STUDIES ON THE EFFECT OF DIET
IN EXPERIMENTAL RENAL DISEASE

a thesis submitted by

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PUBLICATIONS AND PRESENTATIONS OF STUDIES DESCRIBED IN THIS THESIS

1. The effect of dietary protein quality in experimental renal disease
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   Proceedings European Dialysis and Transplant Association:
   22, 921-926 (1985)

2. The effects of varying quantity and quality of dietary protein intake
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   Williams A J, Baker F E, Walls J
   Presented at the Renal Association (1985)

3. The metabolic consequences of differing protein diets in experimental
   renal disease
   Williams A J, Walls J
   Presented at The Medical Research Society (1985)
   Clinical Science 70, 74p (Suppl 13)

4. The effect of varying quantity and quality of dietary protein intake
   in experimental renal disease in rats
   Williams A J, Baker F E, Walls J
   Nephron 46, 83-90 (1987)

5. The metabolic effects of differing dietary protein intakes in
   experimental renal disease
   Williams A J, Walls J

   Williams A J, Walls J
   Presented at the Xth International Congress of Nephrology (1987)

7. The influence of soya protein on the natural history of a
   remnant kidney model in the rat
   Walls J, Williams A J
   Contributions to Nephrology 60, 179-187 (1988)

8. Body composition changes in the subtotally nephrectomised
   rat fed different dietary proteins
   Williams A J, Walls J
   Nephron 51, 384-387 (1989)
SECTION 1

INTRODUCTION
INTRODUCTION

THE EFFECT OF DIET UPON NORMAL RENAL FUNCTION

The initial realisation that the excretory function of the kidney is a variable phenomenon, and that feeding was one factor which could influence renal function, was due in large part to the investigations of Thomas Addis.

Addis, a principal investigator of renal physiology in the early part of this century, documented, in his initial studies, changes in renal function in normal man following administration of adrenaline and pituitrin (Addis T et al, 1918a, 1918b). Further investigations, by Addis and Drury (Addis T, Drury D R, 1923) revealed that the authors' renal clearance of urea was enhanced following ingestion of a meal, or milk, but sugar or whiskey produced no such effect. From these early observations, they concluded that the protein moiety of food was responsible, and to further investigate this possibility, performed similar measurements of urea clearance following ingestion of an amino acid, glutamic acid. Again, a rise in urea clearance was noted.

In the studies of Addis and Drury, the elevation in urea clearance was evident at one and two hours following consumption of milk, and a meal, respectively, and persisted for a further two hours. Hence the initial link between food ingestion and change in renal function had been established.

Urea clearance is a relatively insensitive index of glomerular filtration rate (GFR), being highly dependent upon the urine flow rate. However, following Rehberg's description of creatinine clearance as a
measure of GFR in 1926 (Rehberg P B, 1926), more precise measurements of renal function could be made.

Further important observations regarding the variability of renal function in response to variations in dietary protein were made in a series of experiments undertaken in the laboratory of Norman Jolliffe and Homer Smith in 1931 (Jolliffe N et al, 1931). Their observations were an important landmark in renal physiology, and in essence, showed that creatinine clearance in the dog rose progressively with the quantity of protein present in the diet. A second series of investigations (Shannon J et al, 1932) confirmed their initial findings, and subsequently they showed that, following a high protein meat meal, xylose clearance (an index of GFR) increased by a factor of three, compared to values obtained when a low protein cracker meal diet was supplied.

These observations formed the foundation of a great deal of subsequent research, as, for the first time it was demonstrated that the constituents of the diet were quantitatively important factors in determining the level of renal function, and supported the previous observations of Addis linking GFR and protein ingestion.

Shannon also re-examined experimental data published some four years previously by Deuel (Deuel et al, 1928) and reinforced the observation that urea clearance in man is elevated whilst consuming a high protein diet.

Following the demonstration that diet could influence glomerular filtration rate, a number of investigators subsequently sought to define the mechanisms by which these changes are brought about.

Amongst one of the foremost of Jolliffe and Smith's contemporaries was Robert Pitts, who embarked on a series of studies aimed at elucidating
the possible mechanisms responsible for Shannon's observations. Three years after Jolliffe and Smith's original manuscript, Pitts published experimental findings to support those of Jolliffe (Pitts R, 1935). He also found that xylose clearance in dogs was low whilst maintained on a low protein cracker meal diet, with little change in the post prandial period. However, when a high protein meat diet was substituted, the post absorptive xylose clearance had doubled after 4 weeks, and this value rose by a further 40% in the post prandial state.

The next investigative step that Pitts undertook was to determine if the change in renal function he observed was related to meat per se, or to the quantity of protein ingested. This was performed by feeding the dogs a high protein casein diet, and again a rise in xylose clearance was seen in the post prandial state, and from these observations he postulated that amino acids present in the ingested protein may directly affect kidney function. To examine this possibility, further determinations of xylose clearance following intravenous and subcutaneous infusions of glycine were performed, and a rise was seen within minutes of amino acid administration. From this Pitts concluded that amino acids derived from ingestion of protein may act directly upon the kidney to produce a change in renal function. He discounted urea as being instrumental in producing these changes, as xylose clearance had risen prior to any detectable changes in blood urea.

The next avenue of investigation to be pursued was the effect of protein ingestion upon renal blood flow, as it was reasoned that large changes in glomerular filtration occurring after a meal may well be due to changes in renal blood flow. Van Slyke and colleagues (Van Slyke D et al, 1934) were the initial group of investigators to study this
concept, but at that time, methods available for measuring renal blood flow were very limited. However, a suggestion that changes do occur, was provided by Van Slyke, who documented an increase in renal blood flow following feeding using urea as the marker substance and applying the Fick principle in a uninephrectomised dog (Van Slyke D et al, 1934).

The development of para-aminohippuric acid clearance as a measure of renal plasma flow allowed Pitts to investigate the effects of intravenous glycine on renal haemodynamics and glomerular filtration rate in the dog. Within minutes of commencing an intravenous infusion, an increase in both parameters was evident (Pitts R, 1944). He concluded that a reduction in renal vascular resistance was responsible and that a change in efferent arteriolar tone was the main effector mechanism. Further study into the effects of the protein content of the maintenance diet upon renal haemodynamics, revealed that both renal blood flow and glomerular filtration rate increase, and that renal vascular resistance decreases, with elevation in dietary protein content (Pitts R, 1944).

Further support for the concept of dietary protein as a controlling factor of renal haemodynamics was provided by Hiatt and Hiatt. Whilst investigating the effects of feeding and fasting upon renal function in the harbor seal, they found that feeding increased both renal blood flow (as assessed by diodrast clearance) and glomerular filtration rate, whilst the filtration fraction remained constant (Hiatt E, Hiatt R, 1942). The seal is an unusual mammal, in that access to fresh water is limited, and Hiatt reasoned from this, that the reduction in renal perfusion during fasting was a mechanism by which the seal could conserve water. He also proposed that the
increase in glomerular filtration rate after feeding was an adaptive mechanism whereby the products of protein metabolism would be excreted only at the appropriate time.

Therefore, in the thirty years since the initial observations of Addis, the major effects of diet in relation to renal function in animals had been documented, namely that renal blood flow and glomerular filtration rate both in the basal and post prandial states, were influenced directly by the protein content of the diet.

During the second quarter of this century a number of human studies were also performed (Goldring W et al, 1934, Cope C, 1933) and essentially similar results to the animal studies were obtained. However definitive evidence that dietary protein content could produce changes in renal blood flow and glomerular filtration in man was not forthcoming until 1949 (Pullman T et al, 1949, 1954). Employing the more accurate methods of inulin and PAH clearances as indices of glomerular filtration rate and renal blood flow, this group documented the effects of a low, medium and high protein diet upon renal function in twenty normal subjects. They found that both renal blood flow and glomerular filtration rose with the dietary protein intake, whilst filtration fraction remained stable. Hence dietary protein did seem to exert some influence over renal haemodynamics in man, but the mechanism by which this was effected remained obscure.

Little subsequent research was performed into the effects of diet upon renal function until some twenty years later when O'Connor and Summerill (1976) embarked upon a series of studies. Their efforts were directed towards re-examining the experiments of Shannon and Jolliffe, and Pitts, because they proposed two methodological problems existed in those studies. Renal clearances of marker substances had been measured
whilst animals were maintained in a diuretic state, and the quantity of meat fed to the animals was approximately four times the normal intake, which was felt to be unphysiologically high. To overcome these problems, O'Connor and Summerill measured changes in glomerular filtration rate (creatinine clearance) at normal urine flow rates after a meat meal in three dogs. In addition, they performed similar experiments to Robert Pitts, and documented the change in creatinine clearance following the administration of glycine via a stomach tube (O'Connor W J, Summerill R A, 1976).

The results of O'Connor and Summerill in essence replicated those of previous investigations, in that following both a normal meat meal, and administration of glycine, the glomerular filtration rate rose by approximately 40 per cent, which persisted for up to 6 hours. This phenomenon could not be reproduced by the administration of urea or sulphate in quantities which would be produced by metabolism of the dietary protein. From this observation, together with demonstration by Moustgaard (1948) that the rise in glomerular filtration following amino acid infusion persists, when plasma amino acids have returned to normal levels, and the observation of Rhoades that this phenomenon is not mediated via the renal nerves (Rhoades C P et al, 1934) led O'Connor and Summerill to propose that protein and amino acid ingestion and metabolism may affect renal function. Their postulate included the possibilities of deamination of amino acids by the liver, and the release of gastrointestinal peptide hormones and insulin, which occur following feeding. This line of investigation was to be pursued by a number of laboratories subsequently.

The majority of animal physiological experimentation in the first half of this century had been performed using dogs, but in recent years
the laboratory rat has become more popular by virtue of cost, ease of accessibility to inbred strains, and numbers of animals employed. It was therefore important to determine if the changes in renal function occurring in man and dog are duplicated in the rat following protein ingestion. The dog is a rather unusual animal to study, if reference to the human is made, as, in the laboratory setting, feeding occurs once daily. In contrast, the rat usually consumes food throughout the nocturnal period. In a series of studies performed by Dicker, the renal physiological response to changes in dietary protein in the rat was shown to be similar to that of man and dog (Dicker S E, 1949), and a similar rise in glomerular filtration rate is seen following amino acid infusion (Meyer T W et al, 1983).

With the advent of micropuncture techniques developed using the superficial glomeruli of the Munich Wistar strain of rat, some insight into the factors which govern glomerular function has been possible. Ichikawa applied this technique to investigate the changes in glomerular haemodynamics and function that occur following long term maintenance of rats upon high or low protein diets (Ichikawa I et al, 1980). Their findings showed that whole kidney glomerular filtration and single nephron GFR in animals fed a low protein casein diet were 35% lower than animals fed a high protein casein diet. This appeared to be due to a reduction in both glomerular capillary plasma flow (by 25%) and glomerular capillary ultrafiltration coefficient (by 50%). Contributing factors to the changes in these parameters induced by low protein feeding appeared to be an elevation in both afferent and efferent glomerular arteriolar resistance, and a reduction in the glomerular filtration surface area. Plasma oncotic pressure, and glomerular transcapillary hydraulic pressure gradient, two other
important determinants of glomerular function, were found to be similar in both groups of animals.

In recent years renewed interest in the physiological role of the diet, and especially protein, in determining renal function in man has become apparent. This has been mainly as a result of observations that dietary protein manipulation can be of therapeutic benefit in patients suffering with chronic renal failure (Maschio G et al, 1982, Williams A J et al, 1984). Many of the experimental findings in animals of the previous four decades have been confirmed in man, in that an amino acid infusion will produce an increase in both glomerular filtration rate and effective renal plasma flow (Ter Wee P et al, 1985, Graf H et al, 1983). Also, ingestion of a protein rich meal, will produce a rise in GFR and ERPF, with a reduction in renal vascular resistance (Hostetter T, 1984).

The renal functional and haemodynamic effects of protein and amino acids are apparent, but the underlying mechanisms responsible for these changes are far from clear. However studies undertaken in recent years do provide some indication of possible control and effector mechanisms.
POSSIBLE MECHANISMS BY WHICH PROTEIN INTAKE OR AMINO ACIDS INFLUENCE
RENAL FUNCTION

Increases in dietary protein intake or intravenous infusion of amino acids, either singly or in combination, produce an increase in renal blood flow and glomerular filtration rate in the post prandial or post infusion period, and a high protein intake is associated with a sustained elevation of those parameters. However, what are the possible mechanisms by which these changes may be brought about?

In addressing this question a large number of metabolic and endocrine changes which occur following feeding must be taken into account. To investigate any direct effect amino acids may have upon renal function, Maack perfused the isolated rat kidney with a solution containing a mixture of amino acids, at physiological concentrations of 5 to 20mmol/l (Maack T et al, 1974). His results, in a small number of experiments, showed that the mean inulin clearance of the isolated perfused kidneys did rise by 30%, but this did not achieve statistical significance. Further investigations using this model have revealed that amino acids can effect a reduction in renal vascular tone by up to 35%, but again without any significant change in GFR (Brezis M et al, 1984). The investigators have postulated that the change in renal vascular resistance reflects a direct vasodilatory action of amino acids added to the perfusion medium which is probably related to their role as metabolic substrates in the kidney, as an increase in renal oxygen consumption accompanies the change in renal vascular resistance. These results suggest that amino acids may play some role in affecting renal haemodynamic change but no measurable change in glomerular filtration has yet been found.
The inconclusive evidence for a direct action of amino acids upon glomerular filtration has led to the suggestion that a circulating hormone, released in response to amino acid absorption or infusion may be responsible. Glucagon is a possible candidate for this role. It is released in response to amino acid administration (Pek S et al, 1969), and persistently high levels can be found following ingestion of a protein rich diet (Einstein A et al, 1979). Experimental evidence implicating glucagon as a physiological modulator of renal function however is conflicting. Infusion of large doses of glucagon both intravenously and directly into the renal artery have been shown to elevate both GFR and renal plasma flow in dogs (Johannesen J et al, 1977, Levy M, Starr N, 1972). On the other hand, administration of glucagon in more physiological doses sufficient to produce serum levels in man found in poorly controlled diabetes, had no effect upon renal plasma flow, with only a small change in GFR (Parving H H et al, 1977), and even larger doses sufficient to elevate the blood glucose, produced no change (Elrick H et al, 1958). Further evidence for the lack of a direct effect of glucagon upon GFR, comes from studies by Espinel who documented no change in renal function following infusion of glucagon into the renal artery of the rat (Espinel C H et al, 1976). However, more recent studies do suggest that glucagon may be influential in modulating renal function, in that infusion of physiological doses in the dog will raise the GFR, and larger doses are associated with a rise in renal plasma flow (Palmore W P, 1983). Further support for a role for glucagon was obtained when it was demonstrated that somatostatin, a hormonal inhibitory polypeptide found in stomach, pancreas and the hypothalamus, could abolish the rise in GFR associated with amino acid infusion in rats. This observation has subsequently been confirmed in
man (Meyer TW et al, 1983, Costellino P et al, 1985). Since however, a variety of hormones of pituitary and gastrointestinal origin besides glucagon, may be inhibited by somatostatin, a direct confirmatory role of glucagon in modulation of renal function in response to diet or amino acid infusion has yet to be shown.

The role of vasoactive intestinal polypeptide (VIP), another hormone inhibited by somatostatin, in the physiological modulation of renal function in relation to diet and amino acid infusion has also been investigated. The results are again conflicting in that VIP has been found to both elevate and reduce GFR and renal plasma flow (Espinel CH et al, 1976, Duggan KA, Macdonald GJ, 1987). Further evidence however suggesting that VIP is not instrumental in elevating GFR following protein loading comes from studies showing no change in VIP secretion rate following such dietary manoeuvres (Farrington K et al, 1984).

Further search for a hormonal regulator of renal function, related to amino acid metabolism, has been undertaken by Julia Uranga and colleagues. These investigators have isolated a small glucuronide molecule, glomerulopressin, present in the hepatic venous blood of plasma volume expanded rabbits and toads which, when infused into rats and dogs, will acutely raise GFR (Uranga J, Fuenzalida R, 1975, del Castillo E et al, 1977). This substance is capable of causing smooth muscle contraction which can be abolished by indomethacin, suggesting that prostaglandins may be involved in its mode of action (Uranga J et al, 1979). It has been proposed that the liver produces a substance, perhaps glomerulopressin, in response to increased glucagon levels and raised free amino acids, and this is responsible for the alteration of
renal function seen after protein ingestion and amino acid infusion (Alvestrand A, Bergstrom J, 1984).

The hypothesis that the liver may be intimately involved in hormonally mediating changes in renal function following amino acid infusion and protein ingestion comes from clinical observations in man. In severe liver failure, a reduction in GFR is often found, which can be corrected when hepatic function is restored. Similarly subjects suffering with hepatic cirrhosis do not demonstrate a post prandial increase in GFR (Dratwa M et al, 1987). Corroboration of Uranga's findings and the demonstration that a substance, such as glomerulopressin, is released from the liver following protein ingestion is awaited.

Other evidence indicating that the liver may be involved in the augmentation of renal function following amino acid administration comes from the studies of Lee and Summerill. These investigators have documented the rise in GFR following administration of individual amino acid solutions to conscious dogs via a stomach tube, and found that most amino acids would stimulate a rise in GFR (Lee K, Summerill R A, 1982a, 1982b). The exceptions were L-Cystine and D-Serine, two amino acids which do not enter the general pool of amino nitrogen metabolism. The conclusions reached by Lee and Summerill were that the changes in GFR following amino acid administration could not be ascribed solely to one, or a small number of, amino acids, but rather was due to an increase in urea synthesis, as change in GFR was proportional to urea production rate. However one cannot conclusively ascribe the change in GFR to urea synthesis rate alone, as the authors acknowledge, as effects due to other metabolic processes or substances produced as a result of amino acid metabolism cannot be excluded.
Some investigation has also been directed towards the involvement of prostaglandins in the elevation of GFR following amino acid infusion. The rise in both renal blood flow and glomerular filtration rate induced in man following infusion of an amino acid solution can be completely inhibited by prior administration of indomethacin (Ruilope L et al, 1984, Eisenhauer T et al, 1985). However caution is needed in inferring that prostaglandins play an integral role in this phenomenon. Indomethacin, in addition to its effects upon inhibition of enzymes involved in prostaglandin synthesis, influences the activity of a number of other enzymes, including those concerned with cyclic nucleotide syntheses (Dunn M J, Hood V L, 1977), compounds which themselves may occupy a role in modulating glomerular function (Dworkin L et al, 1983).

In addition to the acute effects of protein ingestion upon renal function, maintaining rats on a high protein diet leads to a sustained increase in GFR (Ichikawa I et al, 1980). Investigating the mechanisms responsible for this, Seney and Wright undertook studies concerning the role of the tubuloglomerular (TG) feedback system in GFR regulation in relation to dietary protein intake (Seney F D, Wright F S, 1985). They found that the higher GFR of rats maintained upon a high protein diet (40% casein) was associated with a 50% reduction in the activity of the tubuloglomerular feedback system compared to animals fed a low protein diet (6% casein), and from this they deduce that the decrease in TG feedback contributes substantially to the elevation in GFR. As the nature of the signal to the macula dense cells effecting TG feedback is not known for certain, only speculation regarding the change associated with high protein feeding can be made.
As it is thought that delivery of sodium to the macula densa is important in TG feedback, increased proximal reabsorption could result in decreased delivery to the sensing mechanism. This hypothesis is supported by the finding of an increase in sodium-hydrogen exchange in brush border vesicles of high protein fed rats (Harris R G et al, 1984). However in this study, early distal tubular sodium concentrations were not determined. The possibility that an increase in urea delivery to the distal tubule was responsible for the changes in TG sensitivity is unlikely, as wide variations in urea concentration have been shown not to influence the TG feedback system (Briggs J P et al, 1980).

The nature of the dietary protein itself may be a factor which influences renal function. The creatinine clearance of subjects ingesting a vegetarian diet ad lib has been shown to be significantly lower than subjects ingesting a normal mixed diet. This was found at both a normal level of mixed protein intake (70g/day), where creatinine clearance of vegetarians was 65% that of the omnivores, and at a reduced level (40g/day) where creatinine clearance was 68% that of the omnivores. (Bosch J P et al, 1983). This was initially thought to be a reflection of an increased creatinine intake derived from meat in the mixed diet. However, measurement of GFR using 51Cr EDTA in vegans and subjects ingesting a mixed diet has produced similar results, showing the GFR of vegans to be 88% that of the omnivorous subjects (Wiseman M J et al, 1987).

Other evidence suggesting that a vegetarian diet may modulate renal function in a manner different from omnivores, comes from a study by Hirschberg (Hirschberg R et al, 1984). They found the post prandial creatinine clearance of four out of nine normal vegetarian subjects to
fall following ingestion of a typical vegetable meal, in contrast to
the usual rise in GFR which follows consumption of meat, or a mixed
meal.

Further evidence suggesting that the nature of the dietary protein
may influence renal function, comes from the observation that
isonitrogenous meals of meat, and a synthetic diet, produce
significantly different magnitudes of change in post prandial GFR in
man (Dhaene M et al, 1987). Indeed, Robert Pitts, some fifty years
previously had demonstrated in the dog that the post prandial rise in
GFR was significantly greater following a meal of meat, compared to a
meal of casein (Pitts R, 1944).

Therefore, some evidence is available to suggest that not all
dietary proteins affect renal function in the same way. Differences in
the amino acid composition of the diets might have been responsible for
the different magnitude of response seen in omnivores and vegetarians.
Other factors, such as the dynamics of amino acid absorption and
hormonal responses following ingestion of different types of protein
are variables which need to be taken into consideration. Therefore,
the effects of dietary protein and amino acids upon renal haemodynamics
have been documented in many studies, but the mechanisms by which these
changes are brought about, be they hormonal, or related to dietary
protein composition, remain elusive.
HARMFUL EFFECTS OF PROTEIN UPON THE KIDNEY

Concurrent with studies in the early part of this century, investigating the effect of dietary protein upon renal physiology, interest was also directed towards the role of diet in the development of glomerulosclerotic lesions and in the aetiology of renal insufficiency. The association of albuminous urine, dropsy and contracted kidneys, signs first described by Richard Bright in 1827, had become a well recognised entity early in this century, but little was known regarding the aetiology of this condition (Bright R, 1836). However it was appreciated that dietary factors, and in particular protein consumption, were important in the treatment of this condition, and this prompted Newburgh to investigate the influence of diet in the aetiology of Bright's disease.

By feeding rabbits diets composed of varying sources of protein such as casein and egg white, at differing levels, he observed the development of interstitial nephritic lesions frequently, but glomerular lesions were more variable. From this he suggested that Bright's disease could be induced in these animals by high protein feeding, and that the kidney injury produced varied with the type of protein eaten. The conclusions drawn by Newburgh (Newburgh L H, 1919, Newburgh L H and Clarkson S, 1923) unfortunately caused confusion and were criticised on a number of points. The frequent, spontaneous occurrence of renal lesions in the rabbit, the nutritional inadequacy of some of the diets, and the fact that the herbivorous rabbit is habituated to a low protein diet and excretes an alkaline urine, made interpretation of his results difficult. In response to these criticisms, Newburgh and Curtis undertook further experiments in the
omnivorous rat, and drew similar conclusions, in that "diets containing 75% dried liver produce a granular kidney in less than one year, but the same amount of casein fed for sixteen months, causes only a moderate tubular injury. The effect of a similar amount of beef muscle is intermediate between the two" (Newburgh L H, Curtis A, 1928). Similar results had previously been reported by Evans and Risley (Evans N, Risley E H, 1925). These observations prompted a number of investigators to examine this phenomenon in more detail. Controversy followed with the appearance of a number of reports that no significant glomerular lesions were to be found in normal rats maintained upon high protein diets for up to 2 years (Osborne T B et al, 1926, Addis T et al, 1926). Newburgh had not found the cause of Bright's disease, but had stimulated interest in what would prove to be an important area of renal pathophysiology.

Theodore Moise was one of Newburgh's contemporary investigators and his views differed with those of Newburgh regarding the development of glomerular lesions and dietary protein intake. His meticulous investigations into the effects of diet upon the development of renal lesions led him to conclude that the age of the animal was an important determinant (Moise T and Smith A, 1927). His studies on the spontaneous glomerular lesions occurring in the rat showed that after one year of feeding a standard laboratory diet, focal glomerular lesions were inconspicuous, but after 500 days were present in a high percentage of animals, and from these observations suggested that the age of the animals rather than the effects of the high protein feeding. Hence, controversy existed regarding the ability of a high protein diet to induce spontaneous glomerular lesions in the normal rat, but the majority of findings support the view that, in young
animals, a high protein intake did not appear to be deleterious. Further studies have however clarified the situation. When food supply is restricted (by 30%) the occurrence of the spontaneous glomerulosclerotic lesion that occurs in the ageing rat (greater than 2 years old) can be delayed (Berg B and Simms H, 1960).

Recent studies have found that the development of the ageing glomerulosclerotic lesion in the rat is also influenced by sex and genetic strain (Bolton W K et al, 1976). Glomerulosclerosis appears to be linked with the development of proteinuria, and an increased filtration of protein macromolecules has been proposed to be instrumental in the genesis of this lesion (Elema J D, Arends A, 1975). The glomerulosclerosis of the ageing rat is qualitatively similar in some respects to the lesion occurring in partially nephrectomised animals and feeding a high protein diet to rats with a reduced renal mass will accelerate the development of glomerulosclerosis, whilst a protein restricted diet exerts an ameliorative effect.

The question of whether different dietary proteins exert differing effects upon renal structure and function however remained unanswered. Newburgh and Curtis's findings of 1928 suggested that liver protein when compared to casein, fed at an unphysiologically high level, did appear to exert a deleterious influence upon the kidney, in terms of the development of sclerotic lesions and glomerular adhesions, but definitive evidence was lacking. Newburgh and Johnston documented results of further studies in which dried liver was fed to young rats for periods up to 1 year. Their conclusions were that a 40% liver protein diet was "nephropathic" for the rat, but feeding lactalbumin, or wheat and soya protein, at the 70% protein level for 1 year resulted in no glomerular injury. From this observation they were led to
conclude that non-protein substances in liver may be damaging the kidney, and in particular they proposed that nucleic acids may be the injurious substance. Subsequent investigations using an extract of liver thought to contain a high concentration of nucleic acid produced renal damage in normal rats (Newburgh L H, Johnston M W, 1931).

Unfortunately the findings of Newburgh and Johnston are difficult to interpret. Histological analysis of the kidneys showed that the lesions induced by the liver diets were mainly those of interstitial fibrosis with round cell infiltration and thickening of the arteriolar walls. No definitive disease of the glomeruli themselves could be detected. Also it required high levels of the liver nucleate diet, much higher than those found in the 40% liver diet, to induce pathological changes within the kidney, and the protein content of this nephropathic diet was not documented.

Hence, some evidence was available to suggest that in the normal rat, a liver diet is more harmful to the kidney than either lactalbumin or wheat and soya. However definitive evidence of this suspicion was not available.

Further studies undertaken by Moise and Smith were directed towards defining the role of the diet in influencing the development of renal lesions in the rat subjected to unilateral nephrectomy. They found that uninephrectomised rats maintained upon a high protein diet (85% casein) had evidence of glomerular thickening and adhesions, whilst animals maintained upon the standard diet (18% casein) had no glomerular changes. Also, examination of the urine of animals on the high protein diet revealed elevated levels of proteinuria and cylinduria (Moise T, Smith A, 1927). Hence, when renal mass is
reduced, a high protein diet appeared to exert a deleterious effect upon the remaining kidney in terms of structure and function. Further studies by Blatherwick using uninephrectomised rats confirmed these findings, and conclusively demonstrated that high protein feeding led to increased mortality from renal insufficiency, proteinuria and glomerular lesions consistent with glomerulosclerosis, whilst animals maintained upon a low protein diet displayed no such abnormalities (Blatherwick N R et al, 1931).

Investigations into the effects of age on the response of the remaining kidney to uninephrectomy demonstrated that this was an important factor, in that young rats (less than 30 days old) tend to develop less severe glomerular lesions in the remaining kidney when maintained on a high protein diet, than adult rats (Smith A et al, 1927).

Active interest was also being directed towards the question of the diet and its effects upon renal size. Watson and Lyon in 1906 first documented that renal weight would increase in the rat maintained upon a high protein diet. Subsequent investigators (Mackay E M et al, 1928) found that the degree of renal hypertrophy induced in normal rats by feeding a high protein diet, was proportional to the quantity of protein present in the diet.

Results from the studies of Moise and Smith using unilaterally nephrectomised rats demonstrated that animals maintained upon a high protein intake developed a greater degree of renal hypertrophy in the remaining kidney; and this was associated with increased levels of proteinuria and a higher incidence of histological glomerular abnormalities (Moise T, Smith A, 1927). Some attention was then paid to investigation of other factors associated with the administration of
a protein rich diet, which may influence the changes in renal size documented experimentally. The possible factors of differing levels of phosphate, acid and urea excretion produced by the higher protein diets, however were excluded in a series of studies in 1926 (Addis T et al, 1926). Some years later it was found that different sources of dietary protein could affect renal size quantitatively, in that a gelatin diet would induce substantially more renal hypertrophy than isonitrogenous diets of albumin or casein (Wilson H, 1933, Mackay E M and Mackay L, 1934). The exact mechanism of this difference however remains unknown.

In further pursuit of links between diet and the pathogenesis of Bright's disease, Chanutin and Ferris in 1932 first described their experimental model in the rat, which possessed most of the features associated with Bright's disease, namely those of hypertension, proteinuria, polyuria and nitrogen retention (Chanutin A, Ferris E B, 1932). They reasoned that this model would approximate renal changes seen in man in Bright's disease, as the loss of functioning renal tissue was common to both. From previous investigations, it was apparent that high levels of dietary protein intake were harmful to the remaining kidney in the uninephrectomised animal, and Chanutin's subsequent experiments were directed towards investigating the effects of different diets upon structure and function in the rat when a larger portion of renal tissue was removed.

In two series of important studies (Chanutin A, 1934, Chanutin A and Ludewig S, 1936), Chanutin describes the effects of meat and liver protein diets, ranging in concentration from 10% to 80%, upon renal function, renal hypertrophy and histological glomerular damage in the rat subjected to approximately 80% nephrectomy. The results may be
summarised as follows, that animals ingesting the higher levels of protein intake showed

1. Larger volumes of more dilute urine
2. Increased levels of proteinuria
3. Increased incidence of hypertension
4. Proportionately greater renal hypertrophy in the remnant renal tissue
5. Increased histological glomerular and tubular damage
6. A higher mortality rate.

