# Effects of Sterile Vesicoureteric Reflux on Renal Growth and Function

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## ABSTRACT

Vesicoureteric reflux (VUR) is predominantly a disorder of childhood associated with urinary infection and renal scarring. It is recognised that, in the absence of urinary infection, scarring can be avoided despite continuing VUR; however, the effects of VUR *per se* on the growing kidney are unknown and this question formed the primary concern of this thesis.

The effects of sterile VUR on renal growth and function in the absence of scarring were investigated in young pigs from soon after birth to about 6 months of age. Two parallel experiments were performed in pig models with either a solitary kidney following unilateral nephrectomy (**Experiment I**) or with paired kidneys (**Experiment II**). In both experiments the effects of VUR were examined in the presence of both normal and abnormal bladder function by including pigs with and without bladder outflow obstruction, as well as appropriate 'non-refluxing' controls.

Bladder function was assessed by urodynamic studies. Renal growth was measured from the weight of the kidneys at the end of the study. Renal function was measured by $^{51}$Cr-Edetic acid glomerular filtration rate (GFR), urinary concentrating ability and renal uptake of $^{99m}$Tc-dimercaptosuccinic acid (DMSA).

Regression analysis was applied separately to the results from each experiment. There was no interaction between VUR and bladder function on any outcome measure and for each of them the 95% confidence intervals were calculated for the effects of VUR.

In **Experiment I**, kidney weight and all parameters of renal function were examined. There was no significant effect of VUR on renal growth or GFR. The renal uptake of $^{99m}$Tc-DMSA was reduced by VUR but was significant only at the 5% level. Urinary concentrating ability was significantly impaired by VUR ($P<0.01$).

In **Experiment II** the effects of VUR on kidney weight and on uptake of $^{99m}$Tc-DMSA were compared in pigs with and without unilateral VUR. There was no significant effect of VUR on relative uptake of $^{99m}$Tc-DMSA or on relative kidney growth.

# PREFACE

This thesis comprises four sections. The clinical relevance of whether or not vesicoureteric reflux (VUR) *per se* affects the kidney and the necessity for an experimental study are revealed in the introductory Section I, which includes a review of the clinical problem of VUR. This is followed in Section II by a description of the pig model with particular regard to comparative aspects of pig and human renal growth and function as well as the unique suitability of the porcine urinary tract for an investigation of VUR. In Section III, as a preliminary to the formal investigation, the outcome measures used to assess the effects of VUR on the kidney, and any experiments which support their use, are described and discussed. The experimental investigation of the effects of VUR on renal growth and function are presented in Section IV.

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THE END

**ENCLOSURES**

1. High pressure sterile vesicoureteral reflux and renal scarring: An experimental study in the pig and minipig. Ransley,P.G., Risdon R.A. and Godley M.L. Reprint from: Contributions to Nephrology, 39, 320-343, (Karger, Basel) 1984.

2. Effects of vesicoureteric reflux on renal growth and function as measured by GFR, plasma creatinine and urinary concentrating ability. An experimental study in the minipig. Ransley P.G., Risdon R.A. and Godley M.L. Reprint from: British Journal of Urology, 60, 193-204, 1987.

3. Effect of unilateral vesicoureteric reflux on renal growth and the uptake of $^{99m}$Tc DMSA by the kidney. An experimental study in the minipig. Godley M.L., Risdon R.A. and Ransley P.G. Reprint from British Journal of Urology, 60, 340-347, 1989.

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"The time has come," the Walrus said,
"To talk of many things:
Of shoes - and ships - and sealing wax -
Of cabbages - and kings -
And why the sea is boiling hot -
And whether pigs have wings."

Lewis Carroll
*"Through the Looking-Glass"*

# Section I

# Introduction

CHAPTER 1

# VESICOURETERIC REFLUX AND RENAL DAMAGE

## 1.1 Vesicoureteric Reflux as an Anomaly

Vesicoureteric reflux (VUR) is the patho-physiological consequence of disordered anatomy and function at the ureterovesical junction.

