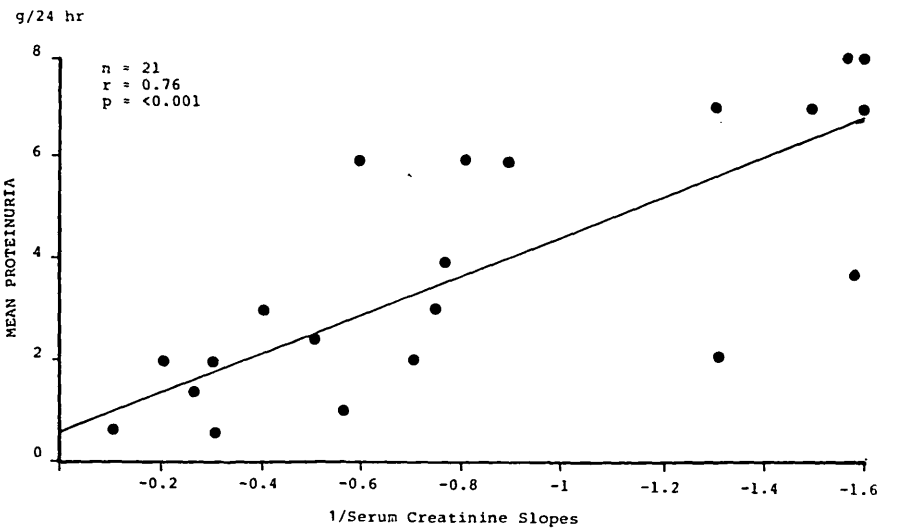


Figure 63 : Correlation between proteinuria and the rate of decline in renal function in patients with chronic glomerulonephritis

(1,08 ± 0,34g/24 hours), ($p < 0,025$) and the PKD patients (1,20 ± 0,35g/24 hours), ($p < 0,025$).

In the chronic glomerulonephritis group, the level of proteinuria (less or more than 2g/24 hours) discriminated between patients with slow (- 0,47 ± - 0,24) and those with fast (- 1,17 ± 0,62) reciprocal of serum creatinine slopes of renal function deterioration ($t = 3,22$, $p < 0,025$).

Proteinuria in this study appeared to be the sole prognostic factor that correlated closely with the outcome of the nephropathy ($r = 0,69$, $p < 0,01$). This correlation was stronger when only patients with chronic GN were studied ($r = 0,76$, $p < 0,001$). (Figure 62,63)

No significant difference was observed between the groups' serum phosphate or blood pressure recordings. These variables failed to correlate with the rate of progression of chronic renal failure.

From this study we confirmed the poor prognostic value of persistent proteinuria in chronic nephropathies.

Experiment II

General observations

Compliance to the diet was good with only 3 patients out of the 39 dropping out of the study. Patients were stable throughout the two experimental periods. Their nitrogen balance, as judged by the urea nitrogen appearance (UNA), was positive 1±0.35 g/24 h and no reduction in serum albumin was observed.

Effect of low protein diet on the progression of chronic renal failure

The renal function at the onset of the observation period (pre-diet) and the baseline reciprocal of serum creatinine slopes were comparable between the four major experimental groups. The introduction of the low protein diet improved the overall rate of decline in renal function of the whole experimental group over the six months observation period ($p < 0,05$) (Figure 64)

However, the response to the dietary protein restriction varied significantly between the experimental groups; patients with chronic tubular disorders (CPN + PKD) had a good response to the diet ($t = 2,53$, $p < 0,025$), while patients with chronic glomerular and vascular disorders (CGN + HNS) had a poor response to the LPD ($t = 1,18$, p = NS). Within these two major groups, patients with chronic pyelonephritis had the best response with a marked slowing of the rate of decline of their renal function on the diet; ($t = 3,26$, $p < 0,025$). On the other hand, patients with hypertensive nephrosclerosis had a poor response to the diet with only one patient out of 9 improving ($t = 0,3$, p = NS). This hypertensive patient with only one kidney, following a unilateral nephrectomy for nephrolithiasis, improved dramatically on the diet with a slope increasing from -2 to +0,72. Finally, patients with chronic glomerulonephritis showed a variable response to the diet ($t = 2,13$ $p < 0,05$) with some patients improving significantly while others remained unaffected. (Figure 65)

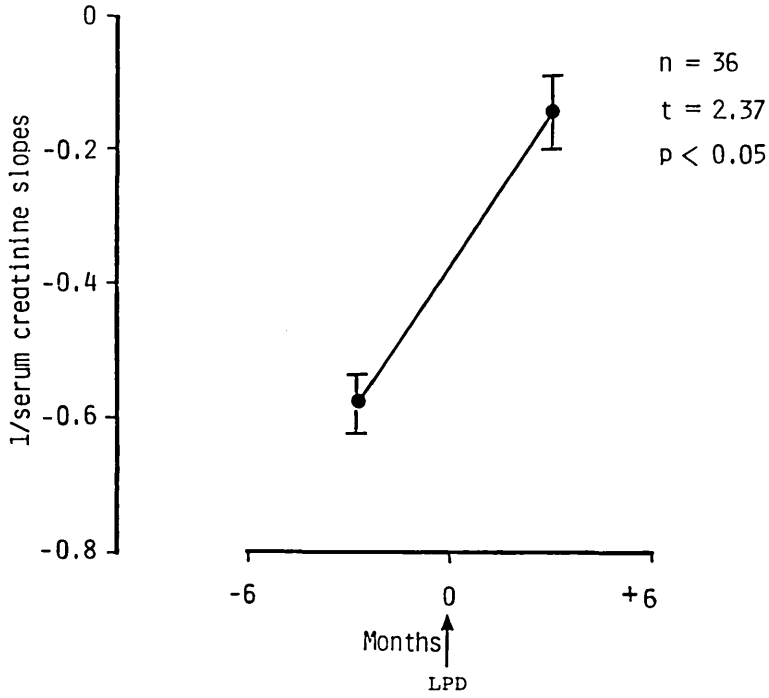


Figure 64 : Overall effect of low protein diet on renal function in patients with chronic renal failure