No difference was found between the diets of liver or meat, when compared at isonitrogenous levels, in terms of renal function or histological damage. However the partially nephrectomised animals, unilaterally nephrectomised animals and intact control animals maintained upon the liver diets had significantly larger kidneys than their counterparts maintained upon the meat diets. Also of interest was the finding that the partially nephrectomised animals maintained upon the 10% protein diets did not develop azotaemia or any marked degree of hypertension, and the renal function, as assessed by urea clearance, was maintained at near normal levels "for a long time". However, doubling the dietary protein intake to 20%, caused a marked increase in the occurrence of histological abnormalities and loss of renal function, and the incidence and severity of the abnormalities described rose incrementally with the dietary protein content.

Hence, from the experimental studies performed some fifty years ago, it was apparent that, in the partially nephrectomised rat, a high protein intake was associated with deleterious effects upon the remaining renal tissue, in terms of both structure and function.
The authors at this time postulated that the injurious effects may be ascribed to the amino acid composition of the protein, an excess of phosphate precursors, or non-protein constituents of the diet avenues of investigation which were to be pursued subsequently.

Three years later, Farr and Smadel undertook to determine the effects of differing dietary protein intakes upon the course of experimentally induced nephrotoxic nephritis in the rat (Farr L E, Smadel J, 1939). The diets consisted of either 5%, 18% or 40% casein, and the results obtained were very similar to those of Chanutin. The mortality of animals ingesting the high protein diet was nearly 90% during the experimental period of eleven months, compared to 30% and 6% for animals ingesting the 18% and 5% protein diets. Those animals on the higher protein intakes also suffered more severe histological renal damage, passed greater quantities of urine with substantially elevated levels of proteinuria.

The effect of reduced intake of dietary protein upon the course of experimental renal disease had now been well documented on three occasions, using two different experimental models of renal disease. However both groups of investigators were cautious in directly extrapolating their experimental findings to apply directly to Bright's disease in man. The aetiology of this condition remained unknown, and whether the diseased human kidney would respond in an analogous manner to that of the rat in response to dietary protein reduction, was also undetermined.

The conclusions that can be drawn from the large amount of experimental evidence from the first half of the century are that high protein feeding in normal animals appears to accelerate the development of glomerulosclerotic lesions which occur as part of the ageing
process. Reduction of renal mass results in the development of similar lesions, and again this can be exacerbated by a high dietary protein intake. The observation that glomerulosclerosis and a reduced functioning renal mass was common to both Bright's disease and Chanutin's experimental model has therefore naturally led to further investigation into the role of diet in the pathogenesis and treatment of renal disease, and the rat remnant nephrectomy model has proved to be a valuable tool in examination of these phenomena.
Until the 1940's the majority of investigations regarding the effects of the diet upon the kidney had been directed towards studying either normal or uninephrectomised animals. This was initially to determine if dietary protein was instrumental in causing Bright's disease. However efforts to produce an experimental model bearing similar histological characteristics to chronic nephritis seen in man, were largely unsuccessful. From the studies of Moise and Smith upon the unilaterally nephrectomised rat, it became apparent that the glomerular lesions induced by high protein feeding in those animals were of a similar nature to the lesions seen in man in chronic nephritis. Subsequent upon these observations, Chanutin and Ferris described their method of producing a model of renal insufficiency in the rat (Chanutin A, Ferris E B, 1932). Until that time there was no animal model available to examine the effects of various dietary and therapeutic manoeuvres upon the pathophysiology of chronic nephritis. They undertook to use the method which was first described by Tuffier in 1889 (Bradford J R, 1899) of reducing renal mass by approximately three quarters. This was performed by partial infarction of the left kidney, by ligation of both poles with linen sutures, and a short time later, performing a right nephrectomy. Their goal was to consistently produce the signs of hypertension, cardiac hypertrophy, polyuria, albuminuria and nitrogen retention that were seen in chronic nephritis. Previous experimenters, in attempting to achieve the goal of Chanutin and Ferris, had produced conflicting and inconsistent results, due mainly to using different species of animals and removing varying quantities of renal tissue. It was found that the goat is able to
survive when only 10% of its renal mass is functioning (Allen F M et al, 1925). However the rabbit requires 30%-40% to survive (Anderson H, 1926). Both the dog (Bradford J R, 1899) and the cat (Bainbridge F A, Beddard A P, 1907) require at least 25% of the original renal mass to sustain life, whilst the rat requires only one sixth (Chanutin A, Ferris E, 1932). In the initial studies undertaken by Chanutin and Ferris in 1932, 75% to 90% of the functioning renal tissue of the rat was excised in a two stage procedure. They found that soon after the second operation to remove renal tissue, the animals excreted large quantities of dilute urine and urine concentration tests demonstrated the inability of those animals to elevate the specific gravity of the urine. Hypertrophy of the remaining renal tissue was found after the second week, as were nitrogen retention and the gradual development of albuminuria. The hypertrophied remnant of renal tissue had a characteristic appearance, a thickened adherent capsule, surrounding pale, oedematous, granular parenchyma. The majority of the animals used in the study either were sacrificed or succumbed to renal insufficiency, prior to the end of the experimental period, but a small number of animals appeared to remain in relatively good health for seven months. Despite their appearance of well being, nitrogen retention, polyuria, hypertension and renal hypertrophy were consistently present. Common to all forms of renal disease is the fundamental loss of nephron units, and it is reasoned from this that the chronic remnant nephrectomy model will approximate conditions of renal insufficiency in man. Much investigation has therefore been performed in defining the functional and structural changes that occur following subtotal nephrectomy.
(a) **FUNCTIONAL RENAL CHANGES FOLLOWING SUBTOTAL NEPHRECTOMY**

Following a reduction in renal mass, an acute rise in the glomerular filtration rate of surviving nephrons occurs within 24 hours (Bank N, Aynedjian H, 1966, Hayslett J et al, 1968, Schultze R et al, 1969). Indeed this functional adaptation is essential if the animal is to survive, but what mechanisms govern this increase in function? Micropuncture studies have given some insight. In uninephrectomised rats a rise in whole kidney GFR, single nephron GFR (SNGFR), and renal plasma flow are found, whilst single nephron filtration fraction (SNFF) remains constant.

Single nephron glomerular filtration rate may be expressed by the equation

\[ \text{SNGFR} = K_f \cdot P_{uf} \]

where \( P_{uf} = \) mean net ultrafiltration pressure

\[ K_f = \text{ultrafiltration coefficient} \]

and \( K_f = k \cdot s \)

where \( k = \) effective hydraulic conductivity of glomerular capillaries

\( s = \) total glomerular capillary surface area

In further dissection of the mechanism of elevated SNGFR values found when renal mass is reduced, Deen determined \( K_f \) in uninephrectomised rats (Deen W M et al, 1974). His findings, in essence, were, that \( K_f \) values were similar to normal animals, and the elevated SNGFR resulted from an increased mean ultrafiltration pressure. This latter parameter, at a constant \( K_f \), is determined by changes in transcapillary hydraulic pressure gradient, systemic protein concentration and glomerular plasma flow. Further micropuncture investigation in the uninephrectomised rat has suggested that the rise
in GFR is due to an elevation in glomerular plasma flow and hydraulic pressure gradient, as systemic protein concentration remained at control values (Bayliss C, Brenner B M, 1978). Concomitant with the functional haemodynamic changes, is an increase in glomerular volume, and glomerular capillary surface area. However the failure of Kf to increase in parallel with glomerular size, as occurs in normal postnatal growth in the rat (Ichikawa I et al, 1979) suggests that glomerular hyperfiltration is associated with early pathological change. The ultrafiltration coefficient (Kf) is the product of the effective hydraulic conductivity (k) and total surface area (s) of the glomerular capillaries. Hence, the failure to observe a rise in Kf could be due to a reduction in the effective surface area available for filtration, a substantial fall in the hydraulic conductivity, or a combination of both.

In further studies, Kaufman duplicated the results of Deen in uninephrectomised rats, and also found that following 75% nephrectomy, the adaptive increase in SNGFR was even greater (Kaufman J M et al, 1974). Four weeks following uninephrectomy, SNGFR rose by 60% over control values, and the increase in renal blood flow paralleled that of GFR, maintaining filtration equilibrium. However a reduction in renal mass to 25% resulted in mean glomerular blood flow rising by 240%, whilst SNGFR increased by 150%. The disproportionate rise in glomerular blood flow, was associated with a fall in filtration fraction from 0.35 to 0.29 (Kaufman J M et al, 1975a). Thus, it appears that in the rat, the increase in SNGFR in response to renal ablation, is governed by the extent to which plasma flow increases in surviving nephrons. Concomitant with an increase in renal plasma flow, a reduction in renal vascular resistance of approximately 50% has been
found in the Munich Wistar rat, one week after five-sixths nephrectomy (Hostetter T H et al, 1981), consequent upon contributions from both afferent and efferent glomerular vasculature. Changes in intrarenal blood flow distribution also occur. Kaufman using a radiolabelled microsphere technique (Kaufman J M et al 1975a), and Carrière using a combination of inert gas washout techniques, autoradiography and renal silicone vascular casts (Carrière S et al, 1973), have demonstrated that in the partially nephrectomised rat there is marked vasodilatation, and in addition to an increase in superficial cortical blood flow, a disproportionately higher blood flow to mid and inner cortex can be seen. These findings have implications for renal tubular function, as medullary blood flow is derived from deep cortical nephrons, and this finding can, at least in part, account for some of the changes in tubular function seen in the partially nephrectomised rat.

Consequent upon the increased solute and water load presented to renal tubules following reduction in renal mass, adaptation of tubular function is required to maintain salt and water homeostasis. Indeed, when renal mass of the rat is experimentally reduced to 15%, sodium balance is maintained, whether the sodium intake is 3 mEq/day or 0.13 mEq/day (Weber H et al, 1975). Due to the large increase in SNGFR in the remnant kidney, proximal tubular sodium delivery is elevated, and the absolute quantity of sodium reabsorbed is increased. This is associated with the histological finding of tubular cell hypertrophy, and an increase in the number of sodium pump sites (Salehmoghaddam S et al, 1985). Distal tubular delivery of sodium is elevated, as is distal sodium reabsorption. However, the increment in SNGFR is so great, that despite an increase in absolute sodium resorption, there is a fall in
fractional reabsorption, and hence the mechanism is counterbalanced, resulting in a rise in the fractional sodium excretion per nephron, maintaining sodium balance. This adaptation in fractional sodium excretion occurs within the first 24 hours following partial nephrectomy, and has been found to rise from 0.7% to 7.0% in partially nephrectomised dogs during this period (Schultze R et al, 1969). Similar adaptive changes in water excretion are seen. The rate of tubular fluid reabsorption is increased markedly, but again, increased fluid delivery to the tubules is elevated such that there is an increase in fluid excretion per nephron, and water homeostasis is maintained. The precise controlling factors that are involved in maintaining sodium balance, and the adaptive increase in fractional sodium excretion are not known at the present time.
Progressive proteinuria is a constant finding in the subtotally nephrectomised rat. This results from an increase in transglomerular flux of macromolecules, consequent upon a reduction in efficiency of the forces which normally exclude protein from the urinary space, i.e. the glomerular fixed negative charge, and size selective properties of the glomerular capillary wall. Suggestions regarding the mechanism of macromolecular transgression across the glomerular capillary barrier have been proposed by Brenner's laboratory.

Studies by Olson, performed in Brenner's laboratory, show that loss of the charge and size selective properties of the glomerular basement membrane which occurs following a reduction in renal mass can be ameliorated in rats fed a low protein diet. They correlate this finding with the observation of an attenuation in the rise of glomerular plasma flow and transcapillary hydraulic pressure gradient in such animals compared to those fed a high protein diet, to suggest that the changes in glomerular permselectivity are a result of direct microvascular injury consequent upon altered glomerular haemodynamics following a reduction in renal mass (Olson J L et al, 1982).

Similar hypotheses concerning the role of altered glomerular haemodynamics have been proposed by Azar, to account for the mesangial expansion and focal glomerulosclerosis that occur in the uninephrectomised rat (Azar S et al, 1977), and by Feld, concerning the failure of autoregulation of deep cortical nephrons and consequent morphological changes that occur in the spontaneously hypertensive rat (Feld L G et al, 1977). Further evidence that alteration in glomerular
haemodynamics affects the porosity of the glomerular filter, was provided by Olivetti by raising glomerular intracapillary hydrostatic pressure with angiotension II infusion. However, in this study, the increased transglomerular flux of proteins appeared to be mediated solely through an increase in pore size, as the electrostatic barrier, as assessed by colloidal iron staining and permeability to variously charged ferritin particles, appeared intact (Olivetti G et al, 1984).

Other evidence has been produced to suggest that changes in glomerular haemodynamics may not be the sole mechanism responsible for alteration in glomerular permselectivity. Giordano has proposed that neutralization of the glomerular charge barrier may occur as a result of circulating natural polycations, and disruption of the electrostatic glomerular barrier would result in proteinuria (Giordano C et al, 1984). This hypothesis was prompted by Mogensen's observation that lysine infusion in normal man will acutely produce proteinuria, but the authors proposed that the mechanism was a reduction in tubular protein reabsorption (Mogensen C et al, 1977), rather than an increase in the amount of protein filtered.

Subsequent studies using homocopolymers of cationic amino acids have shown that intravenous administration of Poly-L-lysine and protamine sulphate, in the rat, will both acutely elevate urinary albumin excretion (Vehaskari V et al, 1982), and that this results from an increase in fractional clearance of albumin rather than a reduction in tubular reabsorption. Further evidence that macromolecular charge is an important factor in determination of proteinuria was presented by Purcell. By chemically increasing the isoelectric point of albumin infused into the rat from the normal value of 4.9 to 7.2-8.2, urinary albumin excretion rose ten-fold, and again this was thought to be due

These findings were supported by studies from Giordano's laboratory demonstrating that Poly-L-histidine and Poly-L-arginine (both cationic amino acid polymers) infusion in both normal and subtotally nephrectomised rats will increase urinary albumin excretion two-fold and ten-fold respectively (Giordano C et al, 1984). Therefore polycationic macromolecules may have the ability to disrupt the electrostatic glomerular barrier and allow albumin to transgress the glomerular wall but demonstration that naturally occurring molecules may act in this manner, as proposed by Giordano, has not yet been shown.

Changes induced in the transglomerular passage of protein molecules which occur in the subtotally nephrectomised rat, are thought to be of importance in the development of the mesangial lesions in this experimental model. An increase in proteinuria in the subtotally nephrectomised rat maintained upon high protein intake, was observed by Lalich, and it was proposed that the increase in transglomerular traffic of proteins may be a stimulus to the development of mesangial thickening and capillary sclerosis seen in this model (Lalich J et al, 1975). Other supportive evidence of this hypothesis comes from the finding that proteinuria precedes the development of focal sclerotic mesangial lesions in the ageing rat, and that in puromycin aminonucleoside induced proteinuria, the sclerotic mesangial lesions appear to be due to an increase in transglomerular protein flux as a result of loss of the glomerular electrostatic barrier (Velosa J et al, 1975, Conset W et al, 1975, Grond J et al, 1982).
Therefore, the progressive proteinuria which accompanies a reduction in renal mass is likely to be due to a reduction in both the size selective and charge selective properties of the glomerular filter, but the exact mechanism for this functional disruption is not known. Also, the increase in transglomerular protein flux itself may contribute to some of the histological abnormalities which develop in this model of renal insufficiency.
Following a reduction in renal mass, the remaining renal tissue undergoes functional adaptation within hours, as shown by increases in GFR and renal blood flow. Structural adaptation also rapidly occurs. The majority of acute investigations examining the renal response to a reduction in renal mass have been undertaken in the unilaterally nephrectomised animal. In animals subjected to greater than a fifty per cent reduction in renal tissue, similar qualitative changes take place, but the complicating factor of local tissue injury renders the unilaterally nephrectomised animal more suitable to acute physiological investigation (Malt R A et al, 1969).

Following the removal of one kidney, hypertrophic changes in the remaining renal tissue begin rapidly. A true increase in dry weight has been observed within 36 hours of contralateral nephrectomy, (Coe F L, Korty P, 1967). This is due to hypertrophic growth, as shown by elevated levels of amino acid incorporation into renal protein, and an increased rate of membrane synthesis (Tomashefsky P, Tannenbaum M, 1969, Toback F G et al, 1974). There is an early marked rise in the ribonucleic acid content of the kidney, which has increased by 33% after 48 hours, and a rapid increase in the number of free ribosomes associated with the elevated rate of protein synthesis. The protein content of the remaining kidney rises in a biphasic manner, with an initial peak in the protein synthetic rate at 24 hours, followed by a second peak occurring 24 to 48 hours later, which declines slowly after 72 hours. A small increase in DNA synthesis is seen in the first two days, but the DNA content of the kidney has risen to 10% at nine days and 25% at two weeks (Malt R A 1969). The disproportionate rise in RNA
and protein content, compared to the DNA suggests that hypertrophy, rather than hyperplasia, is the dominant form of growth.

The elegant microdissection studies of Oliver in 1944, using the remnant rat kidneys provided by Thomas Addis, demonstrated that hypertrophy is the major form of adaptive growth in this situation. Using camera lucida drawings of micro-dissected nephrons, Oliver calculated that the volume of a proximal tubule had increased five times by 30 days following 75% nephrectomy, and the remaining kidney fragment had enlarged so much that its weight was approximately that of a normal kidney. Oliver presented few quantitative measurements, but the loop of Henle also showed a great increase in length and diameter following 75% nephrectomy (Oliver J, 1944). Similar results, demonstrating increased length, diameter and volume of tubules have been shown in the uninephrectomised rat, and ten days following uninephrectomy, proximal tubule cell volume had increased by 76% (Hayslett J P et al, 1968). Tubular cell hypertrophy is associated with an increase in absolute reabsorptive work, probably due largely to the increase in sodium delivery to the tubules. Mitochondrial numbers are elevated in tubular cells, to sustain the increased energy requirements, and also the number of Na-K pump sites are increased in proportion to the protein content of the cell (Salehmoghaddam S et al, 1985).

In addition to tubular hypertrophy occurring when renal mass is reduced, an enlargement of remaining glomeruli also occurs. In five-sixths nephrectomised rats, glomerular volume has been found to increase six fold in a twenty one week period (Shea S M et al, 1978). The increase in size was apparent two weeks post nephrectomy, and increases at a linear rate. Glomerular epithelial cell hypertrophy
accompanies the increase in glomerular size, as does dilatation of glomerular capillaries (Shea S M et al, 1978, Shimamura T, Morrison A B, 1975). In adult animals, it appears that there is no new formation of nephron units (Kaufman J et al, 1975b).

Concomitant with hypertrophy of the formed elements of the kidney, there is an increase in remnant kidney size. Following unilateral nephrectomy, the remaining kidney weight will increase by approximately 50%-80% in four weeks (Hayslett J P et al, 1968, Kaufman J M et al, 1974a). When greater than 50% of the renal tissue is removed, the hypertrophy of the remaining tissue appears to be proportional to the mass ablated. Kaufman et al (1974a) demonstrated that renal mass increased 81% in a four week period in uninephrectomised rats, whilst in animals subjected to 75% renal ablation, the remnant tissue increased in size by 168%. In association with the increased renal hypertrophy seen in 75% renally ablated rats, the calculated single nephron GFR was 47% higher than the uninephrectomised animals. From those observations, Kaufman concluded that the adaptive changes in both structure and function seen in remaining renal tissue are proportional to the amount of tissue removed.

Hence following renal ablation the remaining kidney tissue undergoes a rapid growth which is most marked during the first month post nephrectomy, and is mainly due to hypertrophy. However what are the factors which influence and regulate this compensatory growth?

The age of the animal is an important consideration, as weanling rats demonstrate a greater capacity for compensatory growth than do young adults following renal ablation (Kaufman J et al, 1975). In weanling animals both hypertrophy and hyperplasia contribute to the
growth of the remaining renal tissue, but with advancing age, hyperplasia becomes progressively more limited (Malt R A, 1969).

The availability, and protein content of the diet also influence compensatory hypertrophy. Starvation will reduce renal growth, whilst increasing the protein content of the diet from 15% to 45% will produce a further increment of 20% in compensatory hypertrophy in the unilaterally nephrectomised rat (Halliburton I W, 1969). In Chanutin and Ludewig's experiments using whole dried meat as the dietary protein source for rats that had undergone partial renal ablation, an increase in the dietary protein content from 20% to 40% resulted in a 13% rise in remnant kidney weight (Chanutin A, Ludewig S, 1939). Also Dicker and Shirley observed a similar response to increasing the protein content of a casein diet (Dicker S E, Shirley D G, 1971).

The amino acid composition of the diet may also be an important factor in controlling compensatory renal hypertrophy in the unilaterally nephrectomised rat. Halliburton found that a casein diet supplemented with gelatin would induce 50% more renal hypertrophy than will an isonitrogenous casein diet (Halliburton I W, 1969). Gelatin is an unusual protein in that glycine accounts for approximately one quarter of its amino acid content. Subsequent studies by Halliburton showed that a standard casein diet supplemented with glycine, proline or glutamic acid would induce substantially more renal hypertrophy than supplements of serine or isoleucine. Other evidence that the nature of the dietary protein is important, comes from the studies of Chanutin and Ludewig, who documented that a liver protein diet induced substantially more renal hypertrophy than an isonitrogenous meat protein diet in the subtotally nephrectomised rat (Chanutin A, Ludewig S, 1939).
An increase in the amino acid or protein content of the diet will impose an additional burden of acid and urea to be excreted. Studies involving addition of ammonium chloride to the diet, sufficient to induce acidosis in the uninephrectomised rat, demonstrated a slight increase in compensatory renal hypertrophy, but additions of urea to the diet, had no effect (Halliburton I W, Thomson R I, 1967).

One of the initial hypotheses of the regulation of kidney growth was the work hypothesis, as it was known from the observations of the Mackays and Addis that the protein content of the diet was an important factor in determining renal weight (Mackay E M et al, 1928). It was thought that the increased generation of urea, and the need to excrete more urea, imposed an extra workload upon the kidney and that this induced hypertrophy. However it is now known that urea handling by the kidney does not constitute a significant component of renal energy utilisation. The major process responsible for renal energy consumption is sodium resorption, and it has been thought that this may be a stimulus to renal hypertrophy. However a number of investigations have shown that hypertrophy can be dissociated from tubular reabsorption of sodium (Kiill F et al, 1968, Weinman E J et al, 1973).

Numerous hormones have been implicated in controlling compensatory renal hypertrophy, as following hypophysectomy there is a profound reduction in compensatory growth (Meyer T W et al, 1984, Astarabadi T, 1962), whilst ACTH administration for one week can enhance the rate of compensatory renal growth (Ross J, Goldman J K, 1970).

The observation by Kaufman that the degree of compensatory growth was related to the mass of tissue removed raised the possibility that as a consequence of reduced kidney function, substances may be retained that directly influence renal growth. Following studies undertaken by
Kurnick and Lindsay in 1968, which demonstrated that renal hypertrophy could be induced in a parabiotic mouse whose parabiont had undergone unilateral or bilateral nephrectomy (Kurnick N B, Lindsay P A, 1968). Obertop and Malt examined this situation further using parabiotic rats (Obertop H, Malt R A, 1977). Renal hypertrophy, as detected by an increase in RNA concentration, could be induced in the parabiotic partners of rats that had undergone bilateral nephrectomy, but not in the parabionts of rats that had undergone bilateral ureteral ligation. The inference from these studies was that a humorally transmitted substance was present in those animals which had lost both renal mass and function, as opposed to those animals who had solely lost renal function but with renal mass intact, and that this substance stimulated renal growth in the parabiont.

The existence of such humoral substances, termed renotropins, was suggested by these studies, and subsequent investigations have demonstrated the presence of renotropic factors in uninephrectomised rabbit serum (Yamamoto N et al, 1983) and rat urine (Harris R H et al, 1983). The growth stimulating substance(s) increases significantly within the first 24 hours following partial renal ablation, and appears to be specific for renal tissue. The origin and composition of the renotropin(s) are questions not yet answered.
(d) RENAL HISTOLOGICAL APPEARANCES FOLLOWING SUBTOTAL NEPHRECTOMY

Much investigation has been undertaken into the histological changes occurring in the kidney remnants of partially nephrectomised rats, since the lesions that occur resemble some of the glomerular abnormalities found in a variety of chronic renal diseases in man. The similarity of the lesions might reflect a common shared loss of functioning nephron units. The histological changes occurring are probably a combination of effects reflecting an adaptive increase in glomerular function and size, and pathological abnormalities that occur, perhaps as an ultimate result of the former.

The temporal sequence of events differs quantitatively between a number of studies, due to minor differences in operative procedure and strain of rat employed, but qualitatively there is general agreement upon histological characteristics of the lesions which develop in the remnant kidney of a rat. Prominent changes are seen in the appearance of epithelial cells, mesangium and endothelial cells at an early stage after a reduction in renal mass, and the rapidity of development of the lesions appears to be related to the amount of renal tissue remaining.

The ultimate outcome of glomerular obsolescence resulting from focal, and later global, glomerulosclerosis, is thought to be related to four basic underlying pathological processes which occur following subtotal nephrectomy.

a) Intraglomerular Thrombosis - Endothelial swelling and detachment from the underlying basement membrane, which may result from altered shear stress consequent upon a change in glomerular haemodynamics (Dewey C F et al, 1981), may predispose the endothelial cell to lose its natural thromboresistant qualities (Rennke H, 1986). Microthrombi,
occluding glomerular capillaries, can be seen following subtotal nephrectomy (Purkerson M et al, 1976, Olson J, 1984), and organisation of thrombus will lead to segmental glomerular obsolescence. Anticoagulant administration has been shown to be beneficial in reducing glomerular abnormalities and intraglomerular thrombosis following subtotal nephrectomy, but the exact mechanism by which the ameliorative effect is produced is not known (Purkerson M et al, 1976).

b) Microaneurysm Development - A further phenomenon which may lead to segmental capillary collapse is the organisation of platelets and fibrin that are seen in microaneurysms of intraglomerular capillaries (Dworkin L et al, 1984). The abnormally dilated vascular channels are thought to directly result from the elevated intraglomerular hydrostatic pressure.

c) Mesangial Expansion and (d) Subendothelial Hyaline Deposition - At an early stage following subtotal nephrectomy, endothelial cell detachment from the underlying basement membrane, with exposure of focal areas of lamina rara interna and large cytoplasmic vacuoles can be seen. Loss of foot processes and microvillus formation occurs, and numerous droplets, thought to be lysosomes, can be seen in the attenuated cytoplasm of the epithelial cells (Olson J et al, 1982). In association with these changes are the deposition of eosinophilic, PAS positive material in the subendothelial and mesangial regions which has the light microscopic appearances of hyaline, and immunochemical characteristics of IgM and fibrin related antigens (Rennke H, 1986). Denudation of the glomerular basement membrane, and an increase in large molecular flux, may well be related to the development of these abnormalities, which consequently results in capillary occlusion.
An increased influx of macromolecules into the mesangial compartment is also believed to be responsible for the expansion in matrix which follows subtotal nephrectomy (Olson J et al, 1982). Changes in intracapillary pressures undoubtedly contribute to this macromolecular traffic, and this may also influence mesangial cell metabolism and mitosis.

Whatever the mechanisms responsible, an expansion of the mesangium is a prominent finding following subtotal nephrectomy, and appears to contribute substantially to capillary obliteration and glomerular obsolescence (Purkerson M et al, 1976, Shimamura T, Morrison A B, 1975).

Progression of glomerular pathology occurs by proliferation of mesangial cells and focal enlargement of the PAS positive areas of material within cells. Periglomerular fibrosis and precipitation of coagulated protein material in Bowmans space develops and the once focal nature of the mesangial abnormality expands to involve large areas of mesangial matrix. The expansion of mesangial matrix, together with the reduction in vascular supply to the glomerulus, eventually results in the total replacement of the structure by a mass of fibrotic, PAS positive material.

Tubular changes of dilatation, hypertrophy and hyperplasia are commonly found, reflecting the increase in functional demand (Kenner C H et al, 1985). Numerous protein resorption droplets, most prominent in the proximal tubule, are found representing an increase in the delivery of filtered proteins to the tubular cells. Precipitation of protein also occurs resulting in the formation of tubular casts. Changes of tubular atrophy are also seen with small flat tubular cells lining a small, or non-existent lumen. Inflammatory changes of
interstitial nephritis, with round cell infiltration, are not seen in the chronic stages of the lesion.

The distribution of the glomerular and tubular abnormalities appears to be at random throughout the renal tissue, and severely damaged glomerular remnants may be found in close proximity to relatively normal glomeruli.
FACTORS WHICH MODIFY THE COURSE OF EXPERIMENTAL RENAL DISEASE

a) Protein Intake - Following the initial observations of Chanutin, and Farr and Smadel, that high protein diets are deleterious to renal structure and function in experimental renal disease a number of investigators have subsequently shown that low protein diets can reduce the severity of functional and histological renal damage following subtotal nephrectomy (Kleinknecht C et al, 1979, El Nahas A M et al, 1983, Kenner C H et al, 1985, Hostetter T H et al, 1986). What are the possible underlying mechanisms responsible for this response? One hypothesis, proposed by Brenner in 1982, correlates the changes in glomerular haemodynamics produced by low protein feeding and the ameliorative effects of this diet upon structure and function, and proposes a causative link between the two (Brenner B M et al, 1982). This stems from investigations undertaken in Brenner's laboratory studying glomerular haemodynamics and function in the subtotally nephrectomised rat one week after a reduction in renal mass (Hostetter T H et al, 1981). Their findings demonstrate that subtotally nephrectomised rats fed a protein deficient diet (6%) had significantly lower values for SNGFR than animals fed a standard 24% protein diet. This was associated with lower glomerular plasma flow rates and mean glomerular transcapillary hydraulic pressure differences, which did not differ significantly from normal rats. The low protein diet had therefore abolished the adaptive increase in SNGFR and glomerular plasma flow following a reduction in renal mass, and little change in renal vascular resistance was found.

In addition to the differences in renal haemodynamics and function documented, marked differences in the ultrastructure of the remnant
kidneys was seen. The endothelial cell detachment, attenuation of epithelial cells, foot process fusion, and mesangial expansion, all typical sequelae of a reduction in renal mass were seen in animals maintained upon the 24% protein diet, but the renal ultrastructure of animals maintained upon the low protein exhibited none of these abnormalities.