The ureterovesical junction forms the divide between the upper and lower urinary tracts. The upper urinary tract is of small capacity but allows the kidney to excrete urine continuously at low pressure and to deliver it intermittently into the bladder by frequent ureteric peristalsis[1]. By comparison, the lower urinary tract is a high capacity, low pressure storage system which generates high pressures at intervals for the expulsion of urine. In pathological conditions when the nerve supply to the bladder is abnormal or where there is bladder outflow obstruction abnormal elevation of intravesical pressures may occur, either during urine storage or during voiding[2].

The ureter enters the bladder with an oblique intramural passage through the detrusor muscle and extends in a short submucosal tunnel to open onto the trigone. The ureterovesical junction may be abnormal due to maldevelopment or delayed maturation resulting in so called 'primary' vesicoureteric reflux, or it may be distorted by changes in the bladder wall secondary to bladder pathology; *i.e.* 'secondary reflux'[3]. This crude clinical classification is not

important when considering the pathological effects upon the kidney although it may have a bearing on treatment. The net result of VUR is that it allows the retrograde passage of urine into the upper tracts thus exposing the kidneys to pressure changes usually confined to the lower tract, as well as facilitating the bacterial colonisation of the upper tracts by urinary pathogens[4-7].

The identification of VUR in man and its clinical and experimental investigation was dependent upon the development of contrast radiography in the early 1900s. Prior to this VUR had been detected in experimental studies when agents such as milk and methylene blue had identified the phenomenon in rabbits[8,9]. An examination of human autopsy specimens had founded the presumption first attributed to Galen in 150 A.D. that VUR in man did not occur[10]. The publication of previously undocumented human VUR by Pozzi[11], who in 1893 noted the efflux of urine from the distal segment of a ureter accidentaly severed during pelvic surgery, is testimony to the persistence of this prejudice.

The competence of the ureterovesical junction in preventing retrograde passage of urine is not universal throughout the animal kingdom. In rats and rabbits VUR has been demonstrated as a common observation. It may also occur but with less frequency in dogs, cats and some strains of monkeys, whilst in pigs it is rare[6,12-17]. Gruber[15] associated the inter-species susceptibilty to VUR to natural variations in the length of the intravesical ureter and the relative development of the trigone. These factors are now known to be

implicated in allowing VUR in children[18].

In 1924 in one of the first large studies of micturating cystograms Bumpus[19] reviewed 1,036 cystograms and noted that VUR was related to urinary infection but, with the exception of children, it was always associated with lower tract pathology. At this same time Eisendrath, Katz and Glasser[20] also noted an association between VUR and infection in some patients. These studies were followed in 1930 by a report from Campbell[21] who showed that of 722 cystograms in unanaesthetised children, 12% had VUR but its presence was believed to be associated with some additional abnormality of the urinary tract. No conclusions were made from any of these early studies concerning the significance of VUR as a factor in initiating renal disease. In a review of the literature in 1944 Prather[22] found divergent opinions regarding the occurrence of VUR in the normal urinary tract but concluded that VUR was abnormal in the child. This view gained common acceptance only after accumulated evidence that VUR in apparently normal children was exceptional[23-27]. It was not until 1952 that Hutch[28] recognised the significance of VUR as a major factor in chronic renal failure. In 1960 Hodson and Edwards[29] proposed the existence of a causal relationship between VUR and chronic pyelonephritis after detailing the histories of 20 children with pyelographic segmental scarring who were found to have VUR. These observations were soon confirmed by others[30-32] and founded current attitudes towards the management of children with VUR.

## 1.2 Diagnosis and Grading

The micturating cystourethrogram provides the standard means of assessing VUR when contrast media may be observed to pass into the ureter only or extend into the renal pelvis. Reflux may occur into a radiographically normal ureter or into grossly dilated and distended upper tracts. These radiographic appearances form the basis of a number of 3-, 4- or 5- point classification systems aimed at grading the severity of VUR [33-37].