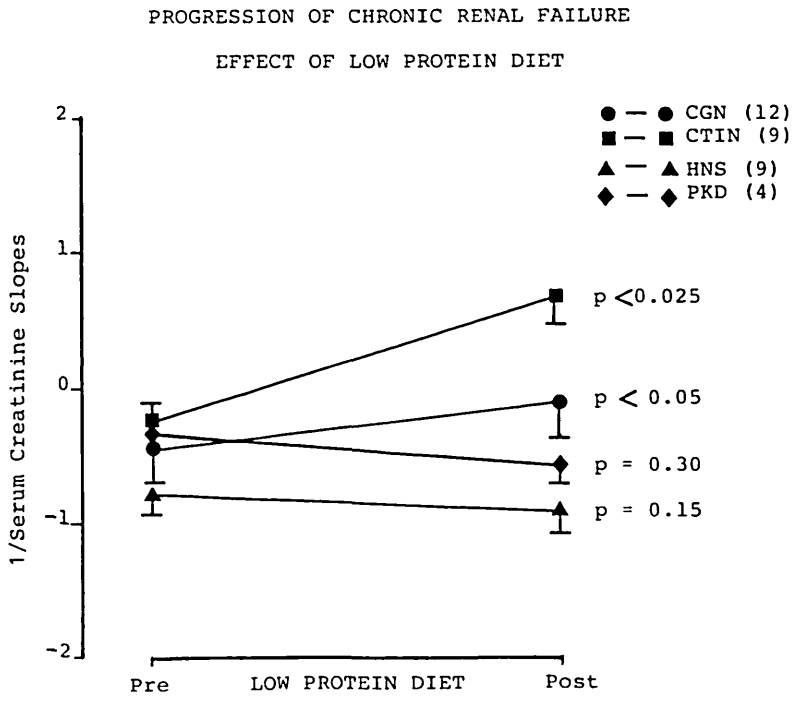


Figure 65 : Differential effect of low protein diet on different nephropathies

Effect of the LPD on proteinuria

A significant reduction in proteinuria was observed in the whole experimental group ($t = 2.63$, $p < 0,025$) (Figure 66). More significantly, in patients with chronic glomerulonephritis, the response of proteinuria to the dietary protein restriction seemed to discriminate between responders and non-responders in regards to improvement in renal function (Figure 67). Finally, there was a close correlation between the reduction of proteinuria on the LPD with the overall improvement in creatinine slopes ($r = 0,76$, $p < 0,01$) (Figure 68).

The low protein diet reduced serum urea and had no significant effects on serum phosphorus and blood pressure control.

From this experiment it became apparent that chronic tubular disorders where glomeruli are relatively spared responded favourably to a low protein diet. While conditions with predominant glomerular or vascular involvement failed to improve on such a diet.

Experiment III

Effect of dietary protein variations on renal plasma flow

Protein loading led to a significant increase in effective renal plasma flow ERPF , ($t = 3.8$, $p < 0,025$) in these patients with chronic renal failure.

Similarly, reduction in dietary protein intake led to a decrease in renal blood flow ($t = 2.4$, $p < 0.05$), although a smaller magnitude (Figure 69). However, the magnitude of the changes in renal blood flow on the two experimental diets varied between individual patients and between experimental subgroups; 4 out of

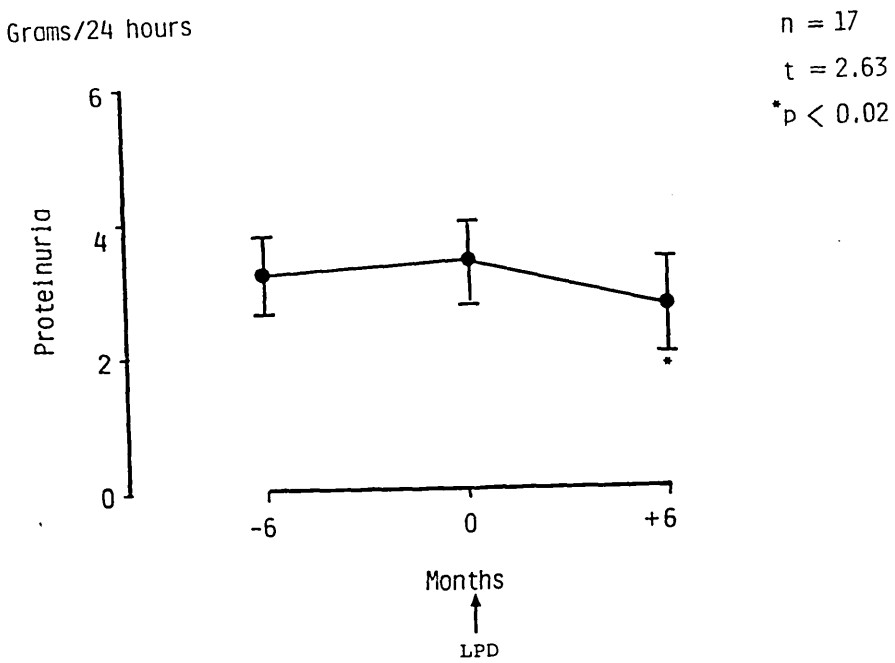


Figure 66 : Effect of low protein diet on proteinuria of patients with chronic renal disorders (Mean±SD).

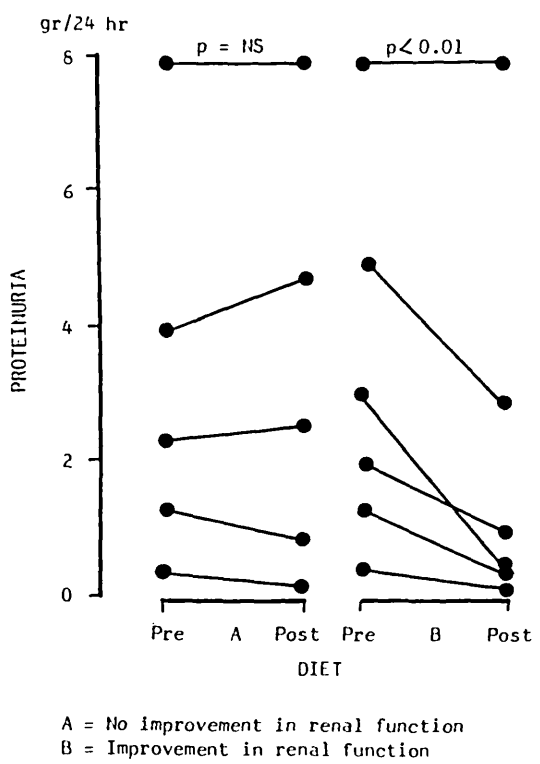


Figure 67 : Response of proteinuria to low protein diet in patients with chronic glomerulonephritis