Brenner proposed that the adaptive increase in function seen following renal mass reduction is itself the injurious factor which produces glomerular abnormalities. By ameliorating the degree of adaption, with low protein feeding, renal function and structure are preserved. The main adaptive factors responsible for the damage being the elevated mean glomerular transcapillary hydraulic pressure difference and glomerular plasma flow rate. The proposed mechanisms for the injurious effects are as a direct result of intraglomerular hypertension upon the capillary network, in an analogous manner to the effects of systemic hypertension; presumably involving mechanical disruption of normal vascular integrity. This suggestion has been subsequently supported by the observation of Anderson that prevention of intraglomerular hypertension in the subtotally nephrectomised rat by administration of enalapril, proteinuria and histological glomerular damage can be ameliorated (Anderson S et al, 1985). Control of systemic hypertension had previously been shown to be beneficial in reducing the degree of histological damage in this model (Purkerson M et al, 1976).

Additional, rather indirect, support for the hyperfiltration hypothesis comes from study of the hypophysectomised subtotally nephrectomised rat. In this model, prevention of the adaptive increase in GFR, renal plasma flow and renal hypertrophy, by hypophysectomy
THE HYPERFILTRATION HYPOTHESIS
(from Brenner B M et al, 1982)

AD LIBITUM
PROTEIN INTAKE

CHRONIC REDUCTION IN RENAL MASS

DIABETES MELLITUS WITH LONGSTANDING HYPERGLYCAEMIA

CHRONIC RENAL VASODILATATION

RENAL PLASMA FLOW

MEAN GLOMERULAR TRANSCAPILLARY HYDRAULIC PRESSURE GRADIENT

GLOMERULAR HYPERFILTRATION

PERMSELECTIVITY CHANGES

DIRECT CELLULAR INJURY

PROTEIN FLUX

CELLULAR PROLIFERATION

ALBUMINURIA

MESANGIAL CELL INJURY

GLOMERULAR SCLEROSIS

MESANGIAL MATRIX OVERPRODUCTION
(Astarabadi T, Essex H, 1953) resulted in amelioration of histological damage to the remnant kidney, a failure of the glomerular transcapillary hydraulic pressure gradient to rise, and a marked reduction in the magnitude of proteinuria (Meyer T W et al, 1984).

The final conclusion reached is that haemodynamic adaptions occurring when nephron number is reduced represents a mechanism for a final common pathway of residual nephron destruction, which may operate when nephron number is reduced, by whatever initial mechanism.

Although the hyperfiltration hypothesis proposed by Brenner has gained widespread support, additional experimental evidence indicates that modification of other parameters may influence the course of renal disease in the subtotally nephrectomised rat, and the adaptive changes in renal haemodynamics may not be the sole mechanism responsible for the injurious effects following subtotal nephrectomy.

b) **Intraglomerular Coagulation** - Observations that intraglomerular thrombosis is a common finding in the subtotally nephrectomised rat, led Purkerson to examine the effects of anticoagulation upon the pathogenesis of experimental renal failure (Purkerson M et al, 1976). Daily heparin administration to subtotally nephrectomised rats resulted in a reduction of the histological abnormalities subsequently developing, and amelioration of hypertension and uraemia. Further investigation by Olson has confirmed this finding (Olson J L 1984). However, in addition to its anticoagulant properties heparin also has other effects of enhancement of the vascular wall negative potential, by virtue of the anionic nature of heparin, and inhibition of renal kallikrein which may lead to a reduction in renin production. In Olson's study, the arterial blood pressure in the treated animals was normal, compared to the untreated hypertensive rats. Hence, the
lowered blood pressure of the treated rats does confuse the situation, as it is not known whether this was cause or effect of reduced renal injury. Further evidence that intraglomerular thrombosis may be an important factor in the development of glomerular lesions in the subtotally nephrectomised rat, comes from the further studies of Purkerson showing that administration of aspirin and dipyridamole are beneficial in retarding the development of histological abnormalities (Purkerson M L et al, 1984). This again was associated with a normalisation of blood pressure, but in both studies, this was thought to be the result of amelioration in renal damage, rather than its cause.

Further evidence suggesting that an elevation in GFR and renal plasma flow may not play as great a role as is proposed by Brenner, has also been produced by the St Louis group. Treatment of subtotally nephrectomised rats with a thromboxane synthesis inhibitor resulted in histological amelioration and reduced levels of proteinuria, despite a GFR and effective renal plasma flow which were 128% and 57% higher than untreated animals (Purkerson M L et al, 1985). This was associated with normal systemic arterial blood pressure, but unfortunately no documentation of glomerular haemodynamics was provided. These observations indicate that platelet/endothelial cell interaction and intraglomerular thrombosis may be important in the development of glomerular lesions in the subtotally nephrectomised rat, and functional and structural amelioration of glomerular damage was evident despite elevation in GFR and renal plasma flow.

c) **Dietary Phosphorus** - The renal toxicity of excess dietary phosphorus has been extensively studied, and several groups have demonstrated the adverse effects of a high dietary phosphorus intake when rats are
subjected to a reduction in renal mass (Haut L et al, 1980, Gimenez L et al, 1982). A fourfold increase in the dietary phosphorus, from the normal intake of 0.5%, to 2.0% in the subtotally nephrectomised rat, results in a marked deterioration of renal function. The mechanism of injury is thought to result from calcium-phosphate deposition in the renal parenchyma, leading to a fibrotic reaction, resulting from the increased phosphate excretion per nephron.

From these observations, Ibels reasoned that a decrease in dietary phosphorus intake may be beneficial in retarding the development of renal insufficiency in the experimental model (Ibels L S et al, 1978). Subsequent studies by this group demonstrated that severe dietary phosphorus restriction prevented the proteinuria, histological damage and death following subtotal renal ablation, however the rats studied on this diet did become severely hypophosphataemic. The question of phosphorus restriction has recently been re-examined, in view of the fact that severe hypophosphataemia will produce a number of changes in animals, namely anorexia and growth retardation (Laouari D et al, 1982). It was unclear from the studies of Ibels' if the beneficial effects of the low phosphorus diet were due to the phosphorus load per se, or to a reduction in other nutrients associated with anorexia and probable reduction in food intake. Controlled experiments performed by Laouari, have demonstrated that moderate phosphorus restriction (0.2%) sufficient to induce mild hypophosphataemia, without anorexia or growth arrest, conferred little or no benefit to rats with reduced renal mass in terms of survival or renal histological damage, and concluded that the protein load is the major factor responsible for renal deterioration in the subtotally nephrectomised rat.
The question concerning the role of phosphorus in the progression of renal disease has been further investigated by Schrier's group. They found an amelioration in experimentally induced renal disease in the subtotally nephrectomised rat following feeding of an aluminium oral phosphate binding agent sufficient to reduce serum phosphate levels, in pair fed animals (Lumlertgul D et al, 1986). The situation, therefore, regarding the precise pathophysiological role of phosphorus in the progression of renal disease remains unresolved at the moment.

d) Dietary Lipids - Manipulation of the dietary lipid intake may also be a factor in affecting the course of renal disease in the subtotally nephrectomised rat. This hypothesis stems from observations that dietary fatty acids are precursors required for prostaglandin synthesis, and this group of complex compounds are involved in regulation of glomerular filtration and platelet-endothelial cell interaction, two mechanisms which are thought to influence the development of experimental renal disease. Barcelli maintained subtotally nephrectomised rats on either a high (safflower oil) or low (beef tallow) linoleic acid diet for a 5 month period (Barcelli U et al, 1982). It was found that animals fed the high linoleic diet did not develop the lesions of progressive glomerulosclerosis or proteinuria, and this was associated with higher renal cortical production of prostaglandin E₂. Barcelli postulated that by altering the linoleic acid concentration of the diet, changes in prostaglandin metabolism occurred in the remnant kidneys which were responsible for the reduction in renal damage. Interpretation of the results is difficult, as the level of dietary lipid supplied in the diet was unusually high (20%) and in addition, increasing the dietary content of linoleic acid is known to affect platelet function and blood pressure.
However, similar results have been obtained using the same experimental conditions (Heifets M et al, 1985). Therefore dietary lipid manipulation does appear beneficial in this model of renal disease, but suggestions regarding the mechanism of action are purely speculative at the moment, as little is known regarding the role of intrarenal prostaglandins in this model of chronic renal insufficiency.
RELATIONSHIP OF DIETARY PROTEIN TO RENAL DISEASE IN MAN

No experimental model of renal insufficiency can replicate the exact pathophysiological changes which occur in human renal disease, as interspecies differences exist in renal size, glomerular function and immune status. In man a large number of primary disease processes can ultimately result in a similar state of pathophysiological dysfunction, namely a reduced quantity of functioning renal tissue. The remnant nephrectomy model of renal insufficiency in the rat however, is a good approximation of changes occurring in a large number of human renal diseases, as common to both is the loss of functioning nephron units. Indeed, conditions in man, such as oligomeganephronia and subtotal renal ablation can progress to terminal renal failure resulting from glomerulosclerosis, as in the rat (Solomon L R et al, 1985).

A well documented, although rather ill understood, phenomenon which often occurs in human renal disease, is a mathematically predictable rate of decline in renal function which will vary from patient to patient. This might infer that once renal tissue has sustained an insult, from whatever initial disease process, subsequent loss of functioning nephron units will proceed at a predetermined rate, and this progression is intrinsic to the kidney rather than dependent upon the continued presence of the initial disease process. Mechanisms responsible for this continued intrinsic progression of renal lesions may be related to haemodynamic changes, as proposed by Brenner, but a number of other factors may also operate.

Protein restricted diets have been used for many years in man in the symptomatic treatment of advanced uraemia. However, the experimental evidence of the previous six decades documenting the
beneficial effects of dietary protein restriction upon the progression of renal lesions in animals, has largely been ignored until recently.

Retrospective analysis of the decline in renal function in patients suffering with chronic renal failure, prescribed a protein restricted diet, has shown that 90% of such patients will derive some benefit, in that the decline in renal function can be lessened or even halted in some cases (Maschio G et al, 1982, Williams A J et al, 1984). A prospective study of dietary protein restriction has confirmed these findings (Rosman J B et al, 1984).

Therefore, the dietary intake of protein appears to be intimately associated with the progression of renal disease in man and further insight into the underlying mechanisms may enhance our understanding of the disease process itself.
CONCLUSIONS

The quantity of dietary protein is undoubtedly an important factor in determination of renal function in the normal animal both in the postprandial and post-absorptive state. When renal mass is reduced experimentally, the amount of protein fed to the animal is also a major factor in determining the severity of the ensuing renal damage, the longevity of the animal and the degree of renal hypertrophy. These experimental observations have important implications for the treatment of renal disease in man.

Knowledge of the precise mechanisms by which dietary protein and amino acids influence renal haemodynamics in the normal and subtotally nephrectomised animal is rather scant at present, but haemodynamic adaptation to a loss of renal tissue does appear to play an important role in both the structural and functional adaptation which follows, and also the degree to which the remaining renal tissue succumbs to injury.

A number of studies previously have suggested that the response of the kidney to feeding different types of dietary protein may differ, indeed experiments performed some fifty years ago, upon subtotally nephrectomised animals, document that the degree of renal hypertrophy may be quantitatively affected by the type of dietary protein. Recent investigations in normal man have also shown that the nature of the dietary protein may be an important factor in determining glomerular filtration. Most of the recent studies investigating the effect of dietary protein upon the structure and function in remnant renal tissue following subtotal nephrectomy have used one type of protein as the source fed to experimental animals, whilst a limited number of other
studies fed a laboratory rat diet, usually composed of proteins from one or two sources. When examining the rat, this is a rather artificial situation, as this animal has evolved as a scavenger, and typically consumes an omnivorous diet. Also, if one extrapolates experimental findings from animals to man, the human consumes many differing types of proteins.

Many questions pertaining to the evolution of renal damage following an experimental reduction in renal mass and its relation to diet remain unanswered. Haemodynamic factors are undoubtedly important, but if differing dietary proteins exert differing influences upon renal function in the normal animal, does a similar phenomenon occur in experimental renal disease, and if this is indeed the case can the course of the disease process be modified?

The majority of studies undertaken have focussed primarily upon aspects of renal pathophysiology in relationship to the diet. A supply of amino acids to the body tissues is an essential prerequisite for normal growth and development, and in states of renal insufficiency many abnormalities of metabolic and endocrine processes have been described, which impair these functions.

The studies described in the following sections have addressed a number of unanswered questions regarding the effects of dietary protein upon the progression of experimental renal disease, and were designed not only to examine the effect of different dietary proteins upon the renal response to subtotal nephrectomy, but also to examine the effects of such diets upon the organism as a whole.
SECTION 2

MATERIALS AND METHODS
MATERIALS AND METHODS

Adult female AS strain Wistar rats (Leicester University) were used throughout the studies described.

ANIMAL HOUSING

Animals were housed in individual cages, 40x25x20cm, with free access to food and tap water, except where stated. Temperature was maintained at 18-22°C, with a 12 hour light/dark cycle.

ANIMAL FEEDING

a) Standard diet

All animals were maintained on the standard rat laboratory diet, in dry pellet form, from weaning. (ERM diet, Labsure Ltd, Croydon.) The amino acid composition of the diet, as supplied by the manufacturers, is given in tables 2.1 and 2.2. The crude protein content of the diet is 16% and the source of the majority of the protein content (10-15% of whole diet protein) is soya meal, and small quantities of wheat, barley and fish meal are added. Trace elements and vitamins are formulated in the diet, and analysis of other components reveals a phosphorus content of 0.7%, sodium 0.7%, crude oil 2.1%, metabolisable energy 2606kcal/kg. The essential amino acid content of the diet is 5.8% (lysine, tryptophan, isoleucine, valine, threonine, methionine, and cystine, histidine, leucine, phenylalanine and tyrosine).

b) Experimental Diets

The experimental diets differed exclusively in the protein source and content. The sources of protein used were casein, and soya (heat
treated, solvent extracted soya flour). The amino acid composition of the protein sources, and the experimental diets (calculated from amino acid composition of crude protein as supplied by the manufacturers, RHM Research Ltd, High Wycombe) are given in tables 2.1 to 2.5. Differences in the amino acid distribution exist, and were most marked for arginine, aspartic acid, proline and glycine and alanine. The amino acid composition of the soya protein was similar to the standard laboratory diet, as the latter was composed largely of soya protein. The essential amino acid content of the casein diets was 22.78%, 11.39% and 5.7%, at 48%, 24% and 12% levels and that of the soya diets was 10.16% and 5.08%, at the 24% and 12% protein levels respectively. All diets exceeded the minimum essential amino acid levels required to support maximal growth in the weanling rat, (4.73%) (From Rama Rao, P B et al, 1959).

The composition of all the experimental diets was adjusted so that the sodium (0.5%) potassium (1.1%) calcium (1.0%) phosphorus (0.5%) metabolisable energy (2867 kcals/kg) vitamin and trace element content were identical (table 2.3).

The experimental diets were formulated as dry powder, and to improve the palatability, and accuracy of food consumption determination, the diet was mixed into a thick paste. Twenty five grams of the dry experimental diet was measured into preweighed earthenware pots (weight approx. 230g, diameter 7cm, height 3cm) to which was added 25ml of deionised water, and thoroughly mixed into a smooth, thick consistent paste. Food was freshly prepared for each animal daily and presented to the animals each morning. Food consumption was determined by reweighing the food containers the following morning (Sartorius balance ±0.5g) and the quantity of dry
PERCENTAGE AMINO ACID COMPOSITION OF EXPERIMENTAL AND LABORATORY DIETS, AND LEVELS REQUIRED FOR MAINTENANCE AND GROWTH

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Level Required for Growth</th>
<th>Level Required for Maintenance</th>
<th>48% Casein</th>
<th>24% Casein</th>
<th>12% Casein</th>
<th>24% Soya</th>
<th>12% Soya</th>
<th>Standard Laboratory Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>0.21</td>
<td>0.07</td>
<td>1.86</td>
<td>0.93</td>
<td>0.47</td>
<td>0.73</td>
<td>0.37</td>
<td>0.40</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.55</td>
<td>0.43</td>
<td>2.36</td>
<td>1.18</td>
<td>0.59</td>
<td>1.06</td>
<td>0.53</td>
<td>0.60</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.69</td>
<td>0.25</td>
<td>4.26</td>
<td>2.13</td>
<td>1.07</td>
<td>1.77</td>
<td>0.89</td>
<td>1.20</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.90</td>
<td>0.14</td>
<td>2.72</td>
<td>1.36</td>
<td>0.68</td>
<td>1.66</td>
<td>0.83</td>
<td>0.70</td>
</tr>
<tr>
<td>Cystine &amp; Methionine</td>
<td>0.49</td>
<td>0.26</td>
<td>1.52</td>
<td>0.76</td>
<td>0.38</td>
<td>0.58</td>
<td>0.29</td>
<td>0.40</td>
</tr>
<tr>
<td>Phenylalanine &amp; Tyrosine</td>
<td>0.72</td>
<td>0.19</td>
<td>4.68</td>
<td>2.34</td>
<td>1.17</td>
<td>1.95</td>
<td>0.97</td>
<td>1.20</td>
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<tr>
<td>Threonine</td>
<td>0.51</td>
<td>0.17</td>
<td>1.64</td>
<td>0.82</td>
<td>0.41</td>
<td>0.93</td>
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<td>0.34</td>
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<tr>
<td>Valine</td>
<td>0.56</td>
<td>0.31</td>
<td>3.10</td>
<td>1.55</td>
<td>0.78</td>
<td>1.14</td>
<td>0.57</td>
<td>0.70</td>
</tr>
<tr>
<td>Amino Acid</td>
<td>Level Required for Growth</td>
<td>Level Required for Maintenance</td>
<td>48% Casein</td>
<td>24% Casein</td>
<td>12% Casein</td>
<td>24% Soya</td>
<td>12% Soya</td>
<td>Standard Laboratory Diet</td>
</tr>
<tr>
<td>-------------</td>
<td>----------------------------</td>
<td>-------------------------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>----------</td>
<td>----------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Arginine</td>
<td>-</td>
<td>-</td>
<td>1.94</td>
<td>0.97</td>
<td>0.49</td>
<td>2.43</td>
<td>1.22</td>
<td>1.00</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>-</td>
<td>-</td>
<td>2.86</td>
<td>1.43</td>
<td>0.72</td>
<td>2.84</td>
<td>1.42</td>
<td>-</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>-</td>
<td>-</td>
<td>8.56</td>
<td>4.28</td>
<td>2.14</td>
<td>4.00</td>
<td>2.00</td>
<td>-</td>
</tr>
<tr>
<td>Proline</td>
<td>-</td>
<td>-</td>
<td>6.14</td>
<td>3.07</td>
<td>1.54</td>
<td>1.29</td>
<td>0.64</td>
<td>-</td>
</tr>
<tr>
<td>Serine</td>
<td>-</td>
<td>-</td>
<td>2.08</td>
<td>1.04</td>
<td>0.52</td>
<td>1.15</td>
<td>0.58</td>
<td>-</td>
</tr>
<tr>
<td>Glycine</td>
<td>-</td>
<td>-</td>
<td>0.92</td>
<td>0.46</td>
<td>0.23</td>
<td>1.10</td>
<td>0.55</td>
<td>0.70</td>
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<tr>
<td>Alanine</td>
<td>-</td>
<td>-</td>
<td>1.34</td>
<td>0.67</td>
<td>0.34</td>
<td>1.17</td>
<td>0.59</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 2.3

**EXPERIMENTAL DIET COMPOSITION (g/100g)**

<table>
<thead>
<tr>
<th></th>
<th>48% Casein</th>
<th>24% Casein</th>
<th>12% Casein</th>
<th>24% Soya</th>
<th>12% Soya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>52.6</td>
<td>26.3</td>
<td>13.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soya</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>51.4</td>
<td>25.7</td>
</tr>
<tr>
<td>Starch</td>
<td>15.1</td>
<td>41.2</td>
<td>54.3</td>
<td>29.1</td>
<td>50.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.2</td>
<td>10.0</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>2.1</td>
<td>1.5</td>
<td>1.2</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Calcium Phosphate</td>
<td>0.5</td>
<td>1.3</td>
<td>1.8</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Salt</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
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<tr>
<td>Magnesium Carbonate</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Potassium Chloride</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>*Vitamin Mix</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Solka Floc</td>
<td>10.9</td>
<td>10.9</td>
<td>10.9</td>
<td>0.5</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* See Table 2.4
### COMPOSITION OF VITAMIN PREMIX (%)

<table>
<thead>
<tr>
<th>Vitamin A (500,000 i.u./g)</th>
<th>0.080</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D₃ (500,000 i.u./g)</td>
<td>0.012</td>
</tr>
<tr>
<td>Vitamin E (500 i.u./g)</td>
<td>0.700</td>
</tr>
<tr>
<td>Menadione</td>
<td>0.010</td>
</tr>
<tr>
<td>Choline Chloride (50%)</td>
<td>12.000</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>0.006</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>0.120</td>
</tr>
<tr>
<td>Calcium D-Pantothenate</td>
<td>0.075</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.030</td>
</tr>
<tr>
<td>Thiamine hydrochloride</td>
<td>0.025</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>0.040</td>
</tr>
<tr>
<td>Cyanocobalamin (0.1% in mannitol)</td>
<td>0.300</td>
</tr>
<tr>
<td>d-Biotin</td>
<td>0.005</td>
</tr>
<tr>
<td>Inositol</td>
<td>3.000</td>
</tr>
<tr>
<td>p-Amino benzoic acid</td>
<td>0.500</td>
</tr>
<tr>
<td>Trace Element Premix*</td>
<td>5.000</td>
</tr>
<tr>
<td>Solka Floc</td>
<td>78.097</td>
</tr>
</tbody>
</table>

### COMPOSITION OF TRACE ELEMENT PREMIX (%)

<table>
<thead>
<tr>
<th>Chromic Chloride</th>
<th>0.1537</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cupric Sulphate</td>
<td>3.9291</td>
</tr>
<tr>
<td>Sodium Fluoride</td>
<td>0.2210</td>
</tr>
<tr>
<td>Potassium Iodide</td>
<td>0.0654</td>
</tr>
<tr>
<td>Ferrous Sulphate</td>
<td>49.7824</td>
</tr>
<tr>
<td>Manganese Sulphate</td>
<td>30.4516</td>
</tr>
<tr>
<td>Sodium Selenite</td>
<td>0.0219</td>
</tr>
<tr>
<td>Zinc Chloride</td>
<td>4.1600</td>
</tr>
<tr>
<td>Solka Floc</td>
<td>11.2149</td>
</tr>
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</table>
PERCENTAGE AMINO ACID COMPOSITION OF CRUDE PROTEIN SOURCES

<table>
<thead>
<tr>
<th></th>
<th>Casein</th>
<th>Soya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>3.55</td>
<td>1.42</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.47</td>
<td>2.07</td>
</tr>
<tr>
<td>Leucine</td>
<td>8.09</td>
<td>3.44</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.19</td>
<td>3.22</td>
</tr>
<tr>
<td>Cystine &amp; Methionine</td>
<td>2.88</td>
<td>1.12</td>
</tr>
<tr>
<td>Phenylalanine &amp; Tyrosine</td>
<td>8.91</td>
<td>3.79</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.11</td>
<td>1.82</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.22</td>
<td>0.66</td>
</tr>
<tr>
<td>Valine</td>
<td>5.88</td>
<td>2.21</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.69</td>
<td>4.72</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>5.42</td>
<td>5.53</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>16.26</td>
<td>7.80</td>
</tr>
<tr>
<td>Proline</td>
<td>11.66</td>
<td>2.51</td>
</tr>
<tr>
<td>Serine</td>
<td>3.97</td>
<td>2.23</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.74</td>
<td>2.14</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.53</td>
<td>2.27</td>
</tr>
</tbody>
</table>
diet consumed could be calculated. Cages were inspected daily for food spillage, but this was found to be negligible. Food consumption was determined daily throughout all study periods.

ANIMAL BODY WEIGHT

Rats were weighed at least three times weekly throughout all studies. (Salter 511 balance ±2.5g)

COLLECTIONS OF SPECIMENS

a) Blood specimens

Blood samples (1.0ml) were collected from the tail vein, by clipping the terminal portion with the rat under light ether anaesthesia. Blood was allowed to drain freely into 1.5ml tubes and allowed to clot at room temperature. The tubes were then subsequently centrifuged for 10 minutes at 3000 rpm and the serum aspirated into separate tubes. Haemostasis was achieved in the clipped tail by applying light cautery.

b) Urine samples

Urine samples were obtained from the animals over a 24 hour period using metabolism cages (Plate 2.1). (Diameter=25cm, height=18cm). Animals were placed in the cages at 9.00 am on the appropriate day, after they had been thoroughly washed in water. The animals were allowed free access to food and water whilst in the metabolism cages, the food container placed on the floor of the cage. Urine was separated from faeces by a conical faecal separator within the cage, and urine collected in plastic containers at the base of the cage, into which had been placed a small quantity of merthiolate, to prevent bacterial growth.
Following a period of 24 hours in the metabolism cage, the urine volume was determined by measuring with a graduated cylinder, and was subsequently centrifuged for 15 minutes to settle the particulate matter that might be present.

Biochemical determinations were then performed upon the fresh urine sample collected in the metabolism cage for 24 hours in any study.

**Biochemical**

a) Urea

Urea was determined using an automated method. Ten milliliters of sample of urine (300μl) were analyzed employing this method.

b) Albunin

Serum albumin was determined using an automated method. Protein was quantified by biuret method.

and

Phosphate was determined using a standard colorimetric method.

Processed materials handbook, Terrytown, New York.

c) Cholesterol and Triglyceride

Serum cholesterol and triglyceride were determined using a Technicon RA-1000 autoanalyzer employing the cholesterol oxidase and glycerol oxidase methods, respectively. Seven-ul samples were required, and the reaction products were measured spectrophotometrically. (RA 1000, Users Handbook, Terrytown, New York.)
Following a period of 24 hours in the metabolism cages, the animals were returned to their normal cages. The urine volume was determined by measurement in a graduated cylinder, and was subsequently centrifuged for 15 minutes at 3000 rpm to remove any particulate matter that might be present.

Biochemical determinations were then performed upon the fresh urine samples. All animals were acclimatised to the metabolism cages for 24 hours on at least two occasions before the start of any study.

**BIOCHEMICAL DETERMINATIONS**

a) **Urea**

Urea was determined in serum and fresh urine using an automated method. (Instrumentation Laboratories, 919 Autoanlyser). Samples of urine (diluted 1:20 with 0.9% saline) and serum (200μl) were analysed employing the urease/glutamate dehydrogenase method.

b) **Albumin and Inorganic Phosphate**

Serum albumin and inorganic phosphate were determined using an automated method, requiring 7.5μl samples. Serum albumin was quantified by employing the bromocresol dye binding method, and phosphate using the phosphomolybdate method, both of which were quantified spectrophotometrically. (Technicon RA 1000, Users Handbook, Tarrytown, New York).

c) **Cholesterol and Triglyceride**

Serum cholesterol and triglyceride were determined using a Technicon RA 1000 autoanalyser employing the cholesterol oxidase and glycerol oxidase methods respectively. Seven μl samples were required, and the reaction products measured spectrophotometrically. (RA 1000 Users Handbook, Tarrytown, New York).
d) **Blood Sugar**

Blood sugar was determined using an autoanalyser (IL 919) employing the glucose oxidase method.

e) **Calcium**

Serum calcium was determined manually using the o-cresolphthalein complexone method. (Sarkar B C, Chauhan U, 1967) (Boehringer Mannheim Diagnostica Ltd). Fifty µl serum samples were mixed with the chromogen, and the absorbence determined spectrophotometrically (SP 30 Spectrophotometer, Pye Unicam, Cambridge).

f) **Urinary Protein**

The protein content of fresh 24 hour urine samples was determined manually using the biuret method (Weichselbaum T E, 1946). The protein in 1.0ml of fresh urine was precipitated with an equal volume of trichloracetic acid, and after mixing, then standing for 10 minutes, was centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted, and the protein precipitate redissolved in biuret reagent, and the absorbence of the solution was determined 30 minutes later against a blank solution, at 540nm. Protein concentration was determined from the absorbence of a standard protein solution (100µl of 60mg/ml protein solution). (Boehringer Mannheim Diagnostica Ltd).

g) **Plasma Amino Acids**

Amino acid concentrations were determined in plasma samples, following deproteinization with an equal volume (400µl) of sulphosalicylic acid (12.5%) containing norleucine (200µmol/l) as the internal standard. Plasma was mixed with sulphosalicylic acid, and following centrifugation at 3000 rpm for 20 minutes, the supernatant was separated, and stored at -20°C until analysis. Amino acids were quantified using an automated high pressure liquid chromatography
system. (Chromaspek, Rank Hilger Ltd). Following column separation of amino acids by ion exchange, detection of amino acid peaks was achieved using the ninhydrin reaction.

h) Serum Insulin

Serum samples (100μl) were stored at -20°C until analysed for insulin. Serum insulin concentrations were determined using a radioimmunoassay method. (Insk-5, Sorin Biomedica, Italy). A fixed amount of added 125I-insulin (γ-emitter) competes with the variable amount of unlabelled insulin in the sample for a fixed number of antibody binding sites. Following 24 hours incubation at 4°C, antibody bound insulin is precipitated with polyethylene glycol, and centrifuged, for 15 minutes at 2000 rpm. The supernatant is aspirated, and the tubes allowed to drain inverted, and then radioactivity in the precipitated antibody-antigen complexes is counted (1280 ultrogamma, LKB Instruments Ltd. Croyden). The larger the amount of unlabelled insulin in the sample, the smaller the amount of radioactively labelled insulin in the precipitated complexes. A standard curve is prepared, using a semilog scale, with insulin concentrations ranging from 10μU/ml to 200μU/ml. The sample concentration is determined from the standard curve, and all estimations are performed in duplicate. The intraassay variation was 17% at 12μU/ml (n=12).

INDIRECT MEASUREMENT OF BLOOD PRESSURE

Indirect measurement of blood pressure was undertaken using a light plethysmographic method. (Swales J D, Tange J D, 1970). This method is able to detect small changes in light translucency, during systole and diastole, by a small photocell. The signal passes from the photocell to a control box, which consists of an amplifier and a
frequency filter. The latter can be tuned to the heart rate of the rat under study (between 350 and 500 beats per minute). From the control box the impulse is fed to an oscilloscope. The rat is anaesthetised lightly with ether, and the tail is passed through a small sphygmomanometer cuff. The transducer is placed on the tail 1-2cm distal from the cuff, and is held lightly in place by a restraining clamp. When the light source is switched on, a pulse appears on the oscilloscope screen. The tail vessels are then occluded by the sphygmomanometer cuff, which is then slowly deflated until the pulse returns to the oscilloscope screen. This is performed as the animal is recovering from the brief period of anaesthesia. The point at which the pulse is first seen to return to the oscilloscope screen when deflating the cuff, is recorded as the systolic blood pressure.