## 1.3 Identification and Prevalence

Vesicoureteric reflux itself is generally discovered during childhood as a result of investigations for other urinary tract problems[38]. The commonest problem leading to identification of VUR is urinary infection and the prevalence of VUR is similar in children who are investigated with overt symptoms as in those with covert bacteriuria identified during screening programmes[39]. In children (aged 2-18 years) the prevalence of VUR is between 18% and 32%[40-43] of those with urinary infections, but it is higher in neonates with rates between 47%[44] and 57%[45]. This last value obtained from screening 1,460 neonates for bacteriuria demonstrates an occurrence of VUR in 0.6% of the neonatal population as a whole. In children without urinary infection the prevalence of VUR is difficult to ascertain. Bailey[46], after reviewing the literature, bravely put a figure of 0.4 to 1.8% of the paediatric population, but favoured the lower value.

## 1.4 Spontaneous Resolution

As an accompaniment of urinary infection, VUR is observed only rarely

in adults[47]. Both this feature of VUR and its greater prevalence in neonates than older children (1.3) are explained by the well documented[38,39] disappearance of VUR during childhood in a high proportion of cases. The grade of VUR influences but does not determine the spontaneous resolution of VUR, the rate of which may be as high as 85% for those with radiographically low grade reflux compared with 41% for those with severe reflux[48].

### 1.5 Renal Scarring

As long ago as 1933, Longcope and Wickenwerder[49] related the radiological and pathological appearances of chronic focal pyelonephritis. These authors noted an association of the disease with childhood infection. It was only after the work of Hodson and Edwards[29] that the significance of the radiological appearance in relation to VUR was appreciated. Nowadays, the classical radiological characteristics of segmental scarring are described as *reflux nephropathy* since this term places an emphasis of association with VUR rather than with infection. In addition, *reflux nephropathy* includes more diffuse renal damage and atrophy of the types usually linked with obstructive uropathies but found on occasions in association with VUR[36].

Although VUR has been identified in the majority of children with radiological signs of *reflux nephropathy* (scarring)[30-33] the converse is not inevitable and the presence of pyelographically normal kidneys with continuing VUR can be a more common observation[36,40-43,50-51].

There is an additional anomaly in that scarring may be observed in children with no demonstrable VUR[41-43;52]; explicable by the spontaneous remission of VUR and by its inconsistent radiological detection in some cases.

It has been presumed that in the majority of cases *reflux nephropathy* is acquired as a result of urinary infection. Although this is supported by animal studies[7,53] evidence from clinical observations is largely circumstantial since pyelographic scarring is usually identified at first presentation. Scarring has been found in 10-26% of children with urinary infection and in 30-60% of those with proven co-existent VUR[41-43,52,54]. Although the appearance of new scars in previously unscarred kidneys is rare they almost always follow a documented urinary infection[55]. That renal scarring can be acquired during life is supported by results from a recent study of 172 children. Renoscintigraphy with $^{99m}$Tc-DMSA indicated that with high grade VUR scarring was present in 60% of older children (>4 years) compared with 40% in children younger than 3 years. In children with low grade VUR the corresponding values were 40% and 20%[56]. In neonates pyelonephritic scars appear to be rare[45,57], although in this age group their radiological detection may be difficult. In a series of 1,460 neonates screened for bacteriuria 14 were found to have urinary infection: of these 8 had VUR, but in none of them was renal scarring identified[45].

It is possible that in a number of children with *reflux nephropathy* the kidneys were abnormal at birth as a consequence of either renal

dysplasia[58] or the occurrence during intra-uterine life of sterile VUR concomitant with an episode of some form of urine outflow obstruction. Cases of fetal VUR are now being identified during pre-natal ultrasound screening and a proportion of them have abnormal kidneys post-natally in the absence of urinary infection[59-61]. It is also of interest that fetal VUR is identified more often in males and that its detection depends upon the observation of dilated upper tracts[59,60]. Where pyelography has demonstrated scarring or parenchymal reduction in neonates or infants less than age 1 year it is invariably associated with high grade VUR with dilated urinary systems and is more common in boys than in girls[36,62,63].