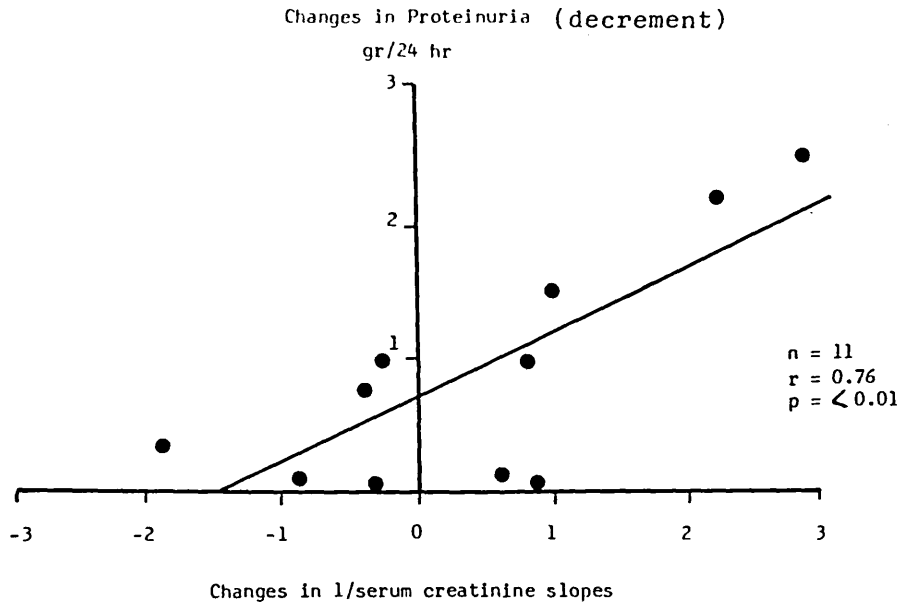


Figure 68 : Correlation between changes in rate of decline in renal function and decrements in proteinuria on a low protein diet

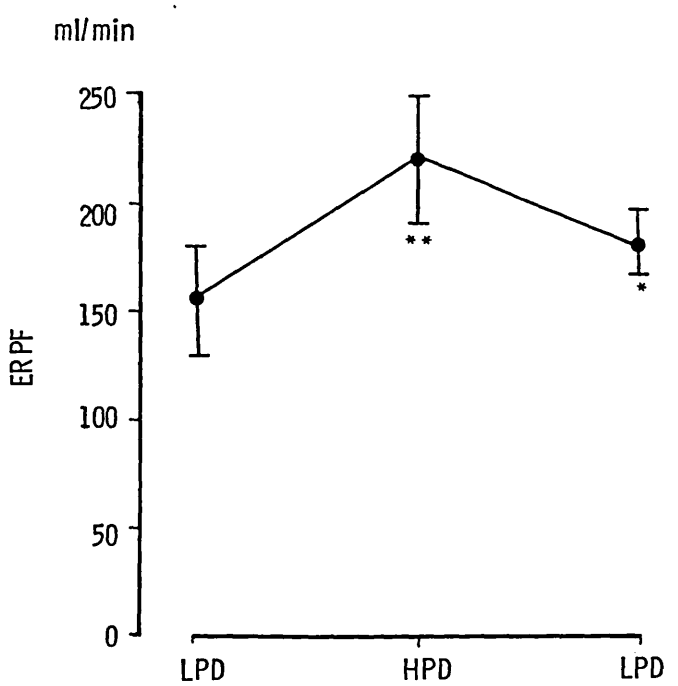


Figure 69 : Effect of acute dietary protein manipulations on effective renal plasma flow in chronic renal diseases - *p<0.05, **p<0.025 (Mean±SEM).

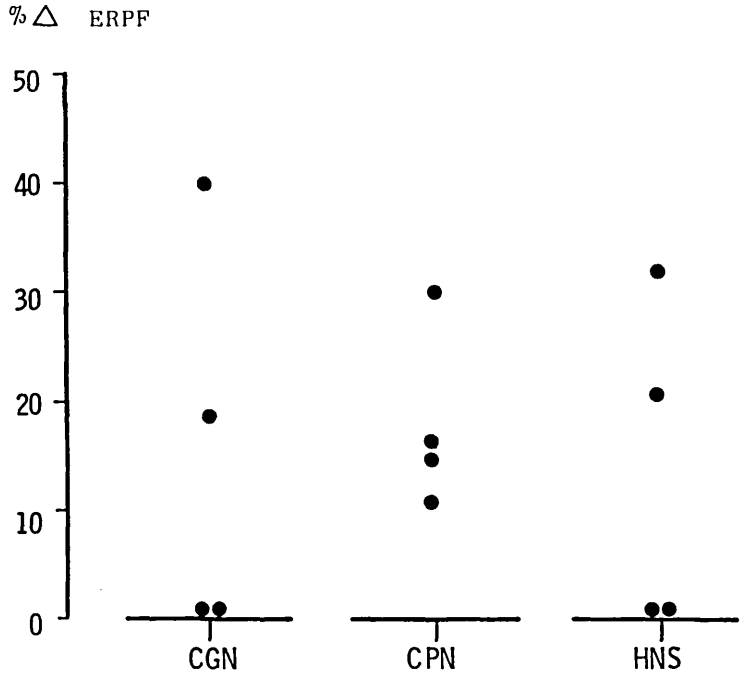


Figure 70 : Percentage decrements in renal plasma flow, in response to a low protein diet, of different nephropathies