OPERATIVE PROCEDURES

i) Creation of Partial Nephrectomy

Rats were anaesthetised using an intraperitoneal injection (2.7 mls/kg body weight) of a 2:1:1 mixture of sterile water, Hypnovel (Midazolam hydrochloride, 5 mg/ml, Roche Pharmaceuticals Ltd) and Hypnorm (Fentanyl citrate, 0.315 mg/ml, Fluanisone, 10 mg/ml, Janssen Pharmaceutica). Anaesthesia was induced within 10 minutes, and persisted for 3 to 6 hours.

The anterior abdominal wall was shaved and sterilised, and the peritoneal cavity exposed through a 5 cm midline incision. The intestine was mobilised and wrapped in gauze soaked in 0.9% saline. The left kidney was identified, and following careful dissection, using an operative microscope, branches of the artery supplying the lower pole of the kidney were ligated using a 6/0 silk suture. The area of
renal ischaemia produced became apparent within a few minutes, with a sharp zone of demarcation separating it from normal renal tissue, and sufficient renal artery branches were ligated (usually one or two) to produce an area of infarction approximately 30% of the kidney (Plates 2.2 and 2.3).

The right kidney was next identified, mobilised by careful dissection, and a 4/0 silk ligature placed around the renal pedicle, which was then divided and the right kidney removed. The right kidney was placed in a pre-weighed pot containing 10% formalin in 0.9% saline, which was reweighed to determine the mass of the right kidney. The intestine was replaced, and the anterior abdominal wall repaired using a 4/0 silk suture, and the skin apposed using a subcuticular 6/0 silk suture.

The animal received a 1.0ml subcutaneous injection of 0.9% saline, and was returned to its cage to recover.

The sham operation consisted of identifying both kidneys in the same manner as previously described, which were then gently mobilised by careful dissection, and the anterior abdominal wall was then repaired, as previously described.

The peri-operative mortality (within 48 hours) in 85 operations was 5%, and no cases of wound dehiscence or infection were encountered.
CREATION OF SUBTOTAL NEPHRECTOMY SHOWING THE LIGATURE OCCLUDING LOWER POLE RENAL ARTERY
LEFT RAT KIDNEY REMOVED ONE WEEK FOLLOWING INFACTION
OF THE LOWER POLE
METHOD USED FOR THE DETERMINATION OF GLOMERULAR FILTRATION RATE AND EFFECTIVE RENAL PLASMA FLOW

a) Glomerular Filtration Rate

Measurements of GFR were undertaken in the conscious animals by determination of inulin clearance. Inulin is a fructose polymer (molecular weight approximately 5200) which is freely filtered across the glomerular capillary walls, and is neither secreted nor reabsorbed by the renal tubules, and by virtue of these characteristics is well suited as a substance with which to determine GFR. The inulin clearance method was first used to determine GFR some fifty years ago and has become the standard method for determining glomerular filtration rate (Richards A N et al, 1934).

The clearance of a substance which is excreted solely by the kidney is the volume of plasma which is completely cleared of that substance per unit time, and can be expressed by the equation

$$\text{Clearance} = \frac{U \times V}{P(t)}$$

where $U =$ urinary concentration of substance

$V =$ urine volume

$P =$ plasma concentration of substance

$t =$ time period of urine collection

Urine collection is required for the determination of the clearance of the substance, and this is performed by the insertion of a urinary catheter into the bladder allowing free drainage of urine into a collecting tube. The volume of urine collected is measured gravimetrically assuming a specific gravity of 1.00. A stable plasma concentration of the substance over the time period of urine collection is required, and this is achieved by an initial loading bolus, sufficient to produce a desired plasma concentration of 0.25mg/ml,
which is calculated assuming the inulin space to be 20% of body weight. A constant infusion of inulin is required to replace the amount excreted in the urine, and the quantity to be infused is calculated assuming a GFR of 2.00ml/min for two kidney animals, and 1.00ml/min for remnant kidney animals. A representative plasma concentration of each clearance period is determined by measurement of inulin concentration in an arterial blood sample taken at the midpoint of each twenty minute collection period.

b) Effective Renal Plasma Flow

The renal clearance of para-aminohippuric acid has been used as a measure of renal plasma flow rate. PAH is a substance which is freely filtered by the glomerulus, and also actively secreted by the renal tubules, and by application of the Fick principle to the determination of PAH clearance, a measurement of renal plasma flow can be made.

Fick Equation

\[
\text{Renal plasma flow} = \frac{U \times V}{A_{PAH} - V_{PAH}}
\]

where

\[U = \text{urine PAH concentration}\]
\[V = \text{urine volume}\]
\[A_{PAH} = \text{arterial PAH concentration}\]
\[V_{PAH} = \text{venous PAH concentration}\]

The denominator in the Fick equation is the arteriovenous difference of PAH. The extraction ratio (arteriovenous difference/arterial concentration) of PAH in the rat is approximately 0.8 (Cortney M A, et al, 1965) demonstrating that the extraction of PAH from plasma is incomplete. The extraction ratio has been assumed to be one for this series of studies, and PAH clearance is the "effective renal plasma flow rate". The extraction ratio was not determined on each study, as it would necessitate further major operative intervention of
placement of a renal venous catheter, which will severely alter renal haemodynamics, or direct sampling of renal venous blood, which would require further anaesthesia, and laparotomy.

As with measurement of inulin clearance, a steady state plasma concentration is required. This is achieved by a priming bolus of PAH, assuming the PAH space to be 40% of body weight, and the desired plasma concentration to be 0.01mg/ml. A continuous infusion of PAH is also used to replace the quantity excreted in the urine, and this was calculated assuming an effective renal plasma flow rate of 6ml/min for two kidney animals, and 3ml/min for remnant kidney animals.

c) Animal Preparation

The two main methods available for determination of renal function in the rat are the conscious rat model, studied following ether anaesthesia for catheter placement, or the anaesthetised rat which is maintained under anaesthesia, usually barbiturate, throughout the procedure. Both preparations are associated with mild reductions in glomerular filtration rate and renal blood flow, whilst blood pressure is mildly elevated in the former, and depressed in the latter (Walker LA et al, 1983). The conscious rat, studied at 2 1/2 hours following ether anaesthesia, is the preparation used in this series of studies. This preparation was chosen as the requirement for any additional anaesthesia during the study period, with its attendant consequences, is obviated, and also the additional surgical trauma induced by placement of a tracheostomy tube, which is usually required when the rat is maintained under barbiturate anaesthesia, is also avoided.
DETERMINATION OF GLOMERULAR FILTRATION RATE AND EFFECTIVE RENAL PLASMA FLOW

Prior to determination of renal function, the animals were allowed free access to food and water, and all studies were commenced in the morning.

a) Insertion of catheters

The animals were lightly anaesthetised using ether, and the left jugular vein and right carotid artery were exposed through two small vertical neck incisions, and following careful blunt dissection were cannulated with P 50 plastic tubing catheters. (I.D.=0.58mm, O.D.=0.96mm, length=50cm, Portex Ltd, Hythe). The catheters were filled with 0.9% saline solution, and following insertion into the vessels, were exteriorised between the scapulae via tunnels which were created from either side of the neck. The skin incisions were sutured.

A silastic tubing urinary catheter, was inserted into the bladder by direct cannulation of the urethral orifice, under direct vision. Three small additional side holes were created in the terminal 0.5cms of the catheter, to ensure good bladder drainage. (I.D.=0.63mm, O.D.=1.19mm, length=7cm, Dow Corning Ltd. Michigan). The catheter was secured to the external urethral orifice by a single suture, and the position of each catheter was checked to ensure that it was correctly in the bladder when the animal was sacrificed.

During the procedure of insertion of the catheters, which usually lasted approximately 40 minutes, a 1.0ml infusion of 0.9% saline was administered via the venous catheter. After the placement of the catheters, the animals were transferred to plastic tubular restraining holders, (I.D.=4.5cm) with appropriate exit holes for the limbs and catheters and allowed 30-45 minutes for recovery (Plate 2.4).
CONSCIOUS RAT IN RESTRAINING HOLDER DURING RENAL FUNCTION STUDIES

Following the recovery period, a 0.5ml intravenous priming dose of heparin (2.5%, Koch Light Laboratories Ltd., Bucks) and para-aminosalicylic acid (0.2%, Koch Light Laboratories Ltd., Bucks) in 0.9% saline was administered via the femoral catheter and a maintained throughout the experiment. No further drug infusions were given to the animal during the study. Arterial pressure was measured using a micromanometer and was maintained at 100 mmHg throughout the experiment. At the conclusion of the study, urine was collected from the bladder via the catheter and then passed through a filter using a peristaltic pump. Urine samples were analyzed for creatinine, sodium, chloride, and potassium concentrations. Urine flow rate was also determined gravimetrically after having previously determined the weight of the urine collection tube and sample. Determinations of plasma creatinine, PAA, sodium and haematocrit were also performed on the experimental blood samples. Urine volume was determined gravimetrically after having previously determined the weight of the urine collection tube and samples (Mettler H31 balance, 0.001g).
The carotid artery catheter was connected, via a metal adaptor and 3-way tap, to a pressure transducer (Akers AE840) and blood pressure monitor (Cambridge 01058 monitor), in order that the mean arterial blood pressure could be continuously monitored.

b) Method of Performing Clearance Periods

Following the recovery period, a 0.5ml intravenous priming dose of inulin (2.5%, Koch Light Laboratories Ltd, Bucks) and para-aminohippuric acid (0.2%, Koch Light Laboratories Ltd, Bucks) in 0.9% saline was administered via the venous catheter, and a maintenance intravenous infusion of inulin (2.0%) and PAH (0.24%) in 0.9% saline was commenced at a rate of 1.5mls per hour, administered using an infusion pump (Dascon 400, Sandoz Ltd). A 90 minute equilibration period then followed.

Following the equilibration period, 3 x 20 minute clearance periods were performed. Arterial blood samples (approximately 200μl) were drawn at the midpoint of each clearance period into heparinised tubes, from the carotid artery catheter. Following the withdrawal of arterial blood samples, the catheter was gently flushed with 100μl of 0.9% saline to ensure the patency of the catheter. At the start and finish of each clearance period, the bladder was gently massaged to ensure complete emptying via the catheter, and urine was collected in tubes (volume=2.0mls) under mineral oil (approximately 0.5mls) to ensure no evaporative loss of urine.

Determinations of plasma inulin, PAH, sodium and haematocrit were performed on the arterial blood samples. Urine volume was determined gravimetrically after having previously determined the weight of the urine collection tube and mineral oil (Mettler H31 balance±0.001g). Determinations of inulin, PAH and sodium were also performed on the
urine samples. Mean blood pressure values were recorded at the midpoint of each clearance period. Urine and plasma samples for determination of inulin, PAH and sodium were stored at -20°C until time of analysis.

Following the third clearance period, the animal was sacrificed, whilst under ether anaesthesia, by exsanguination via the carotid artery catheter into heparinised tubes. The kidneys were carefully dissected free of surrounding tissue, bisected, and placed in a prepared container of 10% formalin.

CHEMICAL DETERMINATIONS

1) Inulin

Inulin concentrations were determined in samples of plasma and urine using the anthrone micromethod (Führ J et al, 1955).

Plasma (25µl) and urine samples (25µl of urine diluted 1:10 with distilled water) were deproteinised using 50µl of zinc sulphate (10%), sodium hydroxide (0.5M) and 100µl of distilled water. After centrifugation at 3000 rpm for 10 minutes, 25µl of supernatant was mixed with ice cold anthrone reagent. (200mg anthrone, 125ml concentrated sulphuric acid, 50mls distilled water). This was then incubated for exactly 10 minutes at 55°C in a water bath, and the tubes then placed in an ice bath. A standard solution of inulin (0.5mg/ml) was treated in exactly the same way as the samples.

Inulin reacts to form a blue-green compound with anthrone, and the absorbance of colour formed in the samples was determined against a plasma blank using 10mm quartz microcuvettes in the spectrophotometer (SP 30, Pye Unicam Ltd, Cambridge) at 550nm.
Inulin concentrations in the samples were determined using a standard curve (range 0.1 to 1.0mg/ml inulin). The interassay coefficient of variation was 4.1% (n=10).

2) **PAH**

PAH concentrations in plasma and urine were determined using the method of Smith (Smith H W et al, 1945).

Plasma (50µl) and urine samples (50µl of urine diluted 1:100 with distilled water) were deproteinised using a solution of cadmium sulphate (3.4%), sodium hydroxide (1.1N) and water. The solution was mixed, and centrifuged at 3000 rpm for 10 minutes, and 500µl of supernatant was sequentially mixed with acidified sodium nitrite (0.1%), ammonium sulphamate (0.5%) and N-ethyline diamine dihydrochloride (0.1%). The PAH reacts to form a pink coloured complex which develops with the latter reagent, and the absorbance of this solution was determined using a spectrophotometer at 540nm. A standard solution of PAH (0.02mg/ml) was used, and treated in exactly the same way as the samples. The PAH concentration of the samples was determined using a standard curve, which was linear up to a PAH concentration of 0.03mg/ml. The interassay coefficient of variation was 6.9%. (n=10).

3) **Sodium**

The sodium concentration of plasma and urine samples were determined by flame photometry (Corning 480) using 5µl samples diluted 1:400 with lithium solution (Corning).

4) **Haematocrit**

The haematocrit of arterial blood samples was determined using microhaematocrit tubes following centrifugation for 5 minutes. (Hawksley Ltd, microhaematocrit tubes and centrifuge).
Determination of Inulin and PAH clearances

The clearance of inulin and PAH were calculated using the standard equation

$$\text{Clearance} = \frac{U \times V}{P \times t}$$

where $U$ = urinary concentration of substance (mg/ml)

$V$ = urinary volume excreted during clearance period (ml)

$P$ = plasma concentration of substance (mg/ml)

$t$ = time period of urine collection (=20 minutes)

Clearance values were obtained for each of the three consecutive time periods, and the mean value calculated. The maximum tubular secretory capacity of PAH occurs at a plasma concentration of 0.02mg/ml and in none of the clearance periods was this plasma concentration exceeded.

Determination of Fractional Sodium Excretion

The fractional sodium excretion is the percentage of the filtered load of sodium which is excreted in the urine. This was calculated from a standard equation, where

$$\text{FE}_{\text{Na}}(\%) = \frac{U_{\text{Na}} \times V}{\text{GFR} \times P_{\text{Na}} \times t}$$

where

$U_{\text{Na}} \times V$ = amount of sodium excreted in the urine during one clearance period

GFR = glomerular filtration rate, (Inulin clearance, ml/min)

$P_{\text{Na}}$ = concentration of sodium in the plasma

$t$ = time period of urine collection (20 minutes)

The $\text{FE}_{\text{Na}}$ for each clearance period was determined and the mean value for each animal calculated.
Determination of Renal Vascular Resistance

Whole kidney vascular resistance was determined from values of mean blood pressure, effective renal plasma flow and haematocrit.

Renal vascular resistance = \( \frac{\text{BP}}{\text{ERPF} / (1 - \text{Hct.})} \) (mmHg/ml/min)

where

BP = Mean Blood Pressure (mmHg)

ERPF = Effective Renal Plasma Flow (mls/min)

Hct = Haematocrit

The total renal vascular resistance was determined for each clearance period, and the mean value calculated.

HISTOLOGICAL ANALYSIS

All kidneys to be examined histologically were bisected, to ensure good penetration of fixative, and fixed in 10% formalin in 0.9% saline. The half kidneys were embedded in paraffin wax, and 2-3μ sections cut in the longitudinal plane. (Microtome. Anglia Scientific Instruments Ltd). The kidney sections were subsequently stained with haematoxylin and eosin, and periodic acid Schiff reagents.

Histological sections were examined using a binocular microscope, magnification x 320. (M 15 C, Vickers Instruments Ltd). The specimens were coded, in order that the observer was unaware of the experimental protocol.

a) Glomerular Analysis

All glomeruli in the kidney section were examined, excepting those immediately adjacent to the zone of scar tissue representing the previously infarcted area in the remnant kidneys. The glomeruli were assessed as
i) being structurally normal, with reference to mesangial size, capillary patency and cellularity.

ii) demonstrating evidence of moderate glomerulosclerosis, consisting of an increase in size of the mesangium, evidence of focal deposits of PAS positive material within the mesangium, and reduction in the number of patent capillaries.

iii) evidence of severe glomerulosclerosis, where the structure of the glomerulus had been replaced largely with PAS positive material, severe evidence of intraglomerular capillary thrombosis, and obliteration of capillary lumina.

The number of glomeruli in each category were expressed as a percentage of the total number of glomeruli examined in that section, which in all cases was greater than 75.

b) Tubular Analysis

Tubular morphology was quantitatively assessed on a scale of 0 to 3, where

Grade 0 = lesion not found
Grade 1 = lesion present in <10% of tubules examined
Grade 2 = lesion present in 10% - 50% of tubules examined
Grade 3 = lesion present in >50% of tubules examined.

Tubular atrophy, and tubular dilatation with intratubular proteinaceous cast deposition were graded in this way, and tubules in both cortex and medulla were examined, but the area of tissue immediately adjacent to the scar tissue was not included in the analysis.
CARCASS ANALYSIS

The carcasses of the experimental animals were analysed for water and fat content, using a freeze drying technique and lipid extraction with a Soxhlet apparatus. Following the removal of the kidney(s) after sacrifice, the gastrointestinal tract, from oesophagus to rectum was removed using scissors at the mesenteric margin, and discarded. The carcass was then stored at -20°C in an airtight container until analysis of body water and fat were performed. The carcasses were thawed at +4°C for 18 hours, and weighed. Next, the head was removed by decapitation immediately posterior to the occipital protruberance, in order that the lipid rich central nervous tissue was not included in the fat analysis. Also, the tail, and four paws were separated from the carcass, which was reweighed, to determine the carcass weight upon which measurements of water and fat were performed. The carcass was then divided into approximately 1 cm³ pieces, and passed through a power driven mincer three times, and subsequently thoroughly mixed, to obtain a homogenous finely minced preparation. To determine tissue water, approximately 25g of minced tissue was frozen in liquid nitrogen, in approximately 0.5g pieces. This was then placed in a prepared plastic container (weight approx 7g, diameter = 7cm, height = 5cm.) and weighed to determine the mass of tissue. The sample was then subjected to freeze drying for 72 hours at 3 x 10⁻² atmos. (EF4 Modulyo Freeze Dryer, Edwards Ltd, Crawley) and after this period was reweighed to determine the amount of water removed from the tissue. Previous studies had shown that under these experimental conditions, no further weight loss occurred in the specimen following 72 hours of dehydration. The coefficient of variation of tissue water determination was 0.15%
The dehydrated tissue was stored in airtight containers until measurement of fat was performed (within one week).

Five grams of coarsely minced dry tissue was placed in a cellulose extraction thimble in the Soxhlet apparatus (Plate 2.5). The principle of this apparatus is that a volatile organic solvent is placed in the lower flask, and heated to above its boiling point by a water bath. The vapour then passes through the sidearm to two Liebig condensers connected in series above the Soxhlet chamber, and the condensed solvent collects in the thimble chamber and bathes the dry tissue. Lipid in the tissue is extracted by the solvent, and when the level of solvent reaches the top of the thimble, the sidearm syphon drains the solvent and extracted lipid back into the boiling flask. In this way, the tissue in the thimble is continuously bathed in fat free solvent, and the extracted fat collects in the lower flask. The extraction solvent was petroleum ether (80mls) (boiling point 30-40°C) and the water bath was maintained at 45°C. Each filling cycle lasted approximately two minutes, and the tissue sample was subjected to extraction for six hours. It was previously determined that no further fat was collected following a further six hour period of extraction (n=6). The solvent-fat mixture in the lower flask was heated at 80°C for 30 minutes to remove the petroleum ether, and any residual solvent was removed by drying under a stream of nitrogen. The extracted fat in the lower flask was aspirated into a small weighing boat, and the mass of lipid determined. All determinations of tissue lipid were performed in duplicate. The coefficient of variation of lipid determination was 2.4% (n=6).

The percentage body water was determined directly from measurements performed on aliquots of the minced carcass, and percentage body fat from the mean value of fat content in the
Plate 2.5

SOXHLET CHAMBER USED TO DETERMINE TISSUE LIPID CONTENT

The percentage of water in the fat-free carcass was determined from:

\[
\text{Total carcass water} \times 100 = \frac{\text{Carcass wt - Total carcass fat}}{\text{Carcass wt}}
\]
freeze dried tissue, which was corrected for the previously determined water content. The fat free solids (FFS), representing that portion of the carcass composed mainly of protein and ash, was calculated from

\[ \text{FFS} = \text{Carcass wt} - (\text{carcass wt x % water}) - (\text{carcass wt x % fat}). \]

The percentage of water in the fat free carcass was determined from

\[ \frac{\text{Total carcass water}}{\text{Carcass wt - total carcass fat}} \times 100 \]

**Tissue Protein Analysis**

When the animal was sacrificed, a small portion of the left lobe of the liver (approximately 50mg) was removed and quickly placed on a small piece of aluminium foil (approximately 15-20mg) and rapidly frozen in liquid nitrogen. Tissue samples were subsequently stored at \(-70^\circ\text{C}\) in 1.5ml airtight tubes until final analysis.

a) **Water Content**

The water content of the sample was determined by the weight difference after dehydration by freeze drying.

The tissue sample on the aluminium foil boat, was maintained frozen by surrounding the plastic container in dry ice. The foil containing the tissue sample was removed from the container with forceps, and placed in the balance, and accurately weighed, then returned to the container, and again stored at \(-70^\circ\text{C}\). The weighing procedure lasted approximately 15 seconds, and the tissue remained frozen throughout. Following the initial weighing, the sample was subjected to freeze drying for 24 hours at \(-70^\circ\text{C}\) and \(3 \times 10^{-2}\) atmos to remove all tissue water. (EF4 Modulydo Freeze Dryer, Edwards Ltd. Crawley). This was previously determined to be adequate for total tissue dehydration.
The dehydrated tissue specimen on the foil boat was then reweighed as previously described. The tissue sample was next transferred, using small clean dry forceps, into 1.0ml of 0.1m NaOH, and the foil reweighed so that the wet weight and dry weight of the tissue sample could be calculated. No weight change was found to occur for the aluminium foil throughout the procedure. All weights were determined using a Mettler ME22 balance (+0.005mg), and the intra assay coefficient of variation for determination of tissue water content was 2.8% (n=6).

b) Tissue Protein Content

Tissue protein content was determined using the method of Delaporte, C et al (1976). Following 24 hours swelling in 1.0ml of 0.1m NaOH at room temperature the tissue sample was homogenised in an all glass homogeniser in 0.1m NaOH. The homogenate was next centrifuged (20 minutes at 3000 rpm) and the supernatant collected and added to the initial alkaline extract, and the volume made up to exactly 5.0mls using 0.1m NaOH.

The protein content of the alkaline tissue extract was determined using the Lowry method (Lowry, O H et al, 1951). The method is based upon the ability of a protein-copper complex to reduce Folin-Ciocalteu phenol reagent, and generate a coloured complex which can be quantified spectrophotometrically.

20μl aliquots (in duplicate) of alkaline tissue extract were analysed for protein content, and the sample concentration was determined from a standard curve, using crystalline bovine serum albumin as the standard protein, at concentrations from 10μg/ml to 100μg/ml. The intra assay coefficient of variation was 6.3% (n=6).
STATISTICAL ANALYSIS

All results are expressed as mean ±SEM (standard error of mean) or median and range, where appropriate. One way analysis of variance, analysis of covariance, Student 't' test and Mann Whitney U test were used to make statistical comparisons. Regression analysis was performed by the method of least squares.

Analysis of data comparing more than two groups, with a normal distribution, was performed using analysis of variance, which is indicated by inclusion of the F ratio. The Student 't' test was used to compare two sets of normally distributed values. The Mann Whitney U test, a non parametric test, was used to compare data that did not have a normal distribution, and in these cases median values are given.

Analysis was performed using an SPSS Program (SPSS Inc. Illinois) and Minitab statistics program (Pennsylvania State University) on a PDP 11/23 computer (Comma Hawk 23/1A, Comma Computers Ltd. Brentwood, UK).
SECTION 3

STUDIES INVESTIGATING THE EFFECTS OF DIFFERENT DIETARY PROTEINS UPON RENAL FUNCTION, RENAL MORPHOLOGY AND BODY COMPOSITION IN THE NORMAL AND SUBTOTALLY NEPHRECTOMISED RAT
INTRODUCTION

Modulation of renal function by the quantity of dietary protein was initially recognised by Addis during the early part of this century (Addis T, Drury D, 1923), and it subsequently became apparent from the early extensive investigations of Chanutin that dietary protein intake also appeared influential in the renal response when kidney mass is reduced (Chanutin A, Ludewig S, 1936). The mechanisms by which dietary protein influences normal renal function and the response to injury are complex, but some insight has come from the extensive studies performed in Brenner's laboratory. Many investigations into this topic have employed micropuncture studies, in both normal and subtotally nephrectomised rats, and the experimental limitations of these invasive manoeuvres must be appreciated in interpretation of the results.

A unifying hypothesis involving changes in renal haemodynamics in response to diet and renal injury has emerged from these studies (Brenner B M et al, 1982). In acknowledging this hypothesis many other potentially important controlling factors which may govern the renal response to injury must also be taken into consideration.

The intimate association of diet and the progression of renal pathophysiological change documented both in experimental animals and man, is the foundation of the investigations presented in this thesis. The studies described, were designed to investigate the effects of feeding two different dietary proteins upon renal function and body composition in normal and subtotally nephrectomised rats.

The evidence suggesting that different dietary proteins may qualitatively affect renal pathophysiological change in the subtotally nephrectomised rat comes from two major lines of research, and spans
nearly fifty years. The investigations of Chanutin in 1939 document that the degree of renal hypertrophy in the remnant tissue of the subtotally nephrectomised rat was influenced by the source of dietary protein, and the effects of differing proportions of amino acids present in the diet upon compensatory renal hypertrophy were further studied by Halliburton in 1969. Further evidence presented by Kaysen and Kropp documented dietary supplementation with a single amino acid would affect the renal pathophysiological response to a reduced kidney mass (Kaysen G A, Kropp J, 1983).


The rationale for the choice of dietary proteins used in the studies, casein and soya, stems directly from these observations, as it was reasoned that if the type of protein is influential in determination of GFR and ERPF a reduction in these parameters may exert a beneficial influence in the subtotally nephrectomised rat.

The studies described in Section 3 were designed to examine the effects of an animal derived protein, casein, and a vegetable protein, soya, upon renal function both in short and long term feeding studies in normal animals, and the effects of these diets upon structure and function of remnant renal tissue in the subtotally nephrectomised rat. Consideration solely of aspects of renal function in isolation is to ignore the nutritive effects of a diet upon the organism as a whole, and therefore investigation of body composition in the experimental animals was also undertaken, as malnutrition due to an inadequate diet
is a poor trade off for the preservation of renal function following subtotal nephrectomy.
STUDY 1 EXPERIMENTAL PROTOCOL

THE EFFECT OF DIFFERENT DIETARY PROTEINS UPON RENAL FUNCTION IN THE NORMAL RAT

Thirty rats were randomly allocated into 3 groups

Group 1  n = 10
Group 2  n = 10
Group 3  n = 10

The animals were maintained on the standard laboratory diet (ERM Diet, Labsure Ltd) with free access to food and tap water. Following a short acclimatisation period, the rats were commenced on one of the three experimental diets

Group 1  24% Casein
Group 2  48% Casein
Group 3  24% Soya

The method of feeding, weight determination and measurement of food consumption were as described in section 2.

Following a four week maintenance period on the experimental diets, the animals were placed in metabolism cages for a 24 hour period, for collection of urine specimens. Urine volume, and urinary urea were determined on the fresh samples.

Two weeks later, determination of inulin and PAH clearances and direct measurement of blood pressure were performed in the conscious animals. All studies were commenced in the morning, following free access to food and water overnight.

The rat was anaesthetised using ether, and P50 polyethylene catheters placed in the right carotid artery and left jugular vein,
which were exteriorised through a subcutaneous tunnel between the scapulae. A silastic bladder catheter was also placed. During the operative procedure, a 1ml infusion of 0.9% saline was administered intravenously.

The animal was placed in a restraining holder, and allowed 45 minutes to recover. After a priming dose, inulin and PAH were infused in saline at 1.5ml per hour. Following a 90 minute equilibration period, 3 x 20 minute urine collection periods were performed, and arterial blood samples were drawn at the midpoint of each period. Urine and plasma samples were stored at -20°C until analysis.

Following the final urine collection period, the rat was lightly anaesthetised with ether, exsanguinated via the carotid artery catheter into heparinised tubes and the plasma stored at -20°C until analysis. The abdominal cavity was quickly exposed through a midline incision, the kidneys mobilised, dissected free of tissue, and placed in prepared containers of formalin (approx 10mls of 10% formalin in 0.9% saline). These were subsequently reweighed to determine the renal mass.

The final specimen of blood obtained at sacrifice was subsequently analysed to determine values for plasma urea, creatinine, albumin and serum cholesterol.
RESULTS

STUDY 1 THE EFFECT OF DIFFERENT DIETARY PROTEINS UPON THE RENAL FUNCTION IN THE NORMAL RAT

BODY WEIGHT AND FOOD INTAKE

There was no significant difference in the body weight of the animals in the three groups at the start of the study. (Gp 1, 233±6, Gp 2, 235±4, Gp 3, 232±4,g) (F ratio = 1.08). During the first week following the introduction of the experimental diets, the food consumption of animals fed the soya diet was significantly lower than animals fed the 24% casein diet. This initial reluctance to feed, which was limited to the 3 to 4 day period following introduction of the new diet, was associated with significant weight loss, which was most marked in those animals with the lowest food intake. However, during the remainder of the study, the food intake of animals in group 3 rose, and the body weight of these animals remained stable (Fig 3.1). During the latter half of the study the food intake of animals in group 3 was significantly higher than groups 1 and 2, which did not differ.