## 1.6 Pathogenesis of Renal Scarring: Experimental Studies.

A series of experimental and morphological studies have contributed to the present understanding of the pathogenesis of renal scarring in association with VUR. The factors important in the genesis of scars are intra-renal reflux, urinary infection and intra-vesical pressures. The subject was contentious for many years but is one of profound significance in relation to any treatment policy for VUR.

### 1.6.1 Intra-renal Reflux

Brodeur, Goyer and Melick[64] determined that pyelotubular backflow was responsible for the parenchymal ingress of radiological contrast media sometimes observed in the presence of VUR during cystography. It was Hodson, however, with co-workers Maling, McManamon and Lewis[65], who related this phenomenon to segmental scarring in *reflux*

*nephropathy* and described it as intra-renal reflux.

Intra-renal reflux (IRR) itself demonstrates an unusual characteristic in that it tends to occur in the polar regions of the kidney and less frequently in the mid-zone. This segmental distribution was found by Ransley and Risdon[66] to be due to variation in papillary morphology. These authors showed that fusion between individual lobes (renculi) often resulted in compound papillae allowing IRR and that these papillary variants were found most commonly at the renal poles and were present in 73% of human kidneys[67]. These observations have been corroborated by others[68] and an absence of compound papillae may explain in part why scarring is not an inevitable consequence of VUR, since the susceptibility of a kidney or a segment of kidney, to IRR and hence to scarring is pre-determined during intra-uterine renal development.

### 1.6.2. Infected Urine

Experimental studies in the pig established that VUR of infected urine quickly initiates segmental damage and scarring analogous to human chronic pyelonephritis and that IRR is the probable mediating mechanism[7,65]. Later studies also showed that the development of scars could be aborted by antimicrobial therapy administered within one week of a positive urinary infection[69]. The pathogenesis of renal scars in the monkey in the absence of VUR has been attributed to ascending infection by P-fimbriated bacteria[70] but the significance of this mechanism in the human is yet to be determined.

### 1.6.3. Sterile Urine

The role of sterile VUR and intra-renal reflux in scar formation was less easily defined. From early experiments in the pig with bladder outflow obstruction Hodson and co-workers[65] made the direct association between intra-renal reflux of sterile urine and subsequent scarring. Ransley, Risdon and Godley[71] later defined the extreme urodynamic circumstances necessary in the pig for this to occur and noted that such conditions are only rarely encountered in clinical practice when VUR is combined with bladder outflow obstruction. Similar conclusions were made by Mendoza and Roberts[72] who found, in monkeys with VUR, that sterile renal parenchymal damage arose only when urodynamic abnormalities were of a severity sufficient to cause a delay in renal excretion rate *i.e.* functional renal obstruction. These studies are futher supported by those of the Jorgensen group[73] showing, in a further pig model of VUR, that scarring in the absence of urinary infection occurred only when bladder outflow obstruction caused an abnormal dilatation of the upper tracts. In the clinical context the urodynamic abnormalities causally associated with sterile scars in the pig and monkey models were similar only to those found in children with posterior urethral valves[74], urethral strictures[75], or with severe bladder and sphincter discoordination such as occurs with neurological abnormalities[76,77]. More pertinently Ransley and co-workers[7,71] have shown, in pig models with normal bladder function or with bladder outflow obstruction, that where bladder voiding pressures were normal or of a degree analogous to those associated with primary VUR in children[78], sterile VUR does not induce scarring or histological damage.

## 1.7 Management of VUR

Surgery has proved successful in correcting reflux in over 90% of cases[79]. However, since VUR is a relatively common disorder which resolves spontaneously in the majority of cases (1.4) a conservative approach has been recommended which aims to maintain a constantly sterile urine with chemoprophylaxis[38,39,80-82]. This therapy is not universally adopted and surgery has been advocated for those with high grade reflux[83-85] or those in whom infection control cannot be adequately maintained[80]. The choice of the most appropriate and beneficial form of treatment is not yet resolved and is now being sought in an international prospective clinical trial comparing the outcome of conservative and surgical management[37,86].