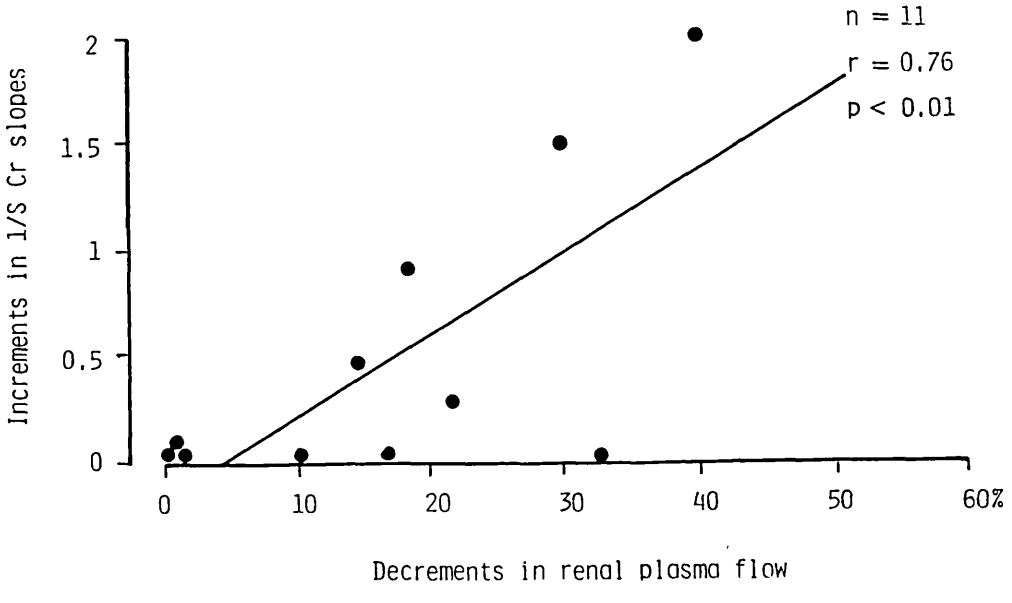


Figure 71 : Correlation between the short term haemodynamic and longterm functional effects of low protein diets

4 patients with chronic tubulo-interstitial diseases responded to the diets while only 2 out of 4 in each of the CGN and HNS groups had a significant variation in their renal perfusion. (Figure 70)

Effect of dietary protein variations on the GFR

None of the 12 patients had a significant change in his glomerular filtration rate during the two experimental periods. This is likely to represent the loss of renal functional reserve of these patients with impaired glomerular filtration rate. Changes in filtration fraction and renal vascular resistance inversely correlated with changes in renal blood flow.

The vascular reactivity of these patients in response to the low protein diet, although over a short period (2 weeks), correlated with their previous response pattern to the same diet over a longer observation time (6 months) ($r = 0.76$, $p < 0.01$) (Figure 71).

From these data it would seem that patients who are capable of reducing their renal plasma flow and increasing their renal vascular resistances in response to a low protein vaso-constricting, diet are the ones who benefited most from it in terms of overall improvement in their renal function.

Discussion

Progression of renal diseases to chronicity with end stage renal failure continues to be a challenge to medical science, as well as a growing social and economical problem in many countries. The mechanisms involved in the progression of nephropathies to chronic renal failure have recently become the focus of major research and

advamces. Brenner et al advocated the compensatory glomerular hyperperfusion and hyperfiltration hypothesis to explain the progressive glomerular scarring that occurs in most forms of end stage renal failure (1981). This interesting concept is based on observations made in the remnant kidney (Deen, 1974) and to a lesser extent in diabetic nephropathy (Hostetter, 1981a) models of experimental renal failure.

Similar observations had been made as early as 1966 by Lubowitz et al who observed the presence of hyperperfused and hyper-filtrating supernephrons in experimental chronic pyelonephritis. However, these authors and others (Rocha, 1973) failed to show supernephrons in experimental glomerulonephritis. Moreso, Blantz and Wilson (1976) have recently observed that in severe progressive experimental nephrotoxic glomerulonephritis, glomerular hypo-perfusion and decreased filtration was the rule. These authors demonstrated that such glomerular vasoconstrictive response to injury is mediated by the α-adrenergic system (Blantz, 1981), while others have established its dependency on thromboxane synthesis (Dunn, 1983).

It would seem, therefore, that glomerular response to renal injury, in experimental renal failure, varies significantly according to the nature of the initial insult; glomeruli when spared at the onset, or when mildly damaged (Allison, 1974) are capable of compensatory, adaptive changes. On the other hand when glomeruli are severely involved at the onset of the nephropathy, they would seem to be incapable of increasing their blood flow and progressive glomerulosclerosis might, in this case, be due to glomerular ischaemia

A low protein diet has been proved beneficial in experimental models where glomerular hyperperfusion predominated; the vasoconstrictor effect of this diet blunted the compensatory hyperperfusion pattern (Hostetter, 1981b) and protected from severe progressive renal failure (Kleinknecht, 1979).

I used a low protein diet to explore the underlying pathophysiology of chronic renal diseases in man. A low protein diet, long known to improve the outcome of chronic renal failure in man (Kluthe,1971), was shown for the first time in my study to have a selective, variable effect on different nephropathies. Patients with predominantly non-glomerular diseases have responded well to the low protein diet. While patients with chronic glomerulonephritis and hypertensive nephrosclerosis failed to improve significantly on the same diet.

Indeed, in hypertensive nephrosclerosis, where the vascular, arteriolar, lesions are diffuse with resulting glomerular ischaemia, a low protein diet had no beneficial influence on the rate of decline of the renal function.

Interestingly, the only patient, in this group, who improved had a single kidney following unilateral nephrectomy. These data, in man, are in keeping with the known haemodynamic patterns of glomeruli in experimental hypertension; Azar et al (1974) have shown glomerular ischaemia and vasoconstriction in the two kidneys hypertension models, while in the one kidney model glomerular vasodilatation overcame the hypertensive ischaemia tendency (Azar, 1977).

In patients with chronic glomerulonephritis, the response to dietary protein restriction was variable and might have reflected the heterogeneity of remnant glomerular morphology and function; such a diet would be expected to have little effect when vascular sclerosis predominated with subsequent glomerular ischaemia. Kincaid-Smith et al (1975) reported a high incidence of vascular sclerosis in chronic glomerulonephritis, these patients would not be expected to improve upon further reduction of their renal blood flow.

On the other hand, when the arteriolar lesions are not predominant in glomerulonephritis, the very nature of the glomerular damage is often heterogenous with possibly focal and segmental hyper-perfusion and hyperfiltration. These patients might be the ones who improved on the low protein diet.

Another potential beneficial effect of dietary protein restriction was the observed reduction in proteinuria. The onset, severity and persistence of proteinuria in chronic glomerular disorders have always been poor prognostic indicators (Cameron, 1979a). Proteinuria in itself has been incriminated in the progression of chronic glomerular scarring (Davies, 1978). Reduction of proteinuria in this study could reflect the improved renal structural damage, or, more likely, represents the reversibility of the glomerular hyperperfusion, hyperfiltration pattern; patients with glomerulonephritis and fixed vascular as well as glomerular structural damage and irreversible perfusion state would have failed to reduce their proteinuria on the low protein diet.

Meanwhile, the patients with reversible hyperperfusion could have decreased their proteinuria and continued to lose their renal function albeit at a much slower rate.