The body weight of animals ingesting the casein diets did not differ significantly at the end of the study. The increment of weight loss sustained by animals ingesting the 24% soya diet in the first week of the study appeared to be the major factor responsible for the lower body weight of animals in this group, compared to groups 1 and 2 (F ratio = 8.65, p<0.01).
BODY WEIGHT AND FOOD INTAKE OF NORMAL RATS FED DIFFERENT PROTEIN DIETS (MEAN±SEM)

Figure 3.1

Body Weight (g)

Food intake (g/day)

Weeks
URINE VOLUME AND URINARY UREA EXCRETION

The twenty four hour urine volume and urinary urea excretion were determined four weeks following introduction of the experimental diets. The urine volume of animals fed the 48% casein diet (28.3±1.5ml/24hrs) was significantly greater than animals maintained upon the 24% protein diets (Gp 1, 19.6±1.4, Gp 3, 21.5±1.9ml/24hrs) (F ratio = 7.8, p<0.01). Values determined for groups 1 and 3, did not differ (p=0.46).

The urinary urea excretion of all groups was significantly different (Gp 1, 13.6±0.6, Gp 2, 23.7±0.7, Gp 3, 10.8±0.7mmol/day) (F ratio = 112.7, p<0.01). Values for animals ingesting the 48% casein diet were approximately double those obtained for animals in groups 1 and 3, and those rats fed the 24% casein diet excreted significantly more urea than animals in group 3 fed the 24% soya diet. The differences in daily urinary urea excretion between the groups were attributable to both differences in urinary urea concentration (Gp 1, 836±49, Gp 2, 991±49, Gp 3, 587±48mmol/l) (p<0.05) and urine volume. A highly significant inverse correlation was found between urine volume and urinary urea concentration within each group (Gp 1, r=-0.93, Gp 2, r=-0.90, Gp 3, r=-0.85).

KIDNEY WEIGHT

The total renal mass of rats in the three dietary groups all differed significantly (Gp 1, 1.666±0.068, Gp 2, 1.857±0.051, Gp 3, 1.400±0.046g). However, as renal mass varies with body weight, correction for this factor by analysis of covariance of the relationship between body mass and renal mass, revealed groups 1 and 3 not to differ (p>0.30). However, the renal weight of animals fed the
high protein 48% casein diet (Gp 2) was found to be significantly greater than animals fed the 24% protein diets (p<0.001). (Gp 1, 0.699±0.019, Gp 2, 0.824±0.022, Gp 3, 0.662±0.021,g/100g body weight).

RENAI FUNCTION STUDIES

a) EFFECTIVE RENAI PLASMA FLOW AND GLOMERULAR FILTRATION RATE

The results of the renal function studies undertaken in the conscious animals, following a period of six weeks consuming the experimental diets, are shown in table 3.1.

The effective renal plasma flow of animals in group 2 fed the high protein diet was significantly greater than animals in groups 1 and 3 (F ratio=4.17, p<0.05) and values for those rats fed the 24% protein diets did not differ (p=0.32). This difference is due in some part to the differences in renal mass between group 2 and groups 1 and 3, as the ERPF per unit renal mass was similar for all groups (F ratio=0.65, p>0.20) and significant correlation was found between ERPF and total renal mass (r=0.63, p<0.001).

The glomerular filtration rate of animals fed the 48% casein diet was significantly greater than rats fed either of the 24% protein diets (F ratio=5.56, p<0.01). No association between GFR and renal mass was found (r=0.29, p>0.10), and the GFR per unit renal mass did not differ significantly between the groups (F ratio=0.14). However, GFR appeared to be highly dependent upon the effective renal plasma flow (mls/min/g.kidney weight) (Gp 1, r=0.52, Gp 2, r=0.62, Gp 3, r=0.74).
RESULTS OF RENAL FUNCTION STUDIES PERFORMED IN NORMAL CONSCIOUS RATS FED DIFFERENT PROTEIN DIETS (MEAN±SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ERPF mls/min/100g body wt</th>
<th>ERPF mls/min/g kidney wt</th>
<th>GFR mls/min</th>
<th>GFR mls/min/g kidney wt</th>
<th>Filtration Fraction %</th>
<th>Renal Vascular Resistance mmHg/ml/min</th>
<th>Renal Vascular Resistance mmHg/ml/min/g kidney wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.85±0.13</td>
<td>4.47±0.20</td>
<td>2.19±0.08</td>
<td>1.35±0.09</td>
<td>30.8±2.7</td>
<td>11.4±0.4</td>
<td>6.82±0.27</td>
</tr>
<tr>
<td>2</td>
<td>3.33±0.14</td>
<td>4.06±0.16</td>
<td>2.60±0.11</td>
<td>1.42±0.08</td>
<td>34.3±1.7</td>
<td>10.0±0.3</td>
<td>5.46±0.27</td>
</tr>
<tr>
<td>3</td>
<td>2.63±0.23</td>
<td>3.98±0.31</td>
<td>1.99±0.18</td>
<td>1.44±0.13</td>
<td>33.7±2.0</td>
<td>15.3±1.2</td>
<td>11.13±1.03</td>
</tr>
</tbody>
</table>

Table 3.1
Figure 3.2
RELATIONSHIP BETWEEN GFR AND ERPF IN NORMAL RATS FED DIFFERENT PROTEIN DIETS
Analysis of covariance of GFR and ERPF revealed the regression slope of group 2 to be significantly different from groups 1 and 3, \((p<0.001)\) (Fig 3.2) indicating that the GFR per unit renal plasma flow in animals fed the high protein diet in group 2 was greater than groups 1 and 3.

No difference was found in the whole kidney filtration fraction of the three dietary groups \((F\text{ ratio}=0.66, p>0.40)\).

c) RENAL VASCULAR RESISTANCE

The renal vascular resistance was derived from values of ERPF, haematocrit and blood pressure (mmHg/ml/min). As the latter two variables were similar for all groups, the major influencing factor of renal vascular resistance was the ERPF, and correspondingly the derived values reflect this measurement to a large extent.

However the renal vascular resistance of all three dietary groups were significantly different from one another \((F\text{ ratio}=12.48, p<0.02)\). Values derived for rats in group 2 fed the 48% casein diet were lower than values for animals in group 1, and rats in group 3 fed the 24% soya diet exhibited significantly higher values than animals in group 1 \((Gp 1, 11.4\pm0.4, Gp 2, 10.3\pm0.3, Gp 3, 15.3\pm1.2, \text{mmHg/ml/min})\).

Correction of the values for the differences in kidney weight again reveals all three groups to differ from one another \((F\text{ ratio}=18.76, p<0.01)\) \((Gp 1, 6.82\pm0.27, Gp 2, 5.46\pm0.27, Gp 3, 11.13\pm1.03, \text{mmHg/ml/min/g. kidney weight})\).

c) HAEMATOCRIT

There was no significant difference in values of haematocrit between any of the three dietary groups \((Gp 1, 0.42\pm0.01, Gp 2, 0.43\pm0.01, Gp 3, 0.42\pm0.01)\) \((F\text{ ratio}=1.21, p>0.10)\).
d) **BLOOD PRESSURE**

The mean blood pressure of animals determined at the end of the operative procedure of catheter placement did not differ between the groups (Gp 1, 111±3, Gp 2, 112±2, Gp 3, 114±2, mmHg) (F ratio=0.61, p>0.20).

Over the ensuing three hours, during the collection of urine and plasma samples, the mean blood pressure of all groups had risen above the immediate post operative value, however, readings for all groups remained comparable and stable (Gp 1, 138±4, Gp 2, 131±4, Gp 3, 135±3, mmHg) (F ratio=1.11, p>0.10).

e) **PLASMA UREA, CREATININE, ALBUMIN AND CHOLESTEROL**

Values of these parameters are given in table 3.2. No significant difference was found between the groups for values of creatinine (F ratio=1.86) or albumin (F ratio=2.98).

The plasma urea of rats consuming the high protein diet in group 2 was significantly greater than those in groups 1 and 3 (Gp 1, 6.9±0.3, Gp 2, 8.3±0.3, Gp 3, 6.5±0.4, mmol/l) (F ratio=8.08, p<0.01).

The cholesterol value of rats in group 3 consuming the soya diet was significantly lower than those groups of animals fed the casein diets (Gp 1, 2.98±0.14, Gp 2, 3.06±0.12, Gp 3, 1.39±0.06 mmol/l) (F ratio=70.27, p<0.001).
Table 3.2

VALUES OF PLASMA UREA, CREATININE, ALBUMIN, AND SERUM CHOLESTEROL DETERMINED AT SACRIFICE IN NORMAL RATS FED DIFFERENT PROTEIN DIETS (MEAN±SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>UREA mmol/l</th>
<th>CREATININE µmol/l</th>
<th>ALBUMIN g/l</th>
<th>CHOLESTEROL mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.9±0.3</td>
<td>60±5</td>
<td>33.3±0.7</td>
<td>2.98±0.14</td>
</tr>
<tr>
<td>2</td>
<td>8.3±0.3</td>
<td>49±5</td>
<td>32.1±0.6</td>
<td>3.06±0.12</td>
</tr>
<tr>
<td>3</td>
<td>6.5±0.4</td>
<td>53±4</td>
<td>32.5±0.6</td>
<td>1.39±0.06</td>
</tr>
</tbody>
</table>
STUDY 2 EXPERIMENTAL PROTOCOL

THE EFFECT OF DIFFERENT DIETARY PROTEINS UPON THE COURSE OF EXPERIMENTALLY INDUCED RENAL DISEASE AND BODY COMPOSITION IN THE SUBTOTALY NEPHRECTOMISED RAT

Thirty one rats (weight 195-245g) were randomly allocated into four groups

Group 1  n = 6
Group 2  n = 5
Group 3  n = 10
Group 4  n = 10

Animals were maintained on the standard laboratory diet as previously described, and at the commencement of the study, were placed in metabolism cages for 24 hrs, for urine collection to determine urinary protein, urine volume and urinary urea. Blood samples (1.0ml) were obtained from the tail vein for determination of serum urea, and the blood pressure was determined indirectly using the tail cuff method.

The animals were then commenced on one of two experimental diets

Group 1  24% casein
Group 2  24% soya
Group 3  24% casein
Group 4  24% soya

The study consisted of an initial six week control period, during which time the normal rats were maintained upon the experimental diets. Following this, animals were subjected to subtotal nephrectomy or sham
operation, and studied for a further ten week period. The method of feeding, recording food consumption and weighing the animals was performed as described in section 2.

Following a four week maintenance period upon the experimental diets the animals were again placed in metabolism cages for 24 hour urine collection to determine urinary protein and urinary urea. Blood samples were also obtained from the tail vein for determination of serum urea, and blood pressure was again determined by the indirect method. These measurements were made in order that any effect of the experimental diet upon these parameters in normal animals could be determined.

Two weeks later, the animals underwent either a subtotal nephrectomy, by removal of the right kidney and partial infarction of the left kidney (approximately 30%) or sham nephrectomy.

Group 1 sham nephrectomy
Group 2 sham nephrectomy
Group 3 subtotal nephrectomy
Group 4 subtotal nephrectomy

The animals were then allowed to recover and continued to be maintained upon the experimental diets.

Following the operative procedures, all the animals were carefully examined daily to ensure the integrity of the wound. In no cases was any wound infection or dehiscence found.

During the course of the study, following the operative procedures, it became apparent that the general condition, body weight and food intake of some animals showed a decline, which usually occurred within a short period of 3-4 days. The condition of those
animals was closely monitored and if it became apparent that the animal was severely unwell, as judged by its generally poor condition, marked lethargy or even inability to move spontaneously, and the animal had lost more than 15% of its body weight in a two day period, the animal was sacrificed in order to prevent unnecessary suffering. This was performed by exsanguination by percutaneous cardiac puncture under ether anaesthesia and the blood obtained was analysed for serum urea. The remnant kidney of the rat was quickly dissected free of surrounding tissue, bisected and placed in 10% formalin in 0.9% saline, for later histological evaluation. A thorough examination of the carcass was then undertaken with special attention being paid to sepsis. In none of the animals examined in this way was any abnormality found excepting for the reduction in renal mass produced operatively.

At four and eight weeks following the operative procedures, blood pressure was measured using the indirect method, and the animals were placed in the metabolism cages for collection of 24 hour urine specimens, to be analysed for urinary protein excretion. Additionally at four weeks following the operative procedure the animals were bled from the tail vein, for determination of serum urea.

During the following 3 week period, measurements of inulin and PAH clearances were performed, and direct measurement of blood pressure made in the conscious animal, as previously described.

Immediately following the last urine collection period, the rat was lightly anaesthetised with ether, and exsanguinated into glass tubes via the carotid artery catheter. The kidney(s) were dissected free of surrounding tissue, placed in prepared containers, which were then reweighed to determine kidney weight.
All catheters were then removed from the carcass, the gastrointestinal tract was removed, and the carcass placed in airtight polyethylene containers and stored at -20°C until carcass analysis was performed.
RESULTS

STUDY 2 THE EFFECT OF DIFFERENT DIETARY PROTEINS UPON THE COURSE OF EXPERIMENTALLY INDUCED RENAL DISEASE AND BODY COMPOSITION IN THE SUBTOTALLY NEPHRECTOMISED RAT

1 FOOD INTAKE

During the initial 6 week control period, animals maintained on the 24% casein diets (Gps 1 and 3) consumed significantly more food than animals on the 24% soya diets. (Total food intake, Casein diet 795±15g, Soya diet 737±17g) (p<0.05) (Fig 3.3). The differences in food consumption were most marked during the initial 2 week period following the introduction of the new diets. Over the remaining four weeks of the control period, food consumption remained at a relatively stable level, between 16 and 19 g/day, for all groups.

Following subtotal nephrectomy the food consumption of both casein and soya fed animals fell significantly. The animals maintained on the casein diets consumed significantly less food than the soya fed animals for the two weeks following reduction in renal mass (p<0.01) however, by four weeks following the operative procedures, the food consumption of both groups had returned to preoperative levels, and remained stable for the duration of the study, and did not differ (p>0.05). In contrast, no fall in food consumption was observed amongst the sham operated control animals in the perioperative period, and following the operative procedures, both groups of animals consumed equivalent quantities of food. During the final 6 weeks of the experimental period, the food consumption of all four groups was similar.
Figure 3.3

FOOD INTAKE OF CONTROL AND SUBTOTALY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS (MEAN±SEM)
There was no significant difference between the body weight of the animals at the start of the study. (Gp 1, 222±6, Gp 2, 211±4, Gp 3, 222±5, Gp 4, 220±5, g) (F ratio=0.71, p>0.10).

During the initial six week control period, rats in groups 1 and 3, fed casein, gained weight at a more rapid rate, which was most marked during the first three weeks, compared to groups 2 and 4. This was coincident with the increased food intake of the casein fed rats following the introduction of the experimental diets. However, at the time of subtotal nephrectomy, there was no difference in the magnitude of weight gain by animals in groups 1 and 2, (Gp 1, 15±2, Gp 2, 9±2, g, p>0.05) or groups 3 and 4 (Gp 3, 18±2, Gp 4, 12±2, g p>0.05). The weight gain per gram of food eaten by all animals fed the soya diet over the initial 6 week period was 74% that of all animals fed the casein diet, but this difference did not achieve significance (Gps 1 + 3, 0.022±0.002, Gps 2 + 4, 0.016±0.002 g weight gain/g food eaten, p>0.05).

There was no difference between the body weight of rats in groups 3 and 4 at the time of the operative procedures of subtotal nephrectomy (Gp 3, 240±4, Gp 4, 229±5, g p>0.05), however, the sham operated controls ingesting the casein were heavier than the control group ingesting soya (Gp 1, 238±4, Gp 2, 217±5, g p<0.05).

Following subtotal nephrectomy significant weight loss, of similar magnitude, was seen in both groups 3 and 4, but neither the magnitude of weight change, nor body weight, of these groups differed significantly for the remainder of the study period. Neither group of animals regained the body weight lost during the first post operative
Figure 3.4

Body weight of control and subtotally nephrectomised rats fed different protein diets (mean±SEM)

Weeks
operative procedure
week, but following this period, body weight remained stable. In contrast, the sham operated control animals in groups 1 and 2 showed a small insignificant weight loss during the first post operative week, which had been regained within two weeks. Following the sham nephrectomy both groups of control animals continued to gain weight at a similar rate for the remaining 12 weeks of the study period (Gp 1, 1.39±0.30, Gp 2, 1.08±0.31, g/week, p>0.40). These values are similar to those obtained for this strain of rat, of similar age, maintained upon the standard laboratory diet (1.60 g/week).

The weight gain per gram of food eaten, by the sham control animals following the operative procedures during the remainder of the study did not differ (Gp 1=0.009, Gp 2=0.012, p>0.40).

There was no difference in body weight of subtotally nephrectomised rats at sacrifice (Gp 3, 208±6, Gp 4, 212±4 g) but control animals ingesting casein were significantly heavier than the soya counterparts (Gp 1, 254±6, Gp 2, 230±7, g, p<0.05). Body weight of control animals was significantly greater than experimental animals at sacrifice, for animals ingesting casein, and soya (p<0.001, p<0.05, respectively).

3 MORTALITY

All animals in the control groups 1 and 2, and subtotally nephrectomised rats fed soya (Gp 4) completed the study, however the survival rate of subtotally nephrectomised rats ingesting casein (Gp 3) was 60% which was significantly lower than group 4 (χ²=4.74, p<0.05). All those animals sacrificed prior to the end of the study showed signs of severe physical illness, but macroscopic examination of the carcass revealed no other cause for their demise apart from renal
insufficiency. Serum urea values determined at sacrifice of the animals which did not survive the study, were significantly higher than surviving animals in group 3 (44.0±4.0, vs 17.0±1.6, mmol/l p<0.001), and also those rats had lost significantly more body weight compared to other animals in group 3 at sacrifice (-30±3.5g).

4 INDIRECT BLOOD PRESSURE MEASUREMENTS

Values for systolic blood pressure of control and subtotally nephrectomised animals measured at weeks 0, 4, 10 and 14 of the study are shown in table 3.3. The blood pressure of control animals did not differ during the study period and remained at normal levels (<130mmHg). Following subtotal nephrectomy, animals in groups 3 and 4 demonstrated an equally significant rise in blood pressure over control values when measured during week 10 (Gp 3, 156±8, Gp 4, 151±7, mmHg) (p<0.01) which remained elevated when determined at week 14 (Gp 3, 163±6, Gp 4, 165±8, mmHg). At neither time did values of animals in groups 3 and 4 differ significantly.

The blood pressure of animals in group 3 not completing the study, determined during week 10, (156±10 mmHg) did not differ from the surviving animals in group 3.

5 URINE VOLUME AND URINARY PROTEIN EXCRETION

The 24 hr urine volume of animals did not differ at the start of the study, but rose significantly in all groups when measured 4 weeks after commencing the hydrated experimental diets (p<0.05) (table 3.4). The urine volume of the sham operated control animals remained stable throughout the study whilst consuming the hydrated diets, and the values of these groups did not differ.
Table 3.3

INDIRECT BLOOD PRESSURE MEASUREMENTS (mmHg) IN CONTROL AND SUBTOTALLY NEPHRECTOMISED RATS PERFORMED AT WEEKS 0, 4, 10 AND 14 (MEAN±SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>WEEK 0</th>
<th>WEEK 4</th>
<th>WEEK 10</th>
<th>WEEK 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1</td>
<td>111±3</td>
<td>116±4</td>
<td>117±5</td>
<td>105±4</td>
</tr>
<tr>
<td>GROUP 2</td>
<td>119±4</td>
<td>118±5</td>
<td>113±6</td>
<td>112±9</td>
</tr>
<tr>
<td>GROUP 3</td>
<td>105±4</td>
<td>110±8</td>
<td>156±8</td>
<td>163±6</td>
</tr>
<tr>
<td>GROUP 4</td>
<td>113±2</td>
<td>112±3</td>
<td>151±7</td>
<td>165±8</td>
</tr>
</tbody>
</table>
Table 3.4

TWENTY FOUR HOUR URINE VOLUME (ml) OF CONTROL AND SUBTOTALY
NEPHRECTOMISED RATS MEASURED AT WEEKS 0, 4, 10 AND 14 (MEAN±SEM)

<table>
<thead>
<tr>
<th></th>
<th>WEEK 0</th>
<th>WEEK 4</th>
<th>WEEK 10</th>
<th>WEEK 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1</td>
<td>9.9±1.1</td>
<td>19.7±1.3</td>
<td>26.5±2.1</td>
<td>23.3±1.1</td>
</tr>
<tr>
<td>GROUP 2</td>
<td>10.0±0.6</td>
<td>19.9±4.2</td>
<td>23.1±4.0</td>
<td>23.1±4.1</td>
</tr>
<tr>
<td>GROUP 3</td>
<td>9.8±1.1</td>
<td>24.0±1.6</td>
<td>41.5±6.3</td>
<td>38.7±4.4</td>
</tr>
<tr>
<td>GROUP 4</td>
<td>8.8±1.4</td>
<td>19.2±0.9</td>
<td>27.4±2.4</td>
<td>22.6±0.9</td>
</tr>
</tbody>
</table>
Following subtotal nephrectomy, values of both groups 3 and 4 increased significantly (p<0.05), and animals in group 3 maintained on the casein diet excreted significantly more urine than animals in group 4 (p<0.05).

Values for urinary protein excretion are depicted in table 3.5. A small rise in urinary protein excretion was seen after commencing the experimental diets in all groups (p<0.01), but throughout the study the urinary protein excretion of the sham operated control animals in groups 1 and 2 did not differ significantly.

Following subtotal nephrectomy, urinary protein excretion of group 3 rose sharply by week 10 (37.5±7.7 to 220±39mg/24 hrs) (p<0.001), and a further, but non significant rise was seen at week 14 (Gp 3, 294±18mg/24 hrs). A similar pattern of increasing proteinuria was documented in group 4 animals, but values obtained were significantly lower than group 3 (Gp 4, 18.3±2.7 to 57.9±9.0 at week 10, 113.8±19.0mg/24 hrs at week 14) (p<0.02, p<0.05 respectively compared to group 3).

6 SERUM UREA, UREA CLEARANCE AND UREA EXCRETION

Values of serum urea for rats in groups 1-4 completing the study are shown in table 3.6. Values for the sham operated control animals in groups 1 and 2 did not differ significantly throughout the study. Values for groups 3 and 4 rose significantly following subtotal nephrectomy when determined during week 10 (Gp 3, 7.1±0.1 to 12.4±1.1mmol/l, p<0.001, Gp 4, 6.1±0.2 to 10.0±0.3mmol/l p<0.001). At the end of the study, the serum urea of the surviving subtotally nephrectomised rats had risen further (Gp 3, 17.0±1.6, Gp 4, 15.6±0.8mmol/l, p<0.05, p<0.001 respectively compared to values
Table 3.5

URINARY PROTEIN EXCRETION (mg/24hrs) OF CONTROL AND SUBTOTALY NEPHRECTOMISED RATS MEASURED AT WEEKS 0, 4, 10 AND 14 (MEAN±SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>WEEK 0</th>
<th>WEEK 4</th>
<th>WEEK 10</th>
<th>WEEK 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.5±0.4</td>
<td>24.2±8.4</td>
<td>23.3±9.5</td>
<td>40.6±9.0</td>
</tr>
<tr>
<td>2</td>
<td>6.4±2.1</td>
<td>25.4±8.6</td>
<td>20.8±8.6</td>
<td>29.9±11.0</td>
</tr>
<tr>
<td>3</td>
<td>7.2±0.6</td>
<td>37.5±7.7</td>
<td>220.0±39.0</td>
<td>294.0±18.0</td>
</tr>
<tr>
<td>4</td>
<td>5.6±0.7</td>
<td>18.3±2.7</td>
<td>57.9±9.0</td>
<td>113.8±19.0</td>
</tr>
</tbody>
</table>
Table 3.6

SERUM UREA VALUES (mmol/l) IN CONTROL AND SUBTOTALY NEPHRECTOMISED RATS DETERMINED AT WEEKS 0, 4, 10 AND AT SACRIFICE (MEAN±SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>WEEK 0</th>
<th>WEEK 4</th>
<th>WEEK 10</th>
<th>AT SACRIFICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.9±0.4</td>
<td>7.6±0.2</td>
<td>7.8±0.1</td>
<td>7.8±0.1</td>
</tr>
<tr>
<td>2</td>
<td>6.4±0.3</td>
<td>6.9±0.3</td>
<td>7.2±0.5</td>
<td>7.4±0.3</td>
</tr>
<tr>
<td>3</td>
<td>6.8±0.2</td>
<td>7.1±0.1</td>
<td>12.4±1.1</td>
<td>17.0±1.6</td>
</tr>
<tr>
<td>4</td>
<td>6.2±0.2</td>
<td>6.1±0.2</td>
<td>10.0±0.3</td>
<td>15.6±0.8</td>
</tr>
</tbody>
</table>
obtained at week 10) but did not differ significantly from each other.

Analysis of all values of serum urea of animals in groups 3 and 4
determined at sacrifice, including those animals not completing the
study period, revealed group 3 to be significantly greater than group 4
(Gp 3, 22.0, Gp 4, 15.3 mmol/l median values, p<0.05).

Coincident with the rise in serum urea seen at week 10 in groups 3
and 4, a significant fall in urea clearance occurred, between week 4
and week 10, reflecting the reduction in renal function produced by
subtotal nephrectomy (Gp 3, 0.647±0.021 to 0.396±0.027mls/min/100g body
weight, Gp 4, 0.577±0.029 to 0.351±0.026mls/min/100g body weight)
(p<0.001), but the urea clearance of groups 3 and 4 did not differ
significantly.

Values of urea clearance determined for the sham operated control
animals over this period demonstrated a slight rise between week 4 and
week 10, but values for groups 1 and 2 did not differ significantly (Gp
1, 0.578±0.039 to 0.688±0.015mls/min/100g body weight, Gp 2,
0.558±0.047 to 0.638±0.086mls/min/100g body weight).

The urea excretion (mmol/day) of the four groups of rats
determined at the start of the study, whilst consuming the standard
laboratory diet (16% protein) did not differ (F ratio=0.94) (table
3.7). However, the urea excretion of all groups had risen
significantly when measured four weeks following the introduction of
the higher protein (24%) experimental casein and soya diets (p<0.01,
for all groups). Values for animals maintained upon the casein diets
were significantly greater than those fed the soya diets (F
ratio=14.26, p<0.05). Significant correlation was found within each
dietary group for daily food intake and 24 hour urinary urea excretion
(24% casein r=0.54, 24% soya r=0.49). However, the regression line
Table 3.7

URINARY UREA EXCRETION (mmol/100g body weight/24 hours) OF
CONTROL AND SUBTOTALLY NEPHRECTOMISED RATS DETERMINED AT
WEEKS 0, 4 AND 10 (MEAN ±SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>WEEK 0</th>
<th>WEEK 4</th>
<th>WEEK 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1</td>
<td>3.46±0.22</td>
<td>6.34±0.36</td>
<td>7.73±0.22</td>
</tr>
<tr>
<td>GROUP 2</td>
<td>3.58±0.24</td>
<td>5.48±0.34</td>
<td>5.76±0.61</td>
</tr>
<tr>
<td>GROUP 3</td>
<td>3.01±0.25</td>
<td>6.62±0.21</td>
<td>6.38±0.58</td>
</tr>
<tr>
<td>GROUP 4</td>
<td>2.93±0.28</td>
<td>4.96±0.11</td>
<td>5.00±0.30</td>
</tr>
</tbody>
</table>
derived for rats in groups 1 and 3 fed the casein diet was significantly different from that of groups 2 and 4, fed soya (p<0.001).

When these values were again determined during week 10 of the study, the control animals ingesting the casein diet continued to excrete significantly more urinary urea than those animals fed soya (p<0.001). However, no difference in values for the subtotally nephrectomised rats in groups 3 and 4 was found (p=0.08). The relationship between food intake and daily urinary urea excretion for groups 3 and 4 persisted (r=0.69, r=0.73 respectively).

7 RENAL WEIGHT

The right kidney of animals in groups 3 and 4 was removed 6 weeks after commencing the experimental diets, during the procedure of subtotal nephrectomy. Kidney weight normally varies as a function of body weight, and this was confirmed for values of right kidney weight of groups 3 and 4 (r=0.82, p<0.001). Analysis of covariance of right kidney weight and body weight for these two groups, revealed no significant difference (p>0.30) (Gp 3, r=0.86, Gp 4, r=0.71) (Gp 3, 0.325±0.007 and Gp 4, 0.319±0.010g/100g body weight p>0.10).

The right and left kidneys of the sham operated animals in groups 1 and 2 were removed at sacrifice. The right kidney is usually heavier than the left, and this was confirmed for the sham operated control animals (left kidney weight/right kidney weight, Gp 1, 0.928±0.013, Gp 2, 0.936±0.040, p>0.90). Analysis of covariance of the right kidney weight and body weight of the sham operated control groups 1 and 2 at sacrifice, with the same parameters of groups 3 and 4 obtained after six weeks ingesting the experimental diet, demonstrated that the values
for mean kidney weight for rats fed casein had increased by 25% (p<0.001) whilst a smaller non significant increase of 9% in right kidney weight was found for those rats fed soya (p>0.01). Also, the right kidney weight of animals ingesting casein in group 1 was significantly greater than animals fed soya in group 2 (Gp 1, 0.407±0.009, Gp 2, 0.349±0.009g/100g body weight, p<0.01).

The weight of the remnant left kidney of the subtotally nephrectomised animals in groups 3 and 4 were significantly different (Gp 3, 0.658±0.025, Gp 4, 0.481±0.023,g/100g body weight) (p<0.001). The correlation of kidney weight and body weight could not be demonstrated for the remnant kidneys of these animals (Gp 3, r=0.56, p>0.20, Gp 4, r=0.28, p>0.60), however, a significant association between remnant kidney weight and 24 hour urinary protein excretion was found, as determined during week 10 of the study (r=0.87, p<0.001) (fig 3.5).

The weight of the remnant kidney of animals in group 3 which did not complete the study period was 1.211±0.122g. Although not directly comparable with the remnant kidney weight of animals in groups 3 and 4, as the survival time following subtotal nephrectomy differed, the kidney weight was greater than group 4 animals (1.017±0.048g) and lower than group 3 animals (1.369±0.061g), but in neither case did the difference achieve significance (p>0.05).
RELATIONSHIP BETWEEN REMNANT KIDNEY WEIGHT AND URINARY PROTEIN EXCRETION IN SUBTOTALY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS

![Graph showing the relationship between remnant kidney weight and urinary protein excretion.](image)

- Group 3
- Group 4
8 RENAL FUNCTION STUDIES

a) Glomerular Filtration Rate and Effective Renal Plasma Flow

Results of renal function studies undertaken in the conscious animals are shown in table 3.8.

The effective renal plasma flow (mls/min/100g body wt) of sham operated control animals in group 1 was significantly higher than group 2 (p<0.01). If the results are expressed per gram of renal tissue, the magnitude of the difference between the groups is reduced, due to the larger renal mass of the rats in group 1, but remains significant (p<0.05).