Previous prospective and controlled clinical trials have been few and have presented differing conclusions. In an early trial comparing the outcome after 3 years of conservative or surgical management, Scott and Stansfeld[83] found that the incidence of urinary infection was higher and renal growth rate lower in those who received medical treatment. More recently the Birmingham reflux study group[82,87] showed no significant differences in renal growth or function between children committed to either modes of management for 2 or 5 years. These formal trials have considered only children with high grade VUR and results showed that irrespective of treatment the prognosis for normal renal growth was poor. This is likely to have been influenced by the high incidence of scarring, more often associated with high grade VUR[36], in the children included in these studies. In comparison with these formal trials De Gracia and Brueziere[88] have

reported the outcome on renal growth 10 to 18 years after surgical or medical management of children with low grade VUR. Only those kidneys where VUR disappeared, either spontaneously during medical treatment (24 kidneys) or after anti-reflux surgery (63 kidneys), were compared clearly. The results showed that only kidneys with scars failed to grow normally irrespective of treatment modality.

Despite doubts concerning management, current advice favours the conservative approach and in general it is accepted as the first line of management for those with low grade reflux[38,39]. Since it has been calculated that spontaneous resolution in a population with VUR occurs at about 10% per annum[48] it is axiomatic that large numbers of children will remain with sterile urinary reflux for a number of years. It is not known however whether prolonged reflux has a deleterious effect on renal performance in the absence of urinary pathogens.

### 1.8 Prognosis

There is incontrovertible evidence that *reflux nephropathy* can cause renal dysfunction producing, for example, diminished urinary concentrating ability[89,90], proteinuria[91,92] and decreased glomerular filtration rate[93]. In addition, it is a common cause of hypertension in children and young adults[38]. Since *reflux nephropathy* is irreversible and can be progressive it may take many years for these problems to become manifest, leading in some cases to end stage renal failure. Of those children afflicted with scarring

the proportion developing chronic renal failure is unknown but has been estimated as 5-6%[94]. Bailey and Lynn[95] calculated from the European transplant registry for 1977 that 0.3 to 0.4 children/million of the population/year will present with chronic renal failure resulting from *reflux nephropathy*. An incidence at least 10 to 20 times higher has been suggested for adult women[91]. Whilst these estimates represent large numbers of individuals they account for only a very small proportion of those exposed to VUR during childhood, the true prevalence of which may be larger than has been recognised[96].

Quite apart from its association with urinary infection and its role in the pathogenesis of renal scarring, the possible effects of VUR on renal growth and function are unknown and are the questions addressed by this thesis.

CHAPTER 2

# STERILE VESICOURETERIC REFLUX: PATHOPHYSIOLOGY

The accumulated evidence (**Chapter 3**) from clinical and experimental studies is insufficient to conclude whether VUR *per se* affects long term renal growth and function. This question is relevant to the majority of the population with VUR where scarring is either absent or limited and is especially important to children with sustained VUR in whom the development of scars can be averted by the prevention of infection with chemoprophylaxis.

In the absence of urinary infection any pathophysiological response by the kidney to VUR is likely to be related to the unnatural retrograde fluid dynamics to which the kidney is exposed. In addition, there may be a component of functional obstruction when there is a dilated, tortuous and poorly draining ureter. The questions examined in this thesis concern only the retrograde dynamic effects of VUR. It has been suggested that this so called 'water hammer' effect does harm the kidney[97].