It is important, however, to remember that a low protein diet has systemic, metabolic (Liepa, 1980) and hormonal (Edozien, 1978) effects that could all contribute to its known salutory influences in chronic renal diseases.

I have shown in this study, that the renal vascular reactivity could be severely affected by renal diseases. I would like to believe that the extent of the renal vascular response to dietary protein manipulations might hold the key to the overall response of individual patients to a reduction in protein intake; regardless of the underlying nephropathy, patients who decreased their renal plasma flow on the low protein diet had benefited from such a diet by slowing the rate of the decline in their renal function.

Finally, it would seem from my data that the loss of renal functional reserve is a universal finding in chronic renal diseases; all of the patients, when challenged with a high protein meal diet failed to increase their glomerular filtration rate. Therefore, fixed filtration as well as fixed renal perfusion make the diseased kidney a very limited organ with little scope for improvement.

In conclusion, I believe that although a low protein diet is beneficial in slowing the progression of chronic renal failure,

such a dietary effect depends on the underlying pattern of renal and glomerular perfusion. Patients with increased or reversible glomerular perfusion are likely to be the ones with mild or focal glomerular involvement. Patients with severe vascular and diffuse glomerular diseases, as well as fixed renal perfusion, are unlikely to improve on such a diet.

The therapeutic approach to patients with chronic renal diseases might have to be, in the future, tailored to the underlying nephropathy once its haemodynamic profile has been established.

CHAPTER IV

DISCUSSION (General)

Over the last thirty years our understanding of the pathogenesis of acute nephritides has been greatly advanced by the research pioneered by Dixon and his colleagues (1968). These workers have designed experimental models of acute glomerular diseases in animals where the role of the immune system in the pathogenesis of the nephritis was beyond any doubt (Unanue & Dixon, 1967). From their research and that of others (Germuth & Rodrigues, 1973) it became apparent that acute glomerular disorders were conditions resulting from some aberrations of the host immune system (Peters & Lachmann, 1974). Circulating immune complexes (Germuth, 1967) as well as auto-antibodies have been incriminated in the pathogenesis of acute glomerulonephritis in experimental animals (Glassock, 1968) and by deduction in man (Cameron, 1982). In man, such an assumption has been strengthened by the almost universal findings of immunoglobulins and complement on the glomerular basement membranes of nephritic kidneys (Berger, 1971). Equally suggestive of the importance of the immune system in man has been the observations of an increased predisposition to nephritis in conditions where the host immune environment is altered or deficient; associations were observed between complement deficiencies and the development of glomerulonephritis (Agnello, 1978) and defects of the phagocytic reticuloendothelial system have been described in patients with active glomerular diseases (Lockwood, 1979b) as well as the presence of abnormal circulating lymphocytes (Chatenoud, 1981).

All these observations in experimental animals and in man have focused on the acute phase of glomerulonephritides.

The therapeutic advances, resulting from this research, have attempted to manipulate the host immune response in order to control the acute, immune induced, inflammatory reactions within the glomeruli (Cameron, 1982). Unfortunately in animals as well as in man, unless such therapies were initiated extremely early in the course of the nephritis (Hind, 1983) nephritis continued to progress.

Such progression, although variable from patient to patient, has a relentless character with the time to end stage renal failure and dialysis often predictable to the month (Rutherford, 1977).

When examined, kidneys during the sub-acute and chronic stages of glomerulonephritis showed little inflammatory reaction but predominantly a severely, progressive scarring process involving the glomeruli, vessels and renal tubules. Glomerulosclerosis has therefore replaced the early inflammatory proliferative changes of acute glomerulonephritis. Glomerulosclerosis is therefore the hallmark of chronicity and progressive scarring (Kincaid-Smith 1975).

Little has been known as to the pathogenesis of glomerular sclerosis, and the role played by the immune system in its progression remains to a great extent unknown. Can glomerular scarring proceed regardless of the persistence of the abnormal immune environment that has initiated it? Can such scarring progress after the aberrant immune response has been corrected by immunosuppressive agents? I attempted through this research to answer these questions; in order to do so I have chosen and studied an experimental model of acute, immune mediated, glomerulonephritis that resembles those observed in man. I designed a novel experimental approach in order to subtract

the nephritic kidneys from the abnormal immune environment; as I followed the course of these kidneys in new hosts with different levels of humoral immune response. The nephritis progressed in these recipients of injured kidneys regardless of the titre of circulating antibodies. I concluded that in nephrotoxic nephritis, progression of glomerular and tubular scarring, is independent of the surrounding humoral immunity. Such a conclusion does not exclude a role for, intra-renal, localised immune alterations in the glomerulosclerosis process. Planted antigens on the glomerular basement membrane have been known to induce an immune reaction with in situ formation of immune complexes (Golbus & Wilson, 1979) as well as stimulation of the cellular arm of the immune response (Bhan, 1978). Although a possible contributor of persistent and progressive renal scarring, after an acute glomerulonephritis, the immune system is likely to play a secondary role.

From my data, it seemed that the course of the nephritic kidneys was predetermined by the magnitude of the structural damage and possibly the compensatory changes that occurred during the sub-acute phase of the nephritis.

Indeed, proteinuria during the late stages of nephrotoxic glomerulonephritis was the major prognostic factor determining the progression of the underlying disease.

Proteinuria when fixed must reflect the extent of the structural glomerular and tubular damage, however when reversible it implies that other equally reversible factors must contribute to its expression.

In nephrotoxic nephritis, I observed changes in urinary protein excretion induced by diet; proteinuria varied proportionally to the extent of the dietary protein intake. A reversible component to proteinuria was therefore observed, during the sub-acute and chronic stages of this experimental glomerulonephritis. The factors most likely to contribute to these changes in protein excretion are haemodynamic ones; both glomerular ischaemia (Myers, 1982) as well as glomerular hyperperfusion (Olsen, 1982) have been reported to induce proteinuria.

Therefore, haemodynamic factors might have a role to play in the proteinuria of nephrotoxic nephritis and possibly equally important role in the progression of the renal scarring process.

Recently, others have postulated that the progression of chronic, experimental, renal disorders is mediated by the sub-acute adaptive changes that take place in the glomeruli after an acute reduction in functional renal mass (Brenner, 1982). It has been advanced that the hyperperfusion and hyperfiltration of remnant glomeruli underlied the sclerotic changes that progressively destroy these compensatory units (Hostetter, 1982a). Prevention of hyperperfusion by a low protein diet was shown to protect from glomerular damage (Hostetter, 1981b).