The glomerular filtration rate (mls/min), of control animals ingesting casein was also significantly higher than those animals fed soya (p<0.02), but the difference becomes insignificant if GFR is expressed as a function of renal mass (p>0.05). However, as GFR is dependent upon the number of functioning glomerular units rather than mass per se, the filtration rate per glomerulus of animals fed casein would be significantly greater than animals fed soya, assuming the number of glomeruli per animal to be the same.

Analysis of covariance of the relationship between ERPF and GFR, of the control animals, shows the two groups to be significantly different (p<0.01) (fig 3.6). Also, values derived for renal vascular resistance of group 1 were significantly lower than group 2 (p<0.01).

In contrast to the control animals, there was no significant difference in the ERPF of the subtotally nephrectomised rats in groups 3 and 4 (p=0.22), however the ERPF per unit renal mass of group 3 animals was significantly reduced compared to group 4 (p<0.05). Values for subtotally nephrectomised animals ingesting both casein and soya
RESULTS OF RENAL FUNCTION STUDIES PERFORMED IN CONSCIOUS CONTROL AND SUBTOTALY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS (MEAN±SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>ERPF (mls/min/100g b.w.)</th>
<th>GFR (mls/min)</th>
<th>Filtration Fraction (%)</th>
<th>Fractional Sodium Excretion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>3.58±0.16</td>
<td>2.40±0.16</td>
<td>25.7±2.0</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>Group 2</td>
<td>2.31±0.28</td>
<td>1.68±0.17</td>
<td>34.0±1.6</td>
<td>0.26±0.05</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.84±0.15</td>
<td>0.88±0.15</td>
<td>51.0±4.0</td>
<td>1.15±0.12</td>
</tr>
<tr>
<td>Group 4</td>
<td>1.11±0.15</td>
<td>1.01±0.13</td>
<td>43.0±4.0</td>
<td>0.55±0.07</td>
</tr>
</tbody>
</table>

Table 3.8
**RESULTS OF RENAL FUNCTION STUDIES PERFORMED IN CONSCIOUS CONTROL AND SUBTOTALY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS (MEAN ±SEM)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Urinary Sodium Excretion (n.Mol/min)</th>
<th>Renal Vascular Resistance (mmHg/ml/min)</th>
<th>Mean BP at recovery (mmHg)</th>
<th>Mean BP during clearance periods (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>655±94</td>
<td>9.5±0.5</td>
<td>121±3</td>
<td>147±3</td>
</tr>
<tr>
<td>Group 2</td>
<td>575±67</td>
<td>15.4±2.4</td>
<td>116±10</td>
<td>156±3</td>
</tr>
<tr>
<td>Group 3</td>
<td>1385±300</td>
<td>91.6±28.0</td>
<td>165±8</td>
<td>197±14</td>
</tr>
<tr>
<td>Group 4</td>
<td>977±189</td>
<td>41.9±5.6</td>
<td>154±5</td>
<td>203±3</td>
</tr>
</tbody>
</table>

Table 3.8 contd
Figure 3.6

Relationship of GFR and ERPF in control rats fed different protein diets.
were significantly lower than their respective controls (p<0.001, p<0.01, respectively). However, non-parametric analysis of ERPF of all subtotally nephrectomised rats (values of 0 ascribed to those animals not surviving the study due to severe renal impairment) reveals group 3 to be significantly lower than group 4 (Gp 3, 0.87, Gp 4, 2.43mls/min, medians, p<0.05).

The glomerular filtration rate of the subtotally nephrectomised rats in groups 3 and 4 were significantly lower than the sham operated control animals (Gps 1 and 2) (p<0.001, p<0.01) and although the remnant kidney GFR of the soya fed rats in group 4 was greater than group 3, this did not achieve significance (p>0.05). However, non-parametric analysis of the GFR of all subtotally nephrectomised rats, reveals group 3 to be significantly lower than group 4 (Gp 3, 0.42, Gp 4, 1.12ml/min, median values, p<0.05). The median renal vascular resistance of group 3 (63mmHg/ml/min) was significantly greater than group 4 (37mmHg/ml/min) (p<0.05).

b) Filtration Fraction (Table 3.8)

The filtration fraction of control animals in group 1 fed casein was significantly lower than group 2 (p<0.01). Values obtained for subtotally nephrectomised rats maintained upon the casein diet were greater than comparable animals fed the soya diets, but the difference did not achieve significance (Gp 3, 51.0±4.0, Gp 3, 43.0±4.0, % p>0.05). The filtration fraction of both groups of subtotally nephrectomised rats was greater than their control counterparts, but only between groups 1 and 3 did the difference achieve significance (Gp 1, 25.7±2.0, Gp 3, 51.0±4.0, %, p<0.001) (Gp 2, 34.0±1.6, Gp 4, 43.0±4.0, %, p>0.10).
c) Sodium Excretion (Table 3.8)

There was no significant difference between the values determined for the urinary sodium excretion of groups 1 and 2 (p>0.20) or groups 3 and 4 (p>0.10). However, the subtotally nephrectomised rats ingesting casein had significantly higher values than their control counterparts in group 1 (p<0.05) and a similar trend was observed amongst animals ingesting soya but the difference between groups 2 and 4 did not achieve significance (p>0.10).

d) Fractional Sodium Excretion (Table 3.8)

The fractional excretion of sodium of the subtotally nephrectomised rats ingesting casein and soya were also significantly higher than values obtained in the sham operated control rats in groups 1 and 2 (p<0.001, p<0.01, respectively) and values for rats in group 3 were significantly higher than group 4 (Gp 3, 1.15±0.12, Gp 4, 0.55±0.07,%, p<0.01). There was no difference in values between control rats on the different diets (p>0.10).

e) Direct Blood Pressure Measurement (Table 3.8)

Values for mean blood pressure of subtotally nephrectomised rats in groups 3 and 4 obtained at the end of the recovery period were significantly higher than those of the control animals (Gp 1, 121±3, Gp 3, 165±8mm/Hg, p<0.01, Gp 2, 116±10, Gp 4, 154±5mmHg, p<0.01), but values for groups 1 and 2, and 3 and 4, did not differ (p>0.20, p>0.10 respectively). During the clearance periods, the mean blood pressure of all groups was found to rise, but values for control animals remained significantly lower than subtotally nephrectomised rats (p<0.01).
f) Haematocrit

The haematocrit of both control and subtotally nephrectomised rats ingesting the casein diets did not differ (Gp 1, 0.42±0.01m, Gp 3, 0.39±0.02) (p>0.50), but both groups were significantly lower than values of animals ingesting the soya diets (Gp 2, 0.47±0.01, Gp 4, 0.47±0.01) (p<0.01).

9 APPEARANCE OF THE RENAL TISSUE

a) Macroscopic

The macroscopic appearance of the remnant kidneys from both groups 3 and 4 was qualitatively similar. The lower pole of the kidney was replaced by a small mass of scar tissue, and the remaining renal tissue was sharply demarcated from this. The surface of the kidneys was brown and granular in appearance, but the tissue surrounding the kidney appeared healthy, and no evidence of infection was found.

b) Microscopic

The histological abnormalities described in the remnant kidney tissue of both groups 3 and 4, are qualitatively similar, however the quantitative occurrence of the lesions differ between the groups.

i) Glomeruli (Plates 3.1 to 3.4)

An increase in PAS staining material was to be seen deposited within the mesangial region, and in the least affected glomeruli this was located around the glomerular stalk. In the more severely affected structures, this material was seen throughout the mesangium. Dilatation of capillary loops was common in the glomeruli with mesangium of normal appearance, however in the more severely affected structures, the number of patent capillary loops was decreased as were the diameters of these capillaries. The presence of coagulated protein
Photomicrographs of rat remnant kidney showing moderate glomerulosclerosis, with capillary loop dilatation and deposition of mesangial PAS positive material (PAS stain)
Plate 3.3
PHOTOMICROGRAPH OF RAT REMNANT KIDNEY SHOWING SEVERE GLOMERULOSCLEROSIS, WITH MARKED DISRUPTION OF GLOMERULAR ARCHITECTURE (PAS STAIN)

Plate 3.4
PHOTOMICROGRAPH OF RAT REMNANT KIDNEY SHOWING TUBULAR DILATATION AND INTRATUBULAR PROTEINACEOUS CASTS (PAS STAIN)
in Bowmans space was frequently seen. Severely abnormal glomeruli were to be seen adjacent to normal glomeruli, and there appeared to be no preferential distribution of glomerular abnormalities to any zone in the cortex. In none of the glomeruli was any inflammatory infiltrate seen.

ii) Tubules

Changes of tubular atrophy and dilatation with intratubular cast formation were seen within the remnant kidneys. The diameter of atrophic tubules was severely reduced, and the size of tubular cells lining these lumina was markedly reduced. Cells lining the dilated tubules were often markedly attenuated, and often the whole lumen would be occupied by proteinaceous material. Notable in some proximal tubular cells were the presence of numerous protein resorption droplets, often associated with large quantities of proteinaceous material in the lumen. The distribution of the tubular lesions did not appear to be preferentially localised to any area of the remnant kidney. In none of the sections examined was any interstitial inflammatory infiltrate seen.

iii) Blood Vessels

Histological changes due to hypertension were seen within some arterial vessels. Changes of medial hypertrophy were found, but endothelial cell proliferation or fibrinoid necrosis, histological characteristics of malignant hypertension, were not seen.

10 QUANTITATIVE HISTOLOGICAL CHANGES

Figure 3.7 depicts the quantitative histological findings in the remnant kidneys of animals in groups 3 and 4, and also of animals in group 3 which did not survive the study period.
RESULTS OF HISTOLOGICAL ANALYSIS OF KIDNEY REMNANTS OF
SUBTOTALLY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS

Figure 3.7

Group

Tubular Atrophy

Grade

3

2

1

3

4

+ Group

Tubular Dilatation

Grade

3

2

1

3

4

+ Group

% abnormal glomeruli

Severe Glomerulosclerosis

Moderate Glomerulosclerosis

+ = animals sacrificed prior to end of study period
The remnant kidney of group 3 rats demonstrated significantly more severely sclerosed glomeruli (Gp 3, 19.8±2.9, Gp 4, 2.4±0.7,%) (p<0.001) and glomeruli exhibiting changes of moderate glomerulosclerosis (Gp 3, 46.3±2.2, Gp 4, 30.4±2.4,%) (p<0.001). Ninety per cent of the glomeruli were abnormal in the remnant kidneys of those animals in group 3 which did not complete the study period due to severe renal insufficiency. (Severely affected, 31.3±5.5%, moderately affected, 57.8±5.9,%).

Tubular abnormalities were also more prominent in the remnant kidney of group 3 rats, compared to group 4 (Tubular dilatation mean scores, Gp 3, 2.5±0.2, Gp 4, 0.9±0.2, p<0.001. Tubular atrophy mean scores, Gp 3, 1.2±0.2, Gp 4, 0.6±0.2, p<0.05). Tubular changes present in the renal tissue of animals in group 3 which did not complete the study period were not significantly different from other animals in group 3. (Tubular dilatation, mean score, 2.3±0.3. Tubular atrophy, 1.3±0.3).

Examination of the renal tissue the sham operated control animals in groups 1 and 2, revealed none of the features that were described in the kidneys of the subtotally nephrectomised rats. Glomeruli, tubules and blood vessels were of normal appearance in both groups 1 and 2.

**BODY COMPOSITION ANALYSIS**

Results of determination of body water and body fat are given in fig 3.8. No significant difference was found in the percentage body water of control groups (Gp 1, 58.0±0.3, Gp 2, 58.4±0.6,%, p>0.90) or of the subtotally nephrectomised groups (Gp 3, 67.9±1.01, Gp 4, 66.7±0.4,%, p>0.50). However, values determined for the subtotally
RESULTS OF BODY COMPOSITION ANALYSIS OF CONTROL AND
SUBTOTALLY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS

Figure 3.8

Percentage Body Water  
Percentage Body Fat  
Fat Free Body Solids (g)
nephrectomised rats were significantly greater than their respective controls (p<0.001).

The percentage body fat of the control rats fed casein in group 1 was significantly greater than the control rats fed soya. (Gp 1, 15.7±0.4, Gp 2, 13.6±0.3,%, p<0.01). Values determined for the nephrectomised rats were similar (Gp 3, 3.1±0.9, Gp 4, 4.6±0.4,%, p>0.10). The percentage body fat of both groups 3 and 4 was significantly lower than the control groups (p<0.001).

As would be predicted, an inverse correlation between percentage body water and percentage body fat was found. This correlation only achieved significance for the subtotally nephrectomised animals (Gp 3, r=-0.99, p<0.001, Gp 4, r=-0.79, p<0.01), due to the narrow range of results amongst control animals (Gp 1, r=-0.55, Gp 2, r=-0.31, p>0.05) (fig 3.9).

The weight of fat free solids, representing mainly the protein and mineral ash content of the carcass, did not differ between the control groups (Gp 1, 51.0±1.3, Gp 2, 48.4±2.3,g. p>0.05) or between the subtotally nephrectomised rats (Gp 3, 46.1±1.7, Gp 4, 46.3±0.8,g. p>0.50). Values obtained for groups 3 and 4 were slightly lower than control animals, but the result did not achieve significance (F ratio=2.71, p>0.05). The percentage water of the fat free body weight, an index of the state of hydration of the animal, of both control and subtotally nephrectomised rats did not differ (F ratio=3.47, p>0.05).

During the course of the study, the control animals fed casein consumed more food than the soya fed animals (Gp 1, 2159±20, Gp 2, 2062±5,g, p<0.01) which was mainly due to the initial period of overeating following the introduction of the new diets. Significant correlation was found between the total quantity of food ingested by
Figure 3.9

RELATIONSHIP OF PERCENTAGE BODY WATER AND PERCENTAGE BODY FAT IN CONTROL AND SUBTOTALLY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS
the control animals, and the percentage body fat ($r=0.92$, $p<0.001$). However, no relationship between these parameters was found for the subtotally nephrectomised rats. The percentage body fat in these groups appeared to be more dependent upon the degree of renal dysfunction, in that the more severe the renal impairment, the less amount of fat present in the carcass. A semilogarithmic plot of percentage body fat and GFR is shown in fig 3.10 (Gp 3, $r=0.96$, $p<0.001$, Gp 4, $r=0.71$, $p<0.01$).
Figure 3.10

RELATIONSHIP OF GFR AND PERCENTAGE BODY FAT IN SUBTOTALY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS
SECTION 4

DISCUSSION
DISCUSSION

The results of Study 1 in this section, investigating the effects of feeding two levels of the same dietary protein (48% and 24% casein) to normal rats for a six week period, confirm the observations made in the rat by Dicker (1949) that high protein feeding is associated with the development of an elevation in glomerular filtration rate and renal mass. Similarly the sustained increase in renal plasma flow in high protein fed animals documented by Pitts in 1944 has been confirmed.

With subsequent advances in experimental investigative technique, more elaborate micropuncture studies have recently revealed the lower GFR associated with a reduction in protein intake, to be due to a fall in glomerular capillary plasma flow rate and glomerular capillary ultrafiltration coefficient (Ichikawa I et al 1980), and the major factor responsible appears to be a change in glomerular arteriolar resistance. A reduced renal vascular resistance was documented in the high protein fed rats in this study but some caution is needed when comparing the results with those of Ichikawa. The low protein diet in the micropuncture investigation (6% casein), which was designed to produce chronic malnutrition, was substantially lower than the level employed in the present study, and the vascular resistance was the directly measured glomerular arteriolar resistance, and not the vascular resistance per unit kidney weight as determined in this study. However, the relationship between whole kidney GFR and ERPF in normal animals has been shown in this study, and the elevated GFR and ERPF in animals fed the high protein 48% casein diet was associated with a reduced renal vascular resistance.
Interpretation of these findings is complicated by the concomitant increase in renal mass associated with high protein feeding, and also the finding that the ERPF per unit renal mass of rats fed the 48% casein diet was marginally lower, than animals fed the 24% diet. This may be reconciled to some extent by the observation that high protein feeding will induce hypertrophy of the whole kidney, but this is most marked in the tubular cells of the relatively poorly perfused inner stripe region of the kidney (Bouby N, et al, 1984). As intrarenal blood flow distribution has not been investigated in this study it would be invalid to state that the elevated GFR in the high protein fed rats is entirely dependent upon the raised renal plasma flow, but the linear relationship between ERPF and GFR (fig 3.2) would appear to lend some support to this.

In order to circumvent the renal hypertrophy produced by high protein feeding, Seney and Wright have measured the GFR of rats with a comparable renal mass, that had been fed either high or low protein diets (Seney F D, Wright F S, 1985). Their findings show that even when renal mass is the same, animals maintained on a high protein diet (48% casein) have a significantly greater GFR than low protein fed animals (6% casein) and attributed this to a reduction in the sensitivity of the tubuloglomerular feedback system. Whatever the precise mechanism responsible for the elevated GFR and increase in renal size associated with high protein feeding, the results of this study suggest that an increase in renal plasma flow and reduction in renal vascular resistance are important determinants. However no comment can be made regarding the intrarenal control mechanisms responsible for this.
The GFR, ERPF and renal mass of normal rats fed 24% casein or 24% soya diets for a six week period, show close similarity, and the relationship between GFR and ERPF appears the same. However the renal vascular resistance of the soya fed animals was significantly elevated compared to the casein diet (p<0.02). Some caution must be exercised when interpreting this result, as the renal vascular resistance is a derived value from four measurements, and might to some extent reflect the slightly lower absolute renal mass and ERPF of the soya group. This may be due, in part, to the unexpected failure of animals fed the soya diet to adapt to the introduction of the experimental diet as quickly as other groups, and the concomitant loss of body mass.

Conclusions to be drawn from Study 1 therefore are that short term feeding (six weeks) of a high protein diet to normal rats, produces an increase in GFR, ERPF and renal mass, but that no significant differences in these parameters develop when the source of dietary protein differs but is fed at an isonitrogenous level. However, the renal vascular resistance appears to differ significantly with both the quantity and source of dietary protein.

The results of Study 2 in this section however show that when normal rats, and subtotally nephrectomised rats, were fed isonitrogenous diets of casein or soya for a longer period (16 to 18 weeks), differences in terms of renal function and renal size became apparent between control animals, and in addition a difference in the progression of renal disease was found in the subtotally nephrectomised rats. The method used to produce the model of renal insufficiency in this study, as shown by the reduced GFR and elevated serum urea, replicates the major observations first reported in the rat by Chanutin in 1932; namely, that a reduction in renal mass is followed by the
development of polyuria, proteinuria, renal hypertrophy, nitrogen retention and hypertension. However comparison of the results of a number of parameters of the two dietary groups reveals that there are major differences in the severity of the renal lesion which develops, and the ultimate outcome.

The significantly greater values of proteinuria, renal hypertrophy and polyuria that were found in the subtotally nephrectomised rats fed casein present a similar pattern to that observed some fifty years ago in a study by Chanutin and Ludewig (1936). However in that case the rats were maintained upon a meat diet, and the results were produced by increasing the protein content of the diets by factors of two and four. Similar results have been forthcoming from other groups, documenting that manipulation of the quantity of protein fed to subtotally nephrectomised rats produces differences in mortality, renal hypertrophy, proteinuria and histological damage (Kenner C H et al, 1985, Hostetter T H et al, 1986). In this study a beneficial effect upon these parameters has been found, but the difference in the diets has been the source of dietary protein, rather than its quantity. Analysis of the relationship between the quantity of proteinuria and weight of the remnant renal tissue reveals a close correlation between these two parameters i.e. increased renal hypertrophy in the remnant kidney is associated with increased proteinuria. This trend has been recognised previously, but again the causal factor has been the quantity of protein fed. From this relationship it is tempting to speculate that the increase in size of the remnant tissue and magnitude of proteinuria are linked by a common causal factor.

An amelioration in both functional impairment and structural damage is apparent in those animals fed the soya diet. The GFR and
ERP of the surviving casein fed rats were lower than their soya fed counterparts, but in neither case did this result achieve significance. Examination of the microscopic features of the remnant tissue of the soya fed rats shows that this diet did not prevent the glomerular and tubular lesions associated with subtotal nephrectomy from developing, but rather reduced the frequency of occurrence. The ameliorative effect of the soya diet is also apparent from the proportion of casein fed rats (40%) that were sacrificed prior to the end of the study. The observation that these animals had sustained marked histological damage in the remnant tissue confirms the severity of renal insufficiency in those animals. This is supported by the significantly higher values of serum urea in those rats, but as measurement of the state of hydration of these animals was not performed, some degree of terminal dehydration may have been present. Systemic hypertension, known to have a deleterious effect upon the progression of renal lesions, was found in both groups of nephrectomised rats. However the elevation in the systemic blood pressure was equivalent in both groups. Values of mean blood pressure determined during the clearance periods in this study are marginally elevated compared to some other studies. This is a reflection of the animal model employed, the conscious rat, and the choice of ether anaesthesia, as both experimental conditions tend to result in mild elevation of blood pressure (Walker LA et al, 1983).

In contrast to the results of short term feeding of normal animals in Study 1, the results of long term maintenance of sham operated control rats upon the two experimental diets reveal that differences in renal function were apparent after the eighteen week experimental period. An elevation in GFR and ERP were documented in the casein fed group compared to the soya fed animals, which was associated with a
reduced renal vascular resistance and elevated renal mass. No difference however was found in the urinary protein excretion or renal histological appearance, and histological analysis shows that neither diet appeared to be intrinsically harmful to renal structure or function. The relationship of GFR and ERPF was found to differ between the dietary groups (p<0.01) and the elevated GFR per unit ERPF in the casein fed rats was significantly higher than the soya fed animals.

The main conclusions to be drawn from Study 2 are that prolonged feeding of isonitrogenous soya and casein diets to the normal rat will result in differences in ERPF, GFR, renal vascular resistance and renal mass, and ingestion of these diets by the subtotally nephrectomised animals are associated with major differences in mortality, proteinuria, renal hypertrophy and histological damage.

A tentative hypothesis that might be advanced to account for the differences in outcome of the subtotally nephrectomised animals is a direct extension of the hyperfiltration theory proposed by Brenner. The main proposition is an alteration in glomerular haemodynamics, namely an increase in glomerular capillary plasma flow rate and transcapillary hydraulic pressure gradient that develop following partial nephrectomy, are the self-perpetuating mechanisms by which structural damage ensues. The implication that dietary protein is important in modulating this response has been proposed from observations that dietary protein restriction in the rat remnant model will ameliorate the elevation in these haemodynamic parameters, and this is associated with a reduction in the severity of the ensuing structural damage. Determination of glomerular plasma flow rate and hydraulic pressure gradient require sophisticated micropuncture techniques, and were not available in this study, but differences in
whole kidney plasma flow rate and glomerular filtration rate were observed in the normal rat following long term feeding. Intrarenal blood flow distribution was not measured, but it would be reasonable to assume from these results that the plasma flow rate per glomerulus was elevated in the normal rats fed casein in the long term study. The direct extension of this hypothesis might be that an increase in glomerular plasma flow and hydraulic pressure gradient consequent upon the impaired autoregulatory capacity following subtotal nephrectomy were augmented by long term casein feeding, and this was responsible for the increased structural and functional damage in this group.

However, there are a number of points which emerge from the results obtained which might cast some doubt upon the immediate application of this theory. Firstly, the protein content of the experimental diets fed to the subtotally nephrectomised group were identical, and the initial proposition by Brenner was formed from observations made in subtotally nephrectomised rats made upon different levels of dietary protein intake. Secondly, in normal animals fed the 24% protein diets for six weeks, the only difference in renal haemodynamics observed was in renal vascular resistance, whilst differences in ERPF, GFR and renal size only became apparent during a prolonged period of feeding. Thirdly, as micropuncture studies were not undertaken, it can only be indirectly proposed from the evidence of the equivalent remnant renal plasma flow rate, and disparate levels of remnant renal histological damage, that the plasma flow per functioning glomerulus and hydraulic pressure gradient in the remnant kidneys of the casein fed animals was greater than the soya fed group. Fourthly, differences in renal function which only became apparent during long term feeding of the two diets in normal animals, may be exaggerated due
to alterations in intraglomerular haemodynamics following subtotal nephrectomy, as seen from the disparate levels of proteinuria and mortality at an early stage following the reduction in renal mass.

Therefore, how might the differences in the casein and soya diets be related to the outcome of the subtotally nephrectomised rats? In considering this question there are two main points which merit discussion. Firstly, the composition of the diet presented to the animals may have been instrumental in producing the changes directly, or alternatively a metabolic or hormonal response by the animal to the diet might be implicated.

The physiological role of dietary protein is to provide substrates required for the synthesis of body proteins, and other metabolically important nitrogen containing compounds, and the potential nutritional value of dietary protein resides in its amino acid composition, digestibility and bioavailability, and the nutritional and physiological status of the animal. By virtue of the fact that the two diets contain protein of different chemical composition, the potential nutritive value, influenced by the foregoing factors, will differ. The experiments in this study were not designed to directly examine the digestibility, bioavailability and absorption of amino acids in the diet, but some comment regarding this is required.

Much previous experimental data is available to show that minor differences in the nutritional equivalence of casein and soya exist. The nutritive value of a diet can be assessed by determination of the rate of weight gain in the experimental animal, and this was first appreciated by Osborne in 1919 (Osborne T B et al 1919). In the young rat, it has been subsequently shown that the rate of weight gain bears close correlation to the degree of nitrogen retention, however in adult
animals, as used in this study, weight gain is mainly due to body fat accretion.

Following the introduction of the experimental diets, the food consumption of the casein fed rats was superior to the soya fed groups, and this phenomenon of initial overeating following dietary change is well recognised, and related to the palatability of the diets. (Sclafani A, Springer, 1976). During this period, the rate of weight gain by the casein fed groups increased, and the body weight stabilised at a higher level. The rate of weight gain, and food consumption, of both the casein and soya fed control animals then became comparable for the remainder of the study. This rapid incremental rise in body weight, in association with the initial period of overeating, again a well recognised phenomenon, whereby the animal adjusts its "set point" weight in response to the palatability of the diet (Sclafani A, 1976, Sherry D, 1981). However, following this initial period of dietary acclimatisation, the equivalence of food consumption and weight gain reflects the broad nutritional comparability of the diets (Pope H O et al, 1975, Nagata Y et al, 1981).

More sophisticated studies into the digestion and absorption of nutrients from isonitrogenous casein and soya diets, however, have revealed that the digestibility of soya protein is 80-90% that of casein, the digestibility of which is generally greater than 95% (Henry K et al, 1956, Henry K, Kon S K, 1957, Henry K et al 1961, Hopkins D et al, 1976, Nitzan Z et al 1979). The reasons for the less complete absorption of amino acids from the soya are varied. The susceptibility of parts of the peptide chains to digestive enzymes may be reduced (Munro H M, 1964), and this is supported by the observations of Goldberg and Guggenheim that both the rate of release, and the absolute
quantity, of lysine, methionine and tryptophan absorbed from soya protein is reduced compared to casein (Goldberg A, Guggenheim K 1962). Evidence is therefore available to support the proposition that the absorption of nutrients from the soya diet may have been marginally inferior to that of the casein diet. This is reflected, to some extent, by the observation that the urea excretion rate, the end product of nitrogen metabolism, in control animals fed soya, was slightly less per gram of food consumed than the casein fed animals. However, this difference was not apparent in the subtotally nephrectomised rats. In both control and subtotally nephrectomised rats, the urea excretion rate was greater than normal animals maintained upon the standard laboratory diet (16% protein), suggesting that the amino acid absorption from both experimental diets was superior to the 16% protein diet.

In addition to differences in the digestibility of the diets, differences in their amino acid composition are apparent. The quality of a dietary protein can be expressed as either its ability to support growth in the young animal, or alternatively by the amino acid composition of the protein in relation to the requirements of the animal (Munro H M, 1964). In practice the quality of a dietary protein when assessed by its chemical composition is dependent upon the quantity of the limiting essential amino acid present, and protein quality can be predicted well from its chemical composition (Rama Rao P B et al, 1964). The essential amino acid composition of the experimental diets differed in their balance, but both were in excess of those recommended as minimum values required to support growth in the rat (Rama Rao P B et al, 1959). It will however be apparent, that the quality of a dietary protein as assessed by its amino acid content,
may not be equivalent to its capacity to support growth, if the
digestibility and absorption of the nutrients differ. In addition to
the consideration of the protein quality and content of the diet the
absorption of other nutrients, such as carbohydrate, essential fatty
acids, vitamins and trace elements must be taken into account when
determining the nutritional value of a diet as a whole. A method of
estimating the quality of a protein based upon the whole body
assimilation of the diet is the protein efficiency ratio (PER), which
expresses the rate of growth per unit of food ingested. This method
has the limitation that it has been standardised for the very young
growing rat, fed dietary protein at the 10% level. The nutritive value
of soya protein as examined by this method approximates 70-85% that of
casein (Hopkins D et al, 1976, Henry K et al, 1961, Henry K, Kon S K,
1957, McLaughan J M, Campbell J A, 1969). This is in accord with the
experimental observations made in this study, in that the protein
efficiency ratio of the soya diet was 74% that of the casein diet,
following the initial period of dietary introduction, but after the
sham nephrectomy the PER values of the diets were equivalent. The
growth rate of the control animals is in sharp contrast to the
subsequent maintenance of body weight seen in the subtotally
nephrectomised rats, but more importantly, no difference was seen
between the experimental diets in the uraemic animals. Hence, evidence
is available from previous studies, and from the growth rate and urea
excretion of control animals in this study, to suggest that soya is a
marginally inferior source of protein compared to casein, but the
differences in weight gain by control animals were not significant and
there was no discrepancy in the gross body weight amongst the uraemic
animals.
Therefore, both the pattern and absolute quantity of amino acids and other nutrients absorbed from the two diets may have been a factor influencing the renal function in the long term fed animals and the subtotally nephrectomised rats. The effect of the diets on renal function in normal animals however appears to be more subtle than a simple increase in dietary protein intake. In both studies no difference was found in the renal weight following a six week period ingesting experimental diets, and parameters of renal function in Study 1 revealed equivalent GFR and ERPF. It was only after a prolonged period of maintenance on 24% protein diets that differences in renal function and mass in normal animals became evident. However, in the subtotally nephrectomised rats, differences in proteinuria were evident four weeks following partial nephrectomy. The question concerning the quantity of protein absorbed from the diets and its effect in the subtotally nephrectomised rat, and the metabolic responses, will be more fully investigated and discussed in Section 5.