The concept of glomerular over-perfusion and filtration at the base of progressive glomerulosclerosis has been extended to other experimental nephropathies. In early diabetic nephropathy, in the rat, glomeruli are known to be increased in size with an increased blood flow and filtration rate (Hostetter, 1981a). Similarly,

in experimental pyelonephritis such supernephrons were detected (Lubowitz, 1969), with a possible contributory effect on their ensuing glomerulosclerosis (Cotran, 1982). Glomerulosclerosis in these different experimental models might be the result of abberant glomerular haemodynamics. Similarly, in man, in conditions such as unilateral renal agenesis (Kiprov, 1982), following unilateral nephrectomy (Zuchelli, 1983), in diabetic nephropathy (Hostetter, 1982) and chronic pyelonephritis (Kincaid-Smith, 1975) glomerulosclerosis is frequent during the late stages of the nephritis and might equally reflect the longstanding glomerular haemodynamic adjustments.

However, in the experimental models where glomerular hyperfiltration and hyperperfusion have been demonstrated, the glomeruli have been invariably structurally normal or even hypertrophied at the onset of the disease process. In the remnant kidney model, in the diabetic nephropathy model and in the pyelonephritis model the glomeruli are normal early in the course of the nephropathy leading to a vigorous, prompt, vasoactive response to reduction in renal mass or to the abnormalities of the host metabolic state (Mogenson, 1975).

On the other hand, in experimental glomerulonephritis, where the glomeruli have been severely damaged by the acute inflammatory process, their response might differ, there are some experimental data to suggest that glomeruli in acute (Blantz & Wilson, 1976), sub-acute and chronic (Maddox, 1975) glomerulonephritis behave differently from the non-glomerular nephropathies.

Indeed, in chronic experimental glomerulonephritis, in spite of a marked heterogeneity of glomerular function, observers failed to demonstrate the presence of overperfused supernephrons (Rocha, 1973). Instead, underperfused, vasoconstricted glomeruli were found in chronic nephrotoxic nephritis (Maddox, 1975); in this model, after an acute glomerular injury the single nephron glomerular filtration rate fell significantly as the glomerular arteriolar resistances increased and the glomerular plasma flow was reduced (Blantz & Wilson, 1976). Such severe vasoconstrictive reaction of injured glomeruli contrasted sharply with the marked glomerular vasodilatation of the remnant kidney (Deen, 1974) or the early diabetic kidney (Hostetter, 1982b). In nephrotoxic nephritis, glomerular hypoperfusion and hypofiltration seem to predominate during the sub-acute and chronic stages. Glomerular ischaemia with subsequent tubular underperfusion might be the pathway to glomerulosclerosis and tubular atrophy and calcifications in chronic glomerulonephritis.

My observations substantiated these assumptions, as I confirmed that the remnant kidney can be protected from glomerulosclerosis by a low protein diet while the glomerulonephritis of nephrotoxic nephritis improved on both low and high protein intake. Equally relevant, was the absence of detrimental effect, on the course of nephrotoxic nephritis by unilateral nephrectomy or volume expansion through an increase in dietary salt intake.

Both reduction in renal mass (Steffes, 1978) and increased solute (Lalich, 1975) have been observed to accelerate the course of the nephropathies where glomerular hyperperfusion and hyperfiltration

was predominant; in the sub-total nephrectomy model, the adaptive glomerular changes are proportional to the amount of renal tissue resected (Hayslett, 1979). In the same model of chronic renal failure, diets supplemented in protein (Kleinknecht, 1979) or salt (Lalich, 1975) accelerated the course of the underlying nephropathy. These different manoeuvres, all expected to expand the intravascular volume, would further decrease the glomerular capillary resistances allowing an additional increment in glomerular plasma flow with subsequent accentuated glomerulosclerosis. In my experiments, the failure to observe such a deleterious effect on renal function and morphology of diets rich in protein and salt might imply that the underlying pathophysiology of chronic experimental glomerulonephritis differs from that of the sub-total nephrectomy model. Chronic glomerulonephritis in animals is more likely to be an ischaemic disorder where vascular sclerosis (Ljungquist, 1970) glomerular hypoperfusion and tubular ischaemic damage all contribute to the final functional and morphological collapse.

In the context of glomerular and tubular ischaemia it becomes almost expected that a therapeutic intervention aiming to increase renal blood flow and urine flow would invariably improve the outcome of the nephritis. A high protein diet with its well known plasma expanding capacity and its osmotic diuretic effect improved the course of chronic ischaemic nephrotoxic glomerulonephritis. Blood volume expansion (Wilson, 1967) and increased urinary flow (Cronin, 1978) were shown to be protective in models of ischaemic tubular damage. Interventions combining these two salutory effects had the maximal beneficial

influence on renal tubule collapse after an ischaemic insult (Cronin, 1978). I believe that the very high protein intake in rats with chronic glomerulonephritis, by allowing a glomerular vasodilatation, improved the renal tubule perfusion and prevented further atrophy and tubular obstruction. And in spite of progressing glomerulosclerosis renal tubule protection preserved renal function.

These findings emphasise the close interaction between glomerular and tubular structure in renal disorders; glomerular damage leads to tubular and interstitial injury with ensuing further reduction in glomerular function (Risdon, 1968). Protection of the glomeruli would prevent further tubulointerstitial scarring while the prevention of tubular collapse and obstruction would allow glomerular filtration to proceed. In chronic glomerulonephritis, like in other renal disorders, the pathway to end stage renal failure necessitates the combined glomerular and tubular destruction.

In man, evidence stressing such dual glomerular and tubular roles in the progression of chronic renal failure have been often under estimated.

In chronic glomerulonephritis, it often seems that tubulo-interstitial damage is an important prognostic factor (Muehrcke, 1957). More important however is the observation in chronic glomerular diseases in man of the major role of tubular scarring in the regulation of glomerular filtration; severely affected tubules with interstitial oedema and fibrosis invariably lead to a proportional impairment in renal function (Riemenschneider, 1980).

In chronic pyelonephritis on the other hand, proteinuria has an ominous prognostic significance (Kincaid-Smith, 1979). Patients with pyelonephritis and proteinuria usually display severe glomerulosclerosis even in the absence of systemic hypertension (Senekjian, 1979). These glomerular lesions, it would seem, determine the progression of the nephritis to end stage renal failure well after recurrent infections have been eradicated .

I would like to believe that progressive renal failure proceeds after an acute glomerular injury regardless of the persistence of the initiating events. With loss of functional renal tissue through acute scarring and with the development of vascular arteriolar sclerotic lesions during the sub-acute stages of the disease, the haemodynamic profile of the residual nephrons is established. When the glomerular injury is mild or focal in nature the spared glomeruli would drop their capillary resistances and compensate for the loss of adjacent nephrons by an increased perfusion and filtration. In cases where the glomerular injury is diffuse and severe at the onset with concomittant arteriolar sclerosis, glomeruli would be ischaemic with decreased filtration. These more severely affected glomerulonephritides would invariably have a rapidly progressive course with glomerular sclerosis and severe tubular ischaemia, atrophy, collapse and obstruction. On the other hand, when the less severely damaged glomeruli hyperperfuse and hyperfiltrate, the course of the nephritis would be slower with these compensatory changes maintaining renal function for some time. Similarly in this case the tubulo-interstitial network would continue to be adequately perfused preventing its destruction and prolonging the useful lifespan of the compensatory

nephrons. However, with time glomerular overperfusion and over-filtration would induce glomerulosclerosis in a fashion similar to the one known to occur in the remnant nephropathy model (Hostetter, 1981b). With the onset of glomerulosclerosis, glomerular capillary networks will become obliterated in a segmental followed by a diffuse fashion.

Therefore, I would like to envisage the development of end stage renal failure in chronic glomerulonephritis in a stepwise fashion as follows :

First, in mild glomerulonephritis and in the ones of focal and segmental nature;

Phase 1 : Mild acute, immune induced, glomerulonephritis

Phase 2 : During the late acute and sub-acute stages of the disease, and as some reduction in renal functional mass has occurred, remnant glomeruli decrease their resistances and allow over-perfusion and compensatory hyperfiltration to occur and maintain renal function. This stage of compensatory hyperperfusion and hyperfiltration would persist for weeks, months or years depending on its severity which is directly proportional to the extent of renal tissue loss during phase 1.

Phase 3 : Persistent glomerular and high pressure filtration would progressively lead to focal and segmental glomerulosclerosis.

During this phase the nephron population would behave in a very heterologous fashion, with some glomeruli or segments of glomeruli decreasing their blood flow through capillary loop obliteration by hyalinosis and sclerosis. At this stage, which could last many years, the whole spectrum and range of glomerular plasma flow, pressure and filtration could be observed.

Phase 4 : During the late sub-acute-chronic stages and as proteinuria persists or increases due to more severe structural damage, more and more glomeruli will become sclerosed as the renal haemodynamic profile is moving away from compensatory overperfusion to ischaemic underfiltration. The reduced glomerular capillary flow could progressively lead to renal tubular ischaemia. At this stage renal failure would develop.

Phase 5 : Renal tubular ischaemia would lead to tubular cells swelling and subsequent tubule luminal obstruction. Tubule obstruction and interstitial oedema would lead to further glomerular vasoconstriction with reduction in glomerular plasma flow and filtration.

Phase 6 : At this late stage of glomerular extreme ischaemia glomerular hyalinosis and sclerosis would spread. Similarly ischaemic, atrophic tubules would collapse as the interstitial network becomes fibrotic and scarred, unable to maintain the integrity of the tubulo-interstitium structure. Tubular and interstitial calcifications would develop at this stage.

Renal function would eventually cease and end stage renal failure would occur.

Second, in severe glomerulonephritis of a diffuse nature;

Phase (1) would be more severe with crescent formation. This stage of early glomerular immune mediated damage would be characterised by a severe glomerular vasoconstrictive response.

Such glomerular ischaemia and underfiltration, with renal failure, would be the hallmark of phase (2) in severe glomerulonephritis. This stage occurring early in the course of the disease would be the haemodynamic equivalent of the late phase (4) in milder forms of injury.

The subsequent progression would lead to phases (5) and (6) where further tubular ischaemia, collapse and calcifications would occur with ensuing end stage renal failure.

With this schematic model of progressive, stepwise, renal scarring in mind, therapeutic interventions could be designed to halt or slow the scarring process. It is likely that the reversibility of the disease process would be maximum in the early stages (up to stage 4) and would decrease as the nephritis progresses.

The therapeutic approaches to the management of glomerulonephritis could be tailored to the different phases of the disease :

Phase 1 : During this early stage of an immune induced nephritis, immunosuppression would seem the most appropriate therapeutic intervention. The blunting and attenuation of the acute inflammatory response would prevent further structural damage

and might convert a potentially severe nephropathy to a milder one where remnant nephrons are spared and capable of compensatory adjustments. However, it is likely that heavy immunosuppression should be confined to this very early stage of a glomerulonephritis. Longterm manipulations of the immune system are likely to be fruitless and potentially harmful. This assumption has been recently confirmed in man where the therapeutic efficiency of immunosuppression in rapidly progressive glomerulonephritis was found to be entirely dependent on the early introduction of the treatment (Hind, 1983).

Phase 2 : As glomerular compensatory changes are maintaining renal function at the expense of their longterm survival, manoeuvres aimed at decreasing the magnitude of these haemodynamic changes would seem opportune.

At this early sub-acute, stage, interventions decreasing the glomerular vasodilatory reaction might prevent the longterm glomerulosclerotic changes. A low protein diet with its known glomerular vasoconstrictive effect could prove ideal during this phase of a glomerulonephritis. Observations made in man imply that the early introduction of dietary protein restriction greatly enhanced its therapeutic value (Maschio, 1982). Alternatively pharmacological interventions aimed at reducing renal blood flow might prove equally beneficial.

Therefore as glomerular overperfusion and high pressure filtration predominate dietary and pharmacological manoeuvres aiming at reducing glomerular blood flow should be applied.

Phases 3 and 4 : During the later sub-acute and chronic stages when glomerulosclerosis has occurred with resulting capillary obliteration and tubular ischaemia a different therapeutic approach might be warranted.

It is likely that at this phase dietary and pharmacological glomerular vasodilatation might prove of paramount importance.

A high protein diet or an equivalent α -adrenergic blocker might prevent further glomerular ischaemia and subsequent tubular damage.

It is equally important during this phase to avoid further glomerular ischaemia; prostaglandin inhibitors when used in man, during this stage of nephritis have proved extremely harmful (Kimberly, 1978). Peripheral vasodilators with a similar action on the renal capillary bed would improve overall renal blood flow and would be recommended when glomerular ischaemia predominates. Similarly, the choice of beta-blockers, for the treatment of concomittant hypertension, might be directed to the ones known to increase renal blood flow.