Comparatively little experimental evidence is available concerning the nature of dietary protein in influencing normal renal function, or the response to subtotal nephrectomy. The early experiments of Chanutin and Ludewig (1936) and Halliburton (1969) showed that the degree of renal hypertrophy following partial nephrectomy is influenced by the nature of the diet, to some extent. The former investigators found that comparing meat and liver diets, differences in the degree of compensatory renal hypertrophy were apparent but the quantity of protein provided appeared more influential, however Halliburton showed that by distorting the amino acid balance fed to animals, by gelatin inclusion in the diet at isonitrogenous levels, changes in the degree of renal hypertrophy could be shown. Other evidence that the amino
acid balance in the diet may be important was provided by Kaysen and Kropp (1983) whilst investigating albumin metabolism in the partially nephrectomised rat. An increase in the dietary content of a single amino acid, tryptophan, by 4%, in a 20% protein diet, produced reductions in proteinuria and hypertension in the subtotally nephrectomised rat following an 8-week period. On the other hand in that study there was no apparent difference in histological damage or renal function, as determined by serum urea and creatinine clearance, between the two groups. The evidence of Kaysen and Kropp, and Halliburton, suggests that the balance of amino acids in the diet may be an important factor in modulating the renal response to subtotal nephrectomy, but little other corroborative evidence has been forthcoming. Other studies, performed in man, have shown that the nature of the dietary protein may be important in modulating renal function (Bosch J P et al, 1983, Wiseman M et al, 1987). Further evidence in support of this observation has come from Dhaene, who has documented an altered post prandial renal response to isonitrogenous synthetic diets of differing amino acid composition (Dhaene M et al, 1987).

The conclusions from the results of renal function studies in this section show that long term feeding of isonitrogenous diets of different protein sources influences both renal function and renal size in the normal rat, and also affect the severity of renal disease which subsequently develops in the subtotally nephrectomised rat. The mechanism may be an initial change in haemodynamic response to reduction in renal mass, but the factors in the diet, or in response to the diet, be they associated with absorption, digestion or the quality
of dietary protein, cannot be defined. Studies described in Section 5 are directed towards examining some of these points in further detail.

In addition to changes in renal function and size which occur following a reduction in functioning renal mass, marked changes in body composition also ensue. Many investigations have been undertaken into body compositional alterations associated with uraemia, almost exclusively in man. The characteristic changes include a reduction in body fat and fat free solids, and a relative increase in total body water, with an altered distribution indicated by a relative excess of extracellular fluid (Coles G A, 1972). The similarity of these changes to those encountered in chronic malnutrition have suggested that the alteration in body composition is a result of protein calorie malnutrition, consequent upon anorexia, vomiting, or therapeutic protein restriction (Coles G A, 1972).

Investigation performed in the uraemic rat have yielded similar changes, but it has been suggested from paired feeding studies using both high and low protein diets, that the changes are primarily a result of uraemia per se, rather than undernutrition (Adelman R D, 1981). A reduced weight gain by uraemic pair fed rats compared to control rats has suggested impaired protein utilization in uraemia (Wang M et al, 1976) and Chantier has suggested that a decreased growth rate is attributable to increased caloric requirements in uraemia (Chantier C et al, 1974). Also, metabolic acidosis, a state which is commonly encountered in uraemia, appears to retard growth by depression of nitrogen utilization (May R C et al, 1986). Taken together these findings might be interpreted as indicating that the body composition changes encountered in uraemia occur as a result of alterations in protein and energy metabolism consequent upon the uraemic state per se.
The results of the carcass analysis performed in this study yielded similar results to those encountered previously in rats and man, ie a relative increase in total body water, and decrease in total body fat in the uraemic animals.

A small but significant increase in body fat was found in the control casein fed rats compared to the soya fed animals, as would be expected from the initially rapid weight gain and higher food consumption by this group. However, both groups of control rats continued to gain weight throughout the study, which was in sharp contrast to the uraemic animals. A 10% loss of body weight was observed in these animals following subtotal nephrectomy, which probably reflected both the operative stress, and the reduced food intake which was apparent for the following two week period. However, for the remainder of the study, the food intake of both control animals and uraemic animals was similar, but there was a marked failure to gain weight by the uraemic groups. Therefore as the nutrient intake was similar for control and uraemic animals, and the relationship between total food intake and body fat found in the control animals was not apparent in the uraemic rats, the mechanism responsible for the reduced body fat must relate to the consequences of a reduced renal mass. Also the nature of the protein presented to the uraemic animals in the diet appeared to have no influence in determining body composition, as results obtained for both casein and soya fed nephrectomised animals were similar.

This proposition is supported by the relationship of body fat to the GFR that was documented in the uraemic rats, and there are a number of factors which may be responsible. The low levels of body fat imply a reduced energy store, which might result from insufficient calories
in the diet to sustain normal metabolism. However, in the normal animals both diets contain sufficient energy to promote normal growth and basal metabolism appears not to be altered by uraemia as Adelman and Holliday have documented a normal basal metabolic rate in the uraemic rat (Adelman R D, Holliday M A, 1977). Other alternatives could be related to the abnormalities of carbohydrate and lipid metabolism that are encountered in the uraemic state. Efficient fat deposition requires the functional integrity of action of both lipoprotein lipase and insulin, and the presence of circulating triglyceride rich particles. A state of insulin resistance is commonly found in renal insufficiency (DeFronzo R et al, 1981) as is a reduced activity of lipoprotein lipase (Roulet J et al, 1985) and it has been proposed that the low activity of this enzyme contributes to the poor nutritional state and lesser weight gain of uraemic animals (Roulet J et al, 1981). Alternatively, energy utilization may be impaired in uraemia, or the energy cost of real growth increased, as suggested by Adelman (1981). An increased energy requirement for protein synthesis in the uraemic rats may have been a contributing factor, as both groups of animals excreted significant quantities of protein in the urine. However no relationship between between body fat content and proteinuria could be found.

Whatever the mechanism responsible for the changes in body composition that were found, it appears that reduced renal function, and its attendant metabolic abnormalities, rather than poor nutrient intake, exert the major influence upon body composition.
SECTION 5

FURTHER STUDIES TO INVESTIGATE THE EFFECTS
OF DIFFERENT LEVELS OF DIETARY PROTEINS
UPON RENAL FUNCTION AND METABOLISM IN THE
SUBTOTALLY NEPHRECTOMISED RAT
INTRODUCTION

The major points that emerge from the results obtained in section 3 are that long term feeding of isonitrogenous casein and soya diets to subtotally nephrectomised rats produced marked differences in survival, renal hypertrophy, proteinuria and renal histological damage. Also, long term feeding of these diets to normal animals resulted in alterations in GFR and ERPF, but the short term feeding studies revealed no such difference.

Further examination of this phenomenon in this section has been directed to answer some of the questions posed by these findings. One of the potential mechanisms by which the observed changes were produced might be a direct extension of the "hyperfiltration hypothesis", if feeding the casein diet produced an exaggerated elevation of SNGFR and glomerular capillary plasma flow rate that occur at an early stage following subtotal nephrectomy. The results of Section 3 demonstrate that long term feeding of the casein diet did indeed produce changes in renal function in normal animals, but renal function studies were not performed at an early stage following subtotal nephrectomy.

As previously discussed, by feeding animals different dietary proteins, there inevitably follows the potential complicating issue of differences in digestion and absorption of nutrients from the diet, and as minor differences in the urea excretion rate of rats fed the two isonitrogenous diets were found, this point requires further investigation.

In order to resolve the unanswered questions, the study described in this section has been designed to investigate more fully the changes in renal function that occur following subtotal nephrectomy, by
performing serial measurements of creatinine clearance, as an index of GFR. Also, to further resolve the potentially complicating factor of differential nutrient absorption from the two different dietary protein sources, diets composed of casein and soya at both 24% and 12% inclusion levels have been employed.

Further indices of nutritional status have been examined in this section, as again it is important to ensure that dietary manipulation does not result in malnutrition, and this has been undertaken by examination of the plasma amino acid profile and determination of liver protein content.
Forty six rats (weight 160-215g) were randomly allocated into four experimental groups

Group A  n=11  
Group B  n=12  
Group C  n=12  
Group D  n=11

Conditions of animal housing were as previously described, and the animals were allowed free access to the standard laboratory diet. One week prior to the operative procedures, blood was obtained from the tail vein, under light ether anaesthesia, following an overnight fast, for determination of serum urea, creatinine, calcium, inorganic phosphate, cholesterol, triglyceride, insulin and glucose. The animals were then returned to the cages with continued free access to food and tap water.

One week later, renal mass was reduced by performing a right nephrectomy, and partial infarction of the left kidney as a one stage procedure, as previously described.

The following day, the animals were commenced on one of the four experimental diets. (Tables 2.1-2.5)  

Group A  24% Casein  
Group B  12% Casein  
Group C  24% Soya  
Group D  12% Soya

Food consumption was determined daily, and the animals weighed three times weekly, as previously described.
At two weekly intervals, the animals were placed in metabolism cages for a 24 hour period for urine collection, and the urine volume, urinary protein, urinary urea and creatinine measured. Also at these times, when the animals were removed from the metabolism cages, blood was obtained from the tail vein, under light ether anaesthesia, for determination of serum urea and creatinine. From these measurements, determinations of creatinine clearance, urea clearance and urea excretion could be made.

Animals were placed in the metabolism cages on six occasions, the ultimate urine collection being undertaken during the 12th week of the study.

During the experimental period, the general condition of the animals was closely monitored, as previously, and if the animals demonstrated signs of severe renal insufficiency, to prevent any undue suffering, the animal was sacrificed. The remnant kidney was removed at sacrifice, and placed in 10% formalin in 0.9% saline, for later histological evaluation.

The study was terminated during the 14th week following reduction of renal mass. The animals were fasted, but allowed water ad lib, for 18 hours prior to sacrifice. Under ether anaesthesia, the animals were sacrificed by percutaneous cardiac puncture, and 5-7mls blood samples obtained. One ml blood samples were placed in heparinised tubes, mixed and centrifuged at 3000rpm for 10 minutes. Aliquots of 0.4ml of plasma were deproteinised by mixing with an equal volume of sulphosalicylic acid. Following centrifugation for 10 minutes, the supernatant was aspirated, and stored at -20°C until amino acid analysis was undertaken. In addition to the four experimental groups, fasting plasma amino acid levels were determined in normal animals of
comparable weight, which were maintained upon the standard laboratory diet (n=15). The haematocrit of the final blood specimens was determined using microhaematocrit tubes, following centrifugation for 5 minutes (Hawksley Ltd). The remaining blood sample was allowed to clot at room temperature and centrifuged at 3000rpm for 15 minutes, and the serum aspirated. Aliquots of 0.5ml of serum were stored at -20°C until analysed for immunoreactive insulin. Determinations of serum urea, creatinine, albumin, calcium, inorganic phosphate, cholesterol, triglyceride and glucose were performed on the serum sample as previously described.

Immediately following exsanguination of the animal the abdominal cavity was exposed through a midline incision. The left remnant kidney was removed, dissected free of surrounding tissue, bisected, and placed in a pre-weighed container of 10% formalin. The container was subsequently reweighed to determine the weight of the kidney tissue.

The liver was next carefully dissected free from abdominal structures, and removed. A small portion (approximately 50mg) was removed from the inferior margin of the left lobe using a sharp scalpel, and placed on a small piece of aluminium foil. This was placed inside a 1.5ml plastic tube, the screw cap applied, and the whole immersed in liquid nitrogen. The specimen of liver was frozen within one or two minutes, and was subsequently stored at -70°C until determinations of tissue water and tissue protein were performed.

The remaining liver was then placed in a pre-weighed plastic container (approximately 10g) and the screw cap applied tightly. The whole was then reweighed to determine the liver weight.

Histological analysis of the coded sections of remnant renal
tissue of animals in all experimental groups, and those animals not completing the study, was performed as previously described.

RESULTS

1 FOOD CONSUMPTION

Food consumption of animals in the different dietary groups is depicted in fig 5.1.

During the first week following the introduction of the experimental diets and subtotal nephrectomy, the food intake of all groups was depressed. However, by week 2, the food intake of all groups had risen, and were not significantly different (Gp A, 13.3±0.3, Gp B, 13.0±0.7, Gp C, 14.8±0.6, Gp D, 14.8±1.0, g/day) (F ratio=1.586, p>0.20).

However, by week 4, the food consumption of group D (12% soya) had risen (19.8±3.5, g/day) and was significantly higher than other groups (F ratio=17.29, p<0.001), and remained so for the duration of the study. The food consumption of animals in the other groups remained stable.

2 BODY WEIGHT

The body weight of animals in the different dietary groups at the start of the study did not differ (Gp A, 192±3, Gp B, 184±5, Gp C, 184±3, Gp D, 189±3, g) (F ratio=1.15, p>0.50) (Fig 5.2). Two weeks following the reduction in renal mass, the body weight of animals in group A was significantly greater than other groups (Gp A, 202±3, Gp B, 181±6, Gp C, 187±2, Gp D, 179±3, g) (F ratio=8.57, p<0.01) however, the
Figure 5.1

FOOD CONSUMPTION OF SUBTOTALLY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS (MEAN±SEM)

Weeks

Food intake (g/day)
Figure 5.2

BODY WEIGHT OF SUBTOTALY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS (MEAN ±SEM)

![Graph showing body weight of subtotally nephrectomised rats fed different protein diets.](image)
perioperative weight loss sustained by animals in groups B, C and D had been regained by week 4.

Animals in all groups demonstrated a gradual increase in weight throughout the experimental period, but the body weight of rats in group A was consistently greater than that of group C until week 14 of the study, at which time the weights of all groups were equivalent (Gp A, 209±5, Gp B, 198±8, Gp C, 191±2, Gp D, 200±2,g) (F ratio=2.99, p>0.05).

At the end of the study period, the weight change over the preoperative weight was not significantly different in any of the groups (F ratio=0.91, p>0.05).

The body weights of animals at sacrifice, following withdrawal of food for 18 hours were all lower than when measured in the fed state, but again there was no difference between the groups (Gp A, 196±6, Gp B, 187±7, Gp C, 181±2, Gp D, 193±3,g) (F ratio=2.78, p>0.05).

3 MORTALITY
The survival of animals ingesting the casein diets at the end of the study was significantly lower than animals fed the soya diets (Gp A, 64%, Gp B, 58%, Gp C, 100%, Gp D, 91%, x²=8.62, p<0.05). Severe renal insufficiency was confirmed in all those animals which were sacrificed prior to the end of the study, by the markedly elevated serum urea determined at sacrifice (Gp A, 29.8±2.5, Gp B, 31.3±3.2,mmol/l)
Examination of the carcass revealed no other abnormality as a cause of demise other than renal insufficiency.
4 URINE VOLUME

The urine volume of animals within the different dietary groups remained stable throughout the experimental period, but animals in group A persistently excreted larger volumes of urine than animals in groups C and D (p<0.01, at all points) (table 5.1). Measurement of the urine volume of animals during the 12th week of the study, revealed an elevated value for animals in group A but there was no significant difference between the other groups (Gp A, 31.1±4.0, Gp B, 19.6±2.9, Gp C, 21.2±1.7, Gp D, 19.7±1.0, ml/24hrs) (F ratio=4.75, p<0.01).

5 URINE PROTEIN EXCRETION

All groups demonstrated a progressive rise in urinary protein excretion (Fig 5.3). Values determined for group A at week 2 following reduction in renal mass were significantly higher than other groups (F ratio=3.08, p<0.05), and the magnitude of rise in urinary protein excretion of group A remained significantly higher throughout the study. Animals in group B also excreted more urinary protein than groups C and D, which became significant at 8 weeks (F ratio=5.229, p<0.01) and remained so for the duration of the study. The magnitude in rise in protein excretion of groups C and D did not differ, and at week 12 both were significantly lower than groups A and B (Gp A, 98.7±34.0, Gp B, 63.8±17.7, Gp C, 30.4±5.1, Gp D, 35.5±6.7, mg/24hrs) (F ratio=3.777, p<0.02).

6 SERUM UREA AND UREA EXCRETION

There was no difference in serum urea between groups when determined one week prior to reduction of renal mass, (Gp A, 6.3±0.2, Gp B, 6.0±0.2, Gp C, 6.4±0.3, Gp D, 6.0±0.2, mmol/l) (F ratio=1.173, p>0.30). Two
### Twenty Four Hour Urine Volume (mLs) of Subtotally Nephrectomised Rats Fed Different Protein Diets (Mean±SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>WEEK 2</th>
<th>WEEK 4</th>
<th>WEEK 6</th>
<th>WEEK 8</th>
<th>WEEK 10</th>
<th>WEEK 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>32.3±2.5</td>
<td>27.0±2.9</td>
<td>28.4±2.0</td>
<td>27.4±3.4</td>
<td>28.7±2.4</td>
<td>31.1±4.0</td>
</tr>
<tr>
<td>B</td>
<td>24.9±1.7</td>
<td>22.9±2.6</td>
<td>22.9±1.3</td>
<td>23.1±3.2</td>
<td>22.8±2.6</td>
<td>19.6±2.9</td>
</tr>
<tr>
<td>C</td>
<td>18.7±2.0</td>
<td>15.1±1.5</td>
<td>17.0±1.5</td>
<td>16.4±1.6</td>
<td>18.9±1.7</td>
<td>21.2±1.7</td>
</tr>
<tr>
<td>D</td>
<td>16.4±0.9</td>
<td>17.1±1.7</td>
<td>17.9±2.1</td>
<td>18.3±1.2</td>
<td>19.2±1.4</td>
<td>19.7±1.0</td>
</tr>
</tbody>
</table>
weeks following the subtotal nephrectomy, values for group A were significantly higher than other groups (F ratio=28.65, p<0.001) and values for all groups remained stable until the termination of the study (Gp A, 11.9±1.3, Gp B, 9.4±0.7, Gp C, 9.2±0.2, Gp D, 8.7±0.5, mmol/l) (F ratio=4.053, p<0.05).

The urinary urea excretion (table 5.2) of animals fed the 24% protein diets were significantly higher than those rats fed the same dietary protein at the 12% level. Animals fed casein excreted significantly more urea than those rats fed soya at the same level of dietary protein intake (Gp A and Gp C, p<0.01, Gp B and Gp D, p<0.05, at all points). The differences in urea excretion were contributed to by both differences in urinary volume (Table 5.1) and also urinary urea concentration, values for groups A and D were significantly different from groups B and C (Gp A, 491±38, Gp B, 316±33, Gp C, 338±21, Gp D, 216±11, mmol/l determined at week 12) (F ratio=17.74 p<0.01).

7 CREATININE CLEARANCE

Serial measurements of creatinine clearance are depicted in fig 5.4. Values of groups A and B determined at two weeks following reduction in renal mass were significantly greater than groups C and D (F ratio=4.04, p<0.02), and the values determined for animals in group A were persistently greater than animals fed the soya diets (p<0.05, weeks 4 to 10). However, values of creatinine clearance determined during the 12th week of the study revealed all groups to be similar (F ratio=1.65, p>0.10).
Figure 5.3

URINARY PROTEIN EXCRETION OF SUBTOTALLY NEPHRECTOMISED RATS
FED DIFFERENT PROTEIN DIETS (MEAN±SEM)
### Table 5.2

**Urinary Urea Excretion Values (mmol/24hr) of Subtotally Nephrectomised Rats Fed Different Protein Diets (Mean±SEM)**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>WEEK 4</th>
<th>WEEK 6</th>
<th>WEEK 8</th>
<th>WEEK 10</th>
<th>WEEK 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13.5±2.6</td>
<td>13.9±1.2</td>
<td>11.1±0.9</td>
<td>13.9±0.5</td>
<td>14.4±0.9</td>
</tr>
<tr>
<td>B</td>
<td>5.6±0.5</td>
<td>5.7±0.4</td>
<td>5.5±0.5</td>
<td>6.6±0.5</td>
<td>5.6±0.7</td>
</tr>
<tr>
<td>C</td>
<td>5.4±0.4</td>
<td>6.1±0.3</td>
<td>6.7±0.2</td>
<td>6.9±0.4</td>
<td>6.8±0.3</td>
</tr>
<tr>
<td>D</td>
<td>3.6±0.4</td>
<td>4.0±0.4</td>
<td>4.1±0.3</td>
<td>4.3±0.2</td>
<td>4.4±0.2</td>
</tr>
</tbody>
</table>
There was no significant difference between any of the groups for the parameters of serum albumin, calcium or phosphate, either before (p>0.20, p>0.70, p>0.20) or after subtotal nephrectomy (p>0.20, p>0.30, p>0.15) (Table 5.3). However, the serum albumin determined at sacrifice was significantly lower for each group compared to values obtained at the beginning of the study (p<0.05, for all groups) as were values of serum calcium (p<0.001). There was no difference in the levels of serum phosphate before or after reduction in renal mass.

No significant difference was found between the groups at the start of the study for values of serum cholesterol (p>0.20) or serum triglyceride (p>0.15) (Table 5.4). At the end of the study, the serum cholesterol of group C animals was significantly lower than groups A and B (p<0.01), and values for animals in group D also showed this trend, but did not achieve significance (p>0.10). Serum cholesterol levels of groups A and B were higher at the end of the study, compared to initial values, but the difference did not attain significance (p=0.082, p=0.087, respectively), whilst values for group C at sacrifice were significantly lower than preoperative values (p<0.001). Cholesterol values for animals in group D showed no change (p=0.72).

A significant correlation was found to exist between urinary protein excretion, as documented during week 12 of the study, and the serum cholesterol determined at sacrifice (r=0.79, p<0.001).

Similarly, serum triglyceride values of animals in groups A and B at the end of the study were higher than groups C and D, but only for group C did the difference attain significance (p<0.01) and also, this
Figure 5.4

CREATININE CLEARANCE OF SUBTOTALY NEPHRECTOMISED RATS
FED DIFFERENT PROTEIN DIETS (MEAN±SEM)
Table 5.3
VALUES OF SERUM ALBUMIN, CALCIUM AND PHOSPHATE BEFORE SUBTOTAL NEPHRECTOMY AND AT SACRIFICE (MEAN±SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PRE OPERATIVE VALUES</th>
<th>VALUES AT SACRIFICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERUM ALBUMIN (g/l)</td>
<td>A 38.4±0.5</td>
<td>34.3±1.5</td>
</tr>
<tr>
<td></td>
<td>B 40.0±0.5</td>
<td>35.3±1.3</td>
</tr>
<tr>
<td></td>
<td>C 38.2±0.4</td>
<td>35.4±0.6</td>
</tr>
<tr>
<td></td>
<td>D 39.4±0.3</td>
<td>37.0±0.8</td>
</tr>
<tr>
<td>F RATIO</td>
<td>1.270</td>
<td>1.471</td>
</tr>
<tr>
<td>SERUM CALCIUM (mmol/l)</td>
<td>A 2.51±0.04</td>
<td>2.26±0.01</td>
</tr>
<tr>
<td></td>
<td>B 2.55±0.03</td>
<td>2.27±0.02</td>
</tr>
<tr>
<td></td>
<td>C 2.51±0.05</td>
<td>2.26±0.02</td>
</tr>
<tr>
<td></td>
<td>D 2.53±0.02</td>
<td>2.30±0.01</td>
</tr>
<tr>
<td>F RATIO</td>
<td>0.378</td>
<td>1.106</td>
</tr>
<tr>
<td>SERUM PHOSPHATE (mmol/l)</td>
<td>A 2.18±0.05</td>
<td>2.05±0.40</td>
</tr>
<tr>
<td></td>
<td>B 2.30±0.07</td>
<td>2.76±1.02</td>
</tr>
<tr>
<td></td>
<td>C 2.15±0.06</td>
<td>2.30±0.18</td>
</tr>
<tr>
<td></td>
<td>D 2.17±0.05</td>
<td>2.08±0.10</td>
</tr>
<tr>
<td>F RATIO</td>
<td>1.298</td>
<td>1.888</td>
</tr>
</tbody>
</table>
value was significantly lower than the preoperative level (p<0.01), but there was no significant difference for the other groups.

10 BLOOD GLUCOSE

Values determined for blood glucose are depicted in table 5.4. No significant difference was found between the groups consuming the different diets either before, or after, subtotal nephrectomy (p>0.30, p>0.30, respectively), and values determined at sacrifice were not different from preoperative levels within each group.

11 HAEMATOCRIT

No significant difference was found for values of haematocrit determined at sacrifice for any of the experimental groups (Gp A, 0.48±0.02, Gp B, 0.48±0.02, Gp C, 0.47±0.01, Gp D, 0.48±0.02) (F ratio=0.078, p=0.97).

12 SERUM IMMUNOREACTIVE INSULIN

The fasting serum levels of immunoreactive insulin determined at the start of the study in the normal animals did not differ between the groups (F ratio=2.25, p>0.20). Similarly, values determined in the fasting state at sacrifice did not differ between the groups (F ratio=2.64, p>0.20), and these values were not significantly different to those obtained at the start of the study (p>0.05, for all groups). No correlation was found between immunoreactive insulin and serum triglyceride levels or blood sugar, either preoperatively, or at sacrifice (Table 5.4).
Table 5.4
VALUES OF SERUM CHOLESTEROL, SERUM TRIGLYCERIDE, BLOOD SUGAR AND SERUM INSULIN, BEFORE SUBTOTAL NEPHRECTOMY AND AT SACRIFICE (MEAN±SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PRE OPERATIVE VALUES</th>
<th>VALUES AT SACRIFICE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F RATIO</td>
<td>F RATIO</td>
</tr>
</tbody>
</table>

**SERUM CHOLESTEROL mmol/l**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>VALUE</th>
<th>F RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.33±0.10</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2.44±0.08</td>
<td>1.55</td>
</tr>
<tr>
<td>C</td>
<td>2.52±0.07</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>2.61±0.12</td>
<td></td>
</tr>
</tbody>
</table>

**SERUM TRIGLYCERIDE mmol/l**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>VALUE</th>
<th>F RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.42±0.02</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.55±0.07</td>
<td>1.90</td>
</tr>
<tr>
<td>C</td>
<td>0.42±0.01</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.47±0.05</td>
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</tr>
</tbody>
</table>

**BLOOD SUGAR mmol/l**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>VALUE</th>
<th>F RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.0±0.3</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>6.7±0.4</td>
<td>1.22</td>
</tr>
<tr>
<td>C</td>
<td>6.2±0.2</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>6.7±0.2</td>
<td></td>
</tr>
</tbody>
</table>

**SERUM INSULIN µU/ml**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>VALUE</th>
<th>F RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11.2±0.4</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>10.5±0.5</td>
<td>2.25</td>
</tr>
<tr>
<td>C</td>
<td>9.5±0.7</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>11.9±0.8</td>
<td></td>
</tr>
</tbody>
</table>
13 **HISTOLOGICAL ANALYSIS**

Qualitatively, the lesions observed in the remnant renal tissue of animals in the different groups were identical to those previously described in section 3.

a) **Glomerular lesions**

The quantitative distribution of glomerular lesions of the surviving animals in the four experimental groups, and those animals that were sacrificed prior to the end of the study period, are shown in figure 5.5.

The incidence of both severe and moderate glomerulosclerosis in the renal tissue of those rats fed the casein diets were significantly higher than those rats fed the soya diets (p<0.01). However, no difference was found in the severity of renal damage between either groups A and B, or groups C and D. The renal tissue of those animals sacrificed prior to the end of the study contained significantly more abnormal glomeruli than the surviving animals (p<0.01).

b) **Tubular Lesions**

The incidence of tubular abnormalities found in the renal tissue is shown in figure 5.6. Again, tubular atrophy and tubular dilatation were seen more frequently in the casein fed rats compared to those animals fed soya. (Mean scores, tubular atrophy, Gp A, 2.29±0.29, Gp B, 1.57±0.20, Gp C, 0.58±0.19, Gp D, 0.60±0.16. Tubular dilatation, Gp A, 2.14±0.34, Gp B, 2.00±0.31, Gp C, 0.50±0.19, Gp D, 0.70±0.15) (Casein and Soya diets compared at both levels of protein intake, p<0.01).

There was no significant difference in the incidence of tubular abnormalities between groups A and B, or groups C and D.
RESULTS OF HISTOLOGICAL ANALYSIS OF GLOMERULAR LESIONS IN REMNANT KIDNEY OF SUBTOTALY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS (MEAN ±SEM)

Figure 5.5

Severe Glom. Sclerosis
MOD. Glom. Sclerosis

Percentage glomeruli affected

0 10 20 30 40 50 60 70 80 90 100

A B C D U

U = animals not completing the study
RESULTS OF HISTOLOGICAL ANALYSIS OF TUBULAR LESIONS IN REMNANT KIDNEYS OF SUBTOTALLY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS

Figure 5.6

Tubular Dilatation

Tubular Atrophy

U = Animals not completing the study

Grade

Grade
14 REMNANT KIDNEY MASS

The weight of the remnant renal tissue of animals in groups A and B fed the casein diet was significantly greater than that of those rats fed the soya diets (Gp A, 0.66±0.04, Gp B, 0.59±0.04, Gp C, 0.46±0.01, Gp D, 0.46±0.01, g/100g body weight) (p<0.005). The remnant kidney weight of rats ingesting the 24% casein diet was greater than those rats fed the casein at the 12% level, but the difference did not achieve significance (p>0.20). Values for animals in groups C and D did not differ.

A significant positive relationship was found between the quantity of protein excreted in the urine (as determined during week 12 of the study) and the remnant kidney weight (r=0.75, p<0.001) (fig 5.7).

15 PLASMA AMINO ACIDS

Median values of fasting plasma amino acids of subtotally nephrectomised rats and control rats are given in table 5.5 (Cystine, tryptophan and proline were not included in the total essential and non essential amino acid ratios, due to unavailability of results for all animals).

a) Plasma Essential Amino Acids

The individual plasma essential amino acids did not differ between the nephrectomised groups when compared at the same level of protein intake (p>0.05, for all essential amino acids). However, comparison of the results at the different levels of intake for the same protein, revealed the threonine level of groups C and D to differ (p<0.05). The total plasma essential amino acids (threonine, valine, methionine, isoleucine, leucine, phenylalanine, tyrosine, histidine, lysine) were not significantly different between the four groups (p>0.05). The only
Figure 5.7

RELATIONSHIP OF REMNANT KIDNEY WEIGHT AND PROTEINURIA, IN
SUBTOTALLY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS

Proteinuria (mg/24hrs)

Kidney weight (g/100g body weight)
difference between the nephrectomised rats and the control rats was the plasma threonine of animals in group D (p<0.05). The total essential amino acids of the control rats were similar to the nephrectomised animals. (Table 5.6)

b) Plasma non essential Amino Acids

Plasma glycine levels were significantly higher in animals fed the soya diets, at both 24% and 12% levels, compared to the casein fed rats (p<0.01) and values of animals in group D were significantly greater than group C (p<0.05). The plasma serine level of animals in group D was also significantly higher than the other nephrectomised groups (p<0.05). Values of the remaining nonessential amino acids did not differ between groups. The total plasma nonessential amino acids (serine, glycine, alanine, ornithine, arginine) of animals in group D were greater than other groups, and also the control group. (Table 5.6)

c) Plasma Amino Acid Ratios

The ratios of selected amino acids are given in table 5.6. Values derived for the EAA/NEAA ratio (essential/nonessential) revealed a significantly lower value for animals in group D, compared to other groups (F ratio=4.57, p<0.05). Groups A, B, C and the control animals did not differ (p>0.30).