Phases 5 and 6 : At this stage protection of the renal tubulo-interstitial network might prevent progression to end stage renal failure. Improvement of the peritubular blood flow and increase of the intratubular urine flow might prevent severe structural tubular ischaemia and damage. Protection at this stage could be provided by agents known to increase urine flow and solute excretion as long as a concomittant increase in renal blood flow is achieved (Cronin, 1978). Agents such as prostaglandins (PGE), and frusemide might, during this phase of glomerulonephritis, be indicated and beneficial. On the other hand prostaglandin inhibitors and thiazide diuretics (Patak, 1979) might prove harmful.

At the later stages, prevention of tubulo-interstitial calcifications might be the ultimate way of preventing total tubular obstruction and destruction. A low phosphate diet, known to decrease intra-renal calcifications (Haut, 1980) might at this stage be protective. Similarly, the inhibition of calcium-phosphate precipitation and crystallisation might be equally beneficial (Gimenez, 1982). Finally, agents increasing the urinary calcium excretion and therefore altering the calcium x phosphate product could be similarly appropriate.

As glomerular and tubulo-interstitial scarring proceeds, abnormal protein and collagen synthesis might contribute to the progression of glomerular and interstitial fibrosis (Bohle, 1977). A low protein diet through its known reduction of protein synthesis effect (Waterlow & Jackson, 1981) might prevent further late sclerotic changes.

I believe such a mechanism explains the protection from glomerulosclerosis observed in the nephrotoxic nephritis model when the dietary protein restriction was introduced late in the course of the disease.

It is important to appreciate that such a schematic approach should in no way preclude a more global therapeutic intervention in glomerulonephritis; where more than one therapeutic manoeuvre would be applied simultaneously. Such a multipurpose therapy with glomeruli, vessels and tubules treated and protected simultaneously is likely to prove immensely successful.

Whether a similar approach could be justified in the management of chronic glomerulonephritis in man would depend entirely on the underlying haemodynamic patterns of the glomerular vasculature in response to injury.

My observations in man with chronic renal disorders have addressed this question and I believe go a long way in establishing that renal diseases in man behave in a comparable manner to their experimental counterpart.

I used, in man, a low protein diet and from the response of different nephropathies to such a vasoconstrictive diet I extrapolated as to the nature of the underlying renal haemodynamic pattern.

A low protein diet has been shown in experimental animals (Ichikawa, 1980) and in man (Klahr & Alleyne, 1973) to reduce renal blood flow. I assumed that if, like in animals, human glomeruli were

overperfused they would respond in a favourable way to dietary protein restriction. And if hyperperfusion was harmful in man, its correction by such a diet would improve renal function or at least slow its rate of decline.

On the other hand, if glomeruli are underperfused and ischaemic through vascular sclerosis and renal vasoconstriction, the low protein diet would fail to have a beneficial impact over these nephrons over a short observation period. However, I believe from the animal experimentation, and from longterm studies on low protein therapy of chronic renal failure in man (Giordano, 1982a), that such a diet is beneficial over a long period regardless of its influence on renal haemodynamics; a low protein diet is likely to protect from glomerular and tubulo-interstitial sclerosis through its affect on the metabolism (Edozien, 1978) with decreased protein synthesis (Waterlow & Jackson, 1981) and an overall salutory action on the ageing process (Berg & Simms, 1960).

In the prospective study I established using a low protein diet in patients with glomerular, tubular and hypertensive renal diseases that the response to a low protein diet varied according to the underlying pathology.

Moreso, I believe that the beneficial response of patients with non-glomerular diseases to a reduction in their dietary protein intake reflects the underlying overperfusion and hyperfiltration of remnant glomeruli in these diseases. Patients with glomerular disorders responded variably to the diet, again reflecting the broad spectrum of morphological and functional changes of remnant

glomeruli after an acute glomerular injury. Patients with hyperperfused glomeruli are likely to improve, while the ones with severe acute and sub-acute injuries would tend to be ischaemic and refractory to such therapeutic manoeuvres. This assumption was reinforced by the observation that patients with chronic glomerulonephritis with reversible proteinuria are the ones whose renal function improved on the diet. It is likely that proteinuria in these patients was not the mere reflection of structural damage but reflected concomittant high pressure glomerular filtration. The low protein diet by alleviating this aberrant glomerular haemodynamic pattern decreased the filtration of protein. The remaining patients whose proteinuria was absent or fixed failed to improve on the protein restricted diet.

Similarly patients with hypertensive nephrosclerosis showed a poor response to vasoconstriction implying that here again glomerular ischaemia and underperfusion, might predominate. These data in man fully support previous ones in experimental animals where these different patterns of glomerular haemodynamics were observed in various disease entities.

From my work and that of others, I would like to believe that progression of renal diseases to chronicity involves many different pathways. And that glomeruli in various diseases behave differently depending on whether they were affected by the initial disease process or whether they were spared initially and affected at a later stage. Surely, the whole spectrum of haemodynamic changes must exist in glomeruli of different underlying nephropathies and at different stages of the development of these nephropathies.

It is conceivable that the early hyperfiltrating and hyperperfused glomeruli of renal tubular disorders progressively see their perfusion decreased as glomerulosclerosis develops.

On the other hand, the hypofiltrating, hypoperfused glomeruli of renal glomerular diseases might have intrinsic areas of segmental hyperperfusion and hyperfiltration.

It is clear that both hypo and hyper-perfused glomeruli or segments of glomeruli will sooner or later develop glomerulosclerosis leading to end stage renal failure.

Prevention of either over or under perfusion of the glomeruli is likely to slow the rate of decline in renal function. Dietary or pharmacological interventions in order to reverse the glomerular haemodynamic aberrant state should replace immunosuppression in the management of sub-acute glomerulonephritides.

More important still is the tailoring of the therapeutic approach of chronic renal diseases to the stage of the nephritis with its specific glomerular and tubular changes.

Simultaneous therapeutic protection of glomeruli as well as renal tubules, by combined vasoactive interventions with ones increasing urine flow and solutes excretion, is likely to halt both glomerular sclerosis and tubular atrophy in chronic renal diseases regardless of the underlying nephropathy.

Finally, the optimalisation of the management of chronic renal failure in man might have to await the discovery of the Munich-Wistar man with his superficial glomeruli. Otherwise, man will have to await the development of new technologies that would allow him to probe his deep glomeruli and unravel their mysteries.

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