A similar pattern was observed in the VIL/GAS ratio (valine + isoleucine + leucine/glycine + alanine + serine), in that values of group D animals were significantly lower than all other groups (F ratio=6.51, p<0.05).

Comparison of the V/G ratios (valine/glycine) revealed that for both casein and soya fed groups, animals fed the 12% protein diets were significantly lower than the 24% diets and control animals (F ratio=12.33, p<0.05), but comparison of values of the different diets
### Table 5.5

**RESULTS OF FASTING PLASMA AMINO ACID LEVELS FOR NORMAL RATS FED THE STANDARD LABORATORY DIET, AND SUBTOTALY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS (MEDIAN AND RANGE) (μmol/L)**

<table>
<thead>
<tr>
<th></th>
<th>Standard Laboratory Diet</th>
<th>24% Casein (pmol/L)</th>
<th>24% Soya (pmol/L)</th>
<th>12% Casein (pmol/L)</th>
<th>12% Soya (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>239 (171-330)</td>
<td>223 (155-234)</td>
<td>247 (188-274)</td>
<td>329 (189-477)</td>
<td>384 (220-554)</td>
</tr>
<tr>
<td>Valine</td>
<td>280 (261-369)</td>
<td>297 (197-309)</td>
<td>335 (252-396)</td>
<td>338 (222-373)</td>
<td>318 (143-388)</td>
</tr>
<tr>
<td>Cystine</td>
<td>59 (46-86)</td>
<td>100 (85-121)</td>
<td>59 (33-98)</td>
<td>72 (69-138)</td>
<td>95 (56-132)</td>
</tr>
<tr>
<td>Methionine</td>
<td>51 (39-67)</td>
<td>53 (38-80)</td>
<td>53 (36-71)</td>
<td>52 (26-66)</td>
<td>62 (41-72)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>149 (121-189)</td>
<td>167 (143-192)</td>
<td>168 (123-191)</td>
<td>179 (82-187)</td>
<td>176 (77-215)</td>
</tr>
<tr>
<td>Leucine</td>
<td>229 (164-273)</td>
<td>243 (225-251)</td>
<td>271 (195-288)</td>
<td>259 (140-309)</td>
<td>249 (120-294)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>74 (31-127)</td>
<td>88 (67-94)</td>
<td>81 (48-122)</td>
<td>66 (13-111)</td>
<td>74 (54-137)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>67 (53-79)</td>
<td>66 (42-93)</td>
<td>72 (58-104)</td>
<td>75 (40-87)</td>
<td>69 (47-83)</td>
</tr>
<tr>
<td>Lysine</td>
<td>442 (343-570)</td>
<td>472 (409-545)</td>
<td>458 (329-507)</td>
<td>426 (316-920)</td>
<td>443 (242-585)</td>
</tr>
<tr>
<td>Histidine</td>
<td>82 (65-101)</td>
<td>100 (76-109)</td>
<td>100 (88-115)</td>
<td>102 (72-107)</td>
<td>99 (57-103)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>224 (20-323)</td>
<td>193 (150-222)</td>
<td>182 (53-277)</td>
<td>170 (28-233)</td>
<td>193 (57-345)</td>
</tr>
<tr>
<td>Alanine</td>
<td>491 (253-557)</td>
<td>582 (401-707)</td>
<td>581 (373-652)</td>
<td>574 (321-840)</td>
<td>638 (281-885)</td>
</tr>
<tr>
<td>Glycine</td>
<td>284 (200-388)</td>
<td>233 (151-265)</td>
<td>338 (212-410)</td>
<td>286 (153-499)</td>
<td>532 (307-711)</td>
</tr>
<tr>
<td>Serine</td>
<td>234 (195-280)</td>
<td>226 (173-256)</td>
<td>234 (178-298)</td>
<td>268 (184-364)</td>
<td>360 (247-479)</td>
</tr>
<tr>
<td>Ornithine</td>
<td>138 (27-255)</td>
<td>141 (117-166)</td>
<td>129 (73-154)</td>
<td>112 (49-155)</td>
<td>122 (82-185)</td>
</tr>
<tr>
<td>Arginine</td>
<td>140 (52-186)</td>
<td>172 (87-222)</td>
<td>155 (106-244)</td>
<td>173 (83-245)</td>
<td>167 (128-244)</td>
</tr>
<tr>
<td>Proline</td>
<td>153 (111-175)</td>
<td>167 (116-188)</td>
<td>146 (111-244)</td>
<td>174 (102-199)</td>
<td>187 (114-211)</td>
</tr>
</tbody>
</table>
at the same level of protein intake revealed only groups B and D to differ (p<0.04).

As the absolute quantity of plasma amino acids of group D was significantly greater than other groups, further analysis of the pattern of amino acids was obtained by expression of the amino acid concentration as a percentage of the total plasma amino acids, shown in table 5.7. Analysis of the results in this way shows the control rats, and the nephrectomised groups fed 24% protein diets not to differ. However, the branched chain amino acids (valine, leucine, isoleucine), and histidine of group D animals were all lower than rats fed the 24% diets, whilst glycine, serine and threonine were all significantly elevated.

16 LIVER WEIGHT, LIVER WATER AND PROTEIN CONTENT

Values for the liver weight, percentage water and protein content of animals in the four experimental groups are given in table 5.8.

No significant difference was found between any of the groups for determinations of liver weight (F ratio=1.59, p>0.10) liver protein content (F ratio=1.17, p>0.10) or liver tissue water (F ratio=1.01, p>0.20).
VALUES OF TOTAL PLASMA ESSENTIAL AND NON ESSENTIAL AMINO ACIDS, AND SELECTED AMINO ACID RATIOS, FOR NORMAL RATS FED THE STANDARD LABORATORY DIET AND SUBTOTAIIY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS (MEAN±SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TOTAL ESSENTIAL AMINO ACIDS (µmol/L)</th>
<th>TOTAL NON ESSENTIAL AMINO ACIDS (µmol/L)</th>
<th>EAA/NEAA</th>
<th>VIL/GAS</th>
<th>V_G</th>
<th>Phe/Tyr</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1600±69</td>
<td>1298±86</td>
<td>1.25±0.06</td>
<td>0.70±0.04</td>
<td>1.33±0.12</td>
<td>0.95±0.15</td>
</tr>
<tr>
<td>B</td>
<td>1739±162</td>
<td>1383±170</td>
<td>1.20±0.12</td>
<td>0.60±0.06</td>
<td>0.94±0.10</td>
<td>1.04±0.11</td>
</tr>
<tr>
<td>C</td>
<td>1664±35</td>
<td>1398±48</td>
<td>1.21±0.05</td>
<td>0.69±0.04</td>
<td>1.03±0.08</td>
<td>1.01±0.10</td>
</tr>
<tr>
<td>D</td>
<td>1785±98</td>
<td>1830±98</td>
<td>0.98±0.04</td>
<td>0.48±0.02</td>
<td>0.60±0.04</td>
<td>0.94±0.07</td>
</tr>
<tr>
<td>Standard Laboratory Diet</td>
<td>1596±42</td>
<td>1233±54</td>
<td>1.32±0.05</td>
<td>0.72±0.03</td>
<td>1.08±0.05</td>
<td>1.00±0.11</td>
</tr>
</tbody>
</table>
Table 5.7

VALUES OF INDIVIDUAL FASTING PLASMA AMINO ACIDS, AS A PERCENTAGE OF TOTAL PLASMA AMINO ACIDS FOR NORMAL RATS FED THE STANDARD LABORATORY DIET AND SUBTOTALY NEPHRECTOMISED RATS FED DIFFERENT PROTEIN DIETS (MEAN±SEM)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Standard Laboratory Diet</th>
<th>24% Casein</th>
<th>24% Soya</th>
<th>12% Casein</th>
<th>12% Soya</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>8.9±0.3</td>
<td>7.2±0.3</td>
<td>7.8±0.3</td>
<td>9.7±0.8</td>
<td>10.9±0.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Valine</td>
<td>10.3±0.3</td>
<td>9.8±0.4</td>
<td>10.7±0.5</td>
<td>9.6±0.8</td>
<td>8.6±0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.9±0.1</td>
<td>1.9±0.2</td>
<td>1.8±0.1</td>
<td>1.5±0.2</td>
<td>1.7±0.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.3±0.1</td>
<td>5.8±0.4</td>
<td>5.4±0.2</td>
<td>4.8±0.4</td>
<td>4.6±0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Leucine</td>
<td>8.2±0.2</td>
<td>8.4±0.4</td>
<td>8.5±0.3</td>
<td>7.3±0.6</td>
<td>6.7±0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.7±0.3</td>
<td>2.8±0.2</td>
<td>2.6±0.2</td>
<td>1.9±0.2</td>
<td>2.2±0.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.4±0.1</td>
<td>2.4±0.2</td>
<td>2.4±0.1</td>
<td>2.1±0.1</td>
<td>1.9±0.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lysine</td>
<td>16.6±0.7</td>
<td>16.5±1.1</td>
<td>14.5±0.5</td>
<td>15.9±2.3</td>
<td>12.5±0.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.9±0.1</td>
<td>3.3±0.1</td>
<td>3.3±0.1</td>
<td>2.8±0.1</td>
<td>2.5±0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>4.6±0.7</td>
<td>6.3±0.7</td>
<td>5.7±0.7</td>
<td>5.0±0.4</td>
<td>5.7±0.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>Alanine</td>
<td>15.5±0.7</td>
<td>19.4±0.8</td>
<td>18.1±0.8</td>
<td>18.3±1.3</td>
<td>17.1±1.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Glycine</td>
<td>9.8±0.4</td>
<td>7.6±0.6</td>
<td>10.7±0.5</td>
<td>11.1±1.3</td>
<td>14.8±0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serine</td>
<td>8.5±0.3</td>
<td>7.6±0.4</td>
<td>7.7±0.3</td>
<td>8.0±0.3</td>
<td>10.3±0.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ornithine</td>
<td>4.9±0.5</td>
<td>4.9±0.6</td>
<td>3.9±0.3</td>
<td>3.4±0.3</td>
<td>3.6±0.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.7±0.4</td>
<td>5.3±0.8</td>
<td>5.2±0.4</td>
<td>5.1±0.5</td>
<td>5.1±0.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Proline</td>
<td>5.2±0.3</td>
<td>5.1±0.7</td>
<td>5.2±0.5</td>
<td>4.7±0.7</td>
<td>5.0±0.3</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
Table 5.8

VALUES OF LIVER WEIGHT, LIVER WATER AND LIVER PROTEIN OF SUBTOTALY NEPHRECTOMISED RATS FED DIFFERENT DIETS (MEAN±SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>LIVER WEIGHT (g/100g body weight)</th>
<th>PERCENTAGE WATER</th>
<th>LIVER PROTEIN (g/100g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.81±0.14</td>
<td>68.4±0.4</td>
<td>0.78±0.05</td>
</tr>
<tr>
<td>B</td>
<td>2.74±0.11</td>
<td>68.5±0.4</td>
<td>0.79±0.03</td>
</tr>
<tr>
<td>C</td>
<td>2.69±0.05</td>
<td>67.7±0.6</td>
<td>0.73±0.02</td>
</tr>
<tr>
<td>D</td>
<td>2.70±0.05</td>
<td>68.2±0.4</td>
<td>0.77±0.02</td>
</tr>
</tbody>
</table>
DISCUSSION

The investigations described in Section 5 were designed to investigate the effects of different quality and quantity of dietary protein upon renal function and various metabolic parameters in the subtotally nephrectomised rat.

The results of animals fed the 24% protein diets are in accord with those previously described in Section 3, in that the soya feeding ameliorates the severity of the induced renal lesion compared to casein fed animals. The 12% casein diet was also chosen in order to further investigate one of the potential factors responsible for the disparate outcome of animals fed the 24% protein diets. By virtue of the nature of the diets, the absolute quantity of amino acids absorbed from the soya diet may have been marginally lower than the casein diet, and so by reducing the quantity of casein presented to the animal by 50% it was reasoned that the effects of protein quality might be more fully investigated. Indirect confirmation of this is shown by the broad similarity of urinary urea excretion throughout the study, therefore the response to subtotal nephrectomy of groups B and C would reflect more the balance of amino acids in the diet.

The results of these studies show that those animals fed the soya diets, persistently develop less renal damage as documented by proteinuria and renal histological changes compared to the casein fed rats, which is evident at either level of protein intake. However, the mortality of animals fed the same protein source at different levels was the same, which may appear to be in conflict with previous observations, but this may be reconciled to some extent by consideration of the study duration. Although not strictly comparable
with investigations performed by other laboratories, due to differences in strain and sex of rat, dietary protein source and operative procedure, previous studies have shown that the beneficial effect, in terms of mortality in subtotally nephrectomised rats, fed two levels of protein similar to this study, have only become apparent after a period of 20 to 26 weeks (Kenner C H et al, 1975, Kleinknecht C et al, 1979, El Nahas A M et al, 1983). In this study, the nature of the protein fed appears to be more influential upon the outcome of partially nephrectomised rats rather than the quantity. Despite the similarity of mortality rates examination of other parameters indicative of the severity of the renal lesion, such as proteinuria and histological score, reveal that casein feeding at the higher level tended to produce more severe lesions than those animals fed the 12% diet. This finding is consistent with previous investigations (Chanutin A, Ludewig S, 1936, Farr L E, Smadel J, 1939). However, it is not replicated by groups C and D fed the soya diets. Also the degree of renal hypertrophy shows a similar trend.

Creatinine clearance is considered a relatively insensitive measure of glomerular filtration rate in the rat, mainly due to altered tubular handling of creatinine, which is dependent upon the serum concentration (Namnum P et al, 1983). In the conscious rat, at normal serum creatinine concentrations, creatinine clearance underestimates glomerular filtration rate by approximately 30%, due to tubular reabsorption of creatinine, but at higher levels net tubular secretion of creatinine occurs, resulting in an overestimate of GFR, as determined by inulin clearance. The serum creatinine levels of animals in this study did not differ significantly, and the assumption therefore is made that tubular creatinine handling is equivalent in all
groups, and that the creatinine clearance is a valid reflection of GFR.

Values of creatinine clearance determined in the casein fed animals at two weeks following reduction in renal mass did not differ but both groups were significantly greater than the soya fed animals. However values determined ten weeks later show all four groups of surviving animals to have a similar creatinine clearance. This finding is similar to that described in Section 3, in that despite more severe renal damage in the 24% casein fed group, as indicated by histological analysis and urinary protein excretion, the glomerular filtration rates are equivalent to the soya fed group. A reduction of dietary protein by 50% produced no significant reduction in creatinine clearance, when determined prior to sacrifice, but those groups did tend towards lower values than the 24% fed animals during the middle part of the study.

The observations of the initially higher creatinine clearance and progressive proteinuria in the casein fed rats might well be accommodated into the hyperfiltration postulate of Brenner. And again, it might also be proposed from the equivalent values of creatinine clearance and disparate levels of renal histological damage, that the filtration rate of each functioning nephron unit of the casein fed rats was greater than those animals fed the soya diets. These results suggest that the higher glomerular filtration rate induced in the remnant kidneys of the casein fed rats, documented at an early stage following subtotal nephrectomy, was responsible for the more severe renal damage sustained by these groups. However, association is not necessarily causation, and discussion of other potentially important factors is therefore warranted.
The role of phosphorus intake in the progression of renal disease is a subject of some debate, and so to exclude this potentially variable factor, all diets were formulated to contain the same quantity of phosphorus, (0.5%), and equivalent calcium content (1.0%). In the uraemic rat, the serum phosphorus concentration correlates well with the phosphorus content of the diet (Laouari D et al, 1982) and the serum inorganic phosphate determined at sacrifice revealed no significant difference between the experimental groups. Similarly, serum calcium determinations were all equivalent at sacrifice, and from these observations it can be assumed that inequalities in phosphorus intake were not responsible for the differences produced by the experimental diets.

Systemic hypertension is also known to be an important factor in the progression of renal disease. Measurements of blood pressure were not performed in this study, but the results obtained in Section 3, demonstrate that no difference was apparent in the systemic blood pressure of the subtotally nephrectomised rats fed either casein or soya as a protein source.

The levels of dietary protein chosen in the studies described were all above the recommended minimum protein intake (12%) to support growth in the laboratory rat (Nat Acad Sci Rep 1972). This is an important consideration, as a number of previous studies have documented the beneficial effect of low protein feeding in uraemic rats, but have employed diets extremely low in protein. The benefits of a reduced, but adequate, protein intake are well recognised in this model of progressive renal disease, but chronic malnutrition induced by inadequate nutrient intake is a poor trade off for preservation of renal function. As shown in section 3, a reduced renal mass is
associated with marked changes in body composition, and because of this, examination of some indices of nutritional status were performed, to ensure nutritional equivalence of the diets used.

The plasma amino acid profile is one such index, and is commonly deranged in chronically uraemic states. This is associated with alterations in protein metabolism but the mechanisms responsible are ill defined (Wang M et al, 1976, Kopple J D, 1978). Investigations directed towards elucidating the factors responsible for this altered state of amino acid metabolism have revealed many abnormalities, including reduced tissue amino acid uptake due to peripheral insulin resistance (Arnold W, Holliday M, 1979), a circulating inhibitor to protein synthesis (Cernacek P et al, 1982), hyperparathyroidism (Garber A J, 1983) and changes in polysome-m-RNA function (Grossman S et al, 1977, Zern M A, 1984).

Characteristic alterations in the pattern of plasma amino acids in advanced uraemia in both man and the rat include depression of the essential amino acids, particularly the branched chain group, and elevations in the nonessential amino acids (Wang M et al, 1976, Kopple J D, 1978). These changes are also characteristic of protein malnutrition (Waterlow J, Alleyne G O, 1971, Waterlow J, 1969, Arroyave G, 1970) and probably some of the abnormalities of the uraemic plasma amino acid profile may well be attributed to protein malnutrition (Kopple J D, 1978). The branched chain amino acids (BCAA) are intimately associated with skeletal muscle metabolism, and in states of malnutrition, and uraemia, low levels of BCAA, especially valine, are found. The pathogenesis of this abnormal pattern is unclear, but a reduced total body valine pool (Jones M E et al, 1978) and lower rates of skeletal muscle release (Alvestrand A et al, 1983) are amongst the
potential factors responsible. Whatever the precise mechanism for this abnormal pattern of plasma BCAA found, it is a sensitive index of the state of protein nutrition (Arroyave G, 1970). Essential amino acid supplements have been shown to improve the distribution of both plasma and intracellular amino acids (Alvestrand A et al, 1978) whilst maintenance of rats on inadequate protein diets (5% casein) will distort the plasma amino acid profile even further (Wang M et al, 1976). In comparing the nutritional qualities of differing protein diets, the pattern of amino acids, as Waterlow indicates (Waterlow J, Alleyne G O, 1971) is a superior index of amino acid metabolism, rather than absolute values.

Those animals fed the 12% soya diet demonstrated reductions in some essential amino acids, and elevations in nonessential amino acids, leading to distortion of the EAA/NEAA, VIL/GAS and V/G ratios, which separated those animals from the other nephrectomised groups and the normal animals. A higher food intake was also evident in group D animals, and this can be reconciled by the observation that a reduction in dietary protein to a marginal level is often accompanied by an elevation in food intake (Mercer L et al, 1981). Taken together, these findings indicate that the 12% soya diet was an inferior nutrient source compared to the other diets, and indeed this is probably a reflection of the low sulphur containing amino acid content in that diet, which was present at a marginal level required for maintenance only. An alternative interpretation might suggest a more severe degree of renal insufficiency in those animals, but this group sustained less renal damage, as judged from the histological score, and degree of proteinuria. Also, elevation of the protein intake of soya protein to
24% resulted in plasma amino acid profiles similar to the other nephrectomised groups, and the normal control animals.

No significant differences were found in the pattern of plasma amino acids between the normal animals and groups A, B and C, however, the EAA/NEAA and VIL/GAS ratios were marginally superior in the normal animals, and this is a reflection of the moderate degree of renal insufficiency of the surviving animals. The sensitivity of the plasma amino acid profile as a reflection of nutritional status is well documented, and is further evidenced in this study, but examination of other nutritional indices, such as body weight, serum albumin and liver protein content, revealed no significant differences between the groups. This can be reconciled by the relative insensitivity of liver protein in reflecting nutritional status (Waterlow J, 1969), and the changes in albumin synthesis and catabolism that occur in renal insufficiency similarly result in the relative insensitivity of this parameter (Coles G et al, 1970, Bianchi R et al, 1978).

An important point to emerge from these results is the degree of renal hypertrophy and proteinuria in rats fed the 12% soya diet was significantly lower than the casein fed animals, and it may be argued that this was a direct result of inadequate nutrition. However, increasing the protein content of the soya diet to 24%, to produce a nutritionally equivalent diet to the casein groups, produced no additional increment in proteinuria, renal hypertrophy, renal histological damage or mortality. This lends further support to the hypothesis that the dietary balance of amino acids may be an important factor in the pathogenesis of the induced renal disease in this model.

In addition to the changes in amino acid and protein metabolism
that are evident in uraemia, abnormalities of lipid metabolism also
commonly prevail. Uraemic hypertriglyceridaemia is thought to
relate to defective clearance of lipid rich particles from the
circulation. Abnormalities relating to this include functional
disturbances in tissue and plasma lipase activity (Mordasini F et al,
1977, Kraemer F et al, 1982) a state of peripheral post receptor
insulin resistance (Smith D, De Fronzo R, 1982) and selective

Hyperinsulinaemia is commonly found in uraemic man, but in the uraemic
rat it appears a more variable phenomenon, as both elevated (Maloff B
et al, 1983) and depressed levels (Roullet J et al 1985) have been
recorded. In this study, a reduction in renal mass appeared to
influence little the circulating levels of insulin, however changes in
the pattern of serum lipids were evident.

The hypocholesterolaemic and hypotriglyceridaemic action of soya
protein is a well recognised, but poorly understood, phenomenon.
Implicated causal factors include the arginine/lysine ratio of the
dietary protein (Sugano M et al, 1984), the dynamics of absorption and
biliary excretion of cholesterol and sterols (Vahouny G et al, 1984,
Nagata Y et al, 1982) and hepatic triglyceride and cholesterol output
(Sugano M et al, 1982). In addition to the influence of dietary
protein and renal function upon serum lipids, the quantity of food
taken by the animals has also been shown to exert a positive influence
upon the serum level of cholesterol and triglyceride (Liepa G et al,
1980). Interpretation of the differences found in the levels of serum
lipids between the four groups is therefore difficult, and further
compounded by the positive influence of proteinuria upon the regulation
of hepatic cholesterol output and serum cholesterol levels (Marsh J, Sparks C, 1979).

The hypocholesterolaemic action of the soya diet in normal animals was documented in section 3, and in these experiments the serum lipid levels of the casein fed animals are elevated compared to the soya fed group. The hypolipidaemic action of the soya protein appears most marked in the 24% protein fed group, and the slightly higher values found in the 12% protein group may well be a reflection of the significantly higher food intake of those animals. Also, the magnitude of proteinuria may be influential in determining the serum cholesterol levels, as shown by the correlation of these two parameters.

The role of lipid abnormalities in renal disease does merit some discussion, as a new approach has recently been suggested whereby changes induced in lipoprotein metabolism consequent upon glomerular injury, may themselves be responsible for perpetuation of mesangial cell damage and progressive glomerulosclerosis (Moorhead J et al, 1982). Much of the evidence for this hypothesis is indirect, but some corroboration has come from investigations showing that correction of hyperlipidaemia in the unilaterally nephrectomised obese Zucker rat, by dietary eicosapentaenoic acid and docosahexaenoic acid supplements, appear to reduce the spontaneous renal disease which develops in this model (Ando A et al 1986). Unfortunately, a simple cause and effect hypothesis is not straightforward, as alterations in dietary fatty acid intake are known to exert marked changes in prostaglandin and thromboxane metabolism (Croft K et al, 1984) which themselves are potent modulators of renal function, and almost certainly involved in modulation of the renal response to injury (Barcelli U et al, 1982).
Whether the changes in serum lipids observed in this study are merely attendant consequences of the different dietary proteins fed to the animals, or in fact assume a more important role in the pathogenesis of the development of the renal lesion, is a question which at present cannot be answered.
SECTION 7

CONCLUSIONS
CONCLUSIONS

The experiments presented in this thesis, were designed to investigate an observation made some fifty years ago by one of the early investigators of renal pathophysiology of this century. The statement made by Chanutin, that "the effect of dietary protein on the kidney, therefore, varies with the type of protein fed and with the relative renal reserve" has received little previous investigation, despite its potential importance in relation to the pathophysiology of renal disease (Chanutin A, Ludewig S, 1936). In addition to the academic importance of such a proposition, recent observations of the role of diet in the progression of experimental and human renal disease, and the diverse nature of dietary protein consumed, both by animals and man, illustrates the relevance of further investigation into this observation.

The studies described were designed to investigate the effects of different sources of dietary protein upon aspects of renal function, progression of renal disease, and upon the composition of the animal as a whole, both in the normal and subtotally nephrectomised rat. In this regard it is of paramount importance, when investigating the effects of diet upon any parameter, to ensure both the nutritional adequacy and equivalence of the diets employed. The results obtained in section 5 illustrate this point, that despite the amelioration of renal lesions developing in those animals fed the lower soya protein diet, discrepancies in the amino acid profiles of this group cast some doubt upon the exact nutritional equivalence of that diet compared to others used.
The major observations of the effect of casein and soya based diets upon normal renal function, and the progression of experimental renal disease, made in these studies, illustrate the importance of diet in renal pathophysiology. The studies described were not designed to examine the putative mechanisms by which these differences occurred, but some discussion regarding this is needed.

The "hyperfiltration hypothesis", as proposed by Brenner and colleagues, could well be applied to account for the differences in functional and structural renal damage that were found following subtotal nephrectomy. The influence of the casein diets upon renal blood flow and glomerular filtration rate in normal long term fed animals, and the initially higher creatinine clearance of subtotally nephrectomised rats, are points which would support the application of such a hypothesis. However as initially discussed, it has recently been appreciated that a number of therapeutic manipulations, such as the dietary lipid content, renal prostaglandin modulation, manipulation of the renin-angiotensin system and haemostatic modulation, can all exert an ameliorative effect upon experimentally induced renal disease. A "final common pathway" of haemodynamic modulation in the evolution of glomerulosclerosis has been proposed as a tentative mechanism, but to consider this as the sole possible effector mechanism of glomerular damage would be to ignore the accumulating evidence suggesting other possible influencing factors.

Amongst these is the influence of dietary lipid in experimental glomerulosclerosis. The mechanism responsible for this observation is obscure, but the known modulatory effect of soya protein upon lipid metabolism, and the supporting evidence presented in this study, might suggest a causal link with the renal pathophysiological change. Also,
the prostaglandin group of compounds, intimately involved in the regulation of glomerular haemodynamics, are cyclic derivatives of unsaturated fatty acids, and modulation of prostaglandin metabolism can be achieved by dietary lipid manipulation. Therefore, speculation that the effects of the diets observed were in fact mediated via changes in lipid metabolism produced by the dietary proteins, is not without support from the results presented.

In addition to the secondary changes observed in lipid metabolism, by virtue of the different dietary proteins, the balance of amino acids consumed by the experimental animals was dissimilar. A single study undertaken by Kaysen and Kropp (1983) suggested that manipulation of the dietary content of a single amino acid was sufficient to alter the course of experimentally induced renal disease. The mechanism by which this occurred is purely speculative, but the results presented do support this observation. However, like that study, if the mechanisms are solely attributable to the amino acid balance, the manner by which the observed differences were produced again remains elusive.

The experiments presented in this thesis were designed primarily to investigate the effect of different dietary proteins upon the course of the renal lesion in the subtotally nephrectomised rat. The conclusions reached are that the nature of the diet does appear to be influential in the pathophysiology of this model, and that long term maintenance upon these diets can modulate normal renal function.

Whether a primary haemodynamic basis for the observed changes is important, and if changes in lipid metabolism play an integral role, or if both observations are merely attendant consequences of the different diets, without any direct effect responsible for the observed differences, are lines for further investigation.
SECTION 8

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J Clin Invest 73: 1167-1174
STUDIES OF THE EFFECT OF DIET IN EXPERIMENTAL RENAL DISEASE

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Modulation of renal function and growth by the quantity of dietary protein has long been recognised, but its influence upon the course of experimental renal disease only more recently appreciated. The studies described were designed to investigate the effect of different dietary proteins, casein and soya, in the modulation of renal function in the normal rat, and their effect upon the course of experimentally induced renal insufficiency in the subtotally nephrectomised rat.

Short term feeding studies (6 weeks) of isonitrogenous (24% protein) casein and soya diets produced no difference in glomerular filtration rate (GFR) or effective renal plasma flow (ERPF) in normal rats. However, high protein feeding (48% casein) induced renal hypertrophy and elevations in GFR and ERPF. In contrast, long term feeding studies (18 weeks) of the casein diet resulted in elevated levels of GFR and ERPF compared to an isonitrogenous (24% protein) soya diet. Maintenance of subtotally nephrectomised rats upon soya protein diets resulted in marked amelioration of induced renal disease, in terms of mortality, proteinuria and renal histological damage, when compared to rats maintained upon isonitrogenous 12% and 24% casein diets. Analysis of body composition revealed the carcasses of subtotally nephrectomised rats to be composed of significantly less fat, and have a greater water content than normal animals. This reduction in body fat was proportional to the degree of renal dysfunction, rather than the dietary protein source. Plasma amino acids of subtotally nephrectomised rats fed casein and 24% soya diets were similar, but elevated levels of plasma non essential amino acids were found in rats fed 12% soya. Reduced serum lipid levels were found in both normal and subtotally nephrectomised rats fed soya diets. Potential mechanisms involved in the evolution of renal disease and the role of dietary proteins are discussed.