## 2.1 Potential Pathophysiological Effects of VUR

As discussed earlier (**1.6**) sterile VUR can cause damage in the form of segmental scarring but this occurs only in extreme conditions of abnormal bladder function. The pathogenesis of these sterile scars is unknown. Tubular rupture and parenchymal extravasation of tubular glcycoprotein (Tamm Horsfall protein or uromucoid) has been

implicated[98] but failure to identify these features at all sites of sterile scars suggests that neither is the primary cause[71,99]. What is clear, however, is that there exists a threshold in the pressure related dynamic effect above which scarring occurs. The pertinent question is whether or not VUR presents a hazard below this threshold.

Ransley[100] showed, in the pig, that in the presence of VUR there is direct, and un-attenuated, transmission of intra-vesical pressures to the renal pelvis. He also demonstrated that intra-renal reflux (IRR) can occur at intra-pelvic pressures as low as 10 to 15mm Hg (13-20cm $H_2O$). Other investigators using the pig[65,101] or human autopsy specimens[102] found that higher intra-pelvic pressures of 30 to 35cm $H_2O$ were necessary for the demonstration of IRR. Even these relatively higher pressures are insufficient, in themselves, to generate scarring since an absence of scarring, both macroscopically and histologically, has been demonstrated[7,71] in pig models with elevated intra-vesical pressures, which on occasions approached 90cm $H_2O$. In the absence of scarring, therefore, IRR could provide a mechanism for inducing impaired renal performance by extending the hydrodynamic effects into renal tubules of susceptible papillae. The evidence, however, of direct tubular damage is unconvincing. Since urinary excretion of small molecular weight proteins increases in tubulopathies[103] their increased concentration in urine has been sought in VUR. Bell, Wilkin and Atwell[104] concluded that excretion of certain micro-proteins (retinol binding protein) was elevated in children with VUR but their data indicates that this was associated

only with renal scarring.

Despite the absence of any direct evidence of tubular damage resulting from VUR there is other evidence from Walker, Richard, and Dobson *et al*[89] suggesting that the abilty of the kidney to concentrate urine is affected. In this study urinary concentrating ability was related to the degree of radiological abnormality in children with VUR. The results showed that although the mean maximal urinary concentration (864 mosmoles) for 32 children with VUR but without scars or other radiological abnormality was within the range regarded as normal (*i.e.* >800 mosmoles) it was significantly reduced in comparison with 12 control children without any urological abnormality. The manner in which VUR affects concentrating ability is unknown. The possible or likely mechanisms may include IRR since this may directly affect tubular cells or disrupt countercurrent mechanisms. Other mechanisms quite independent of IRR may be ascribed to the pressure effects of VUR or even to the presence in the renal pelvis of refluxed urine, since this may well be of an inconsonant tonicity to the papillary medulla where the countercurrent multiplier system achieves maximum osmolality. However, any effect of VUR on concentrating ability is not conclusive since in the above study, only 6 weeks were allowed between the last documented urinary infection and the concentrating test and urinary infection alone may reduce concentrating ability for up to 12 weeks after its eradication[105].

Experimental studies indicate that VUR effects could be secondary to

an action on renal blood flow. Orr, Kimbrough and Gillenwater[106] measured renal blood flow by means of an indwelling flow transducer during 233 observations of bladder filling and voiding in 10 dogs. Although they occasionally noted no change in renal artery blood flow when voiding pressures were low, the usual response was a small but sharp drop accompanying the rise in intra-vesical pressure. When urinary reflux was present this response was significantly enhanced in comparison with 'non-refluxing' contralateral kidneys. Renal artery spasm was observed in some instances but only in the presence of reflux and it happened most often when voiding pressures were high. During bladder filling, and in contrast to the observations during voiding, an increase in renal blood flow sometimes occurred. Again this was significantly greater in the presence of VUR.

Other experiments of VUR and renal blood flow have been performed by Helin[107] who used a dye dilution technique to measure renal blood flow in the pig whilst retrograde pressures were applied to the kidney. During the stepwise increase in pressure, blood flow was significantly altered only in kidneys which had been exposed to VUR for 3 to 4 months. The results from 6 pigs showed that blood flow increased with low retrograde pressures (10mm Hg; 14cm $H_2O$) but decreased at higher pressures (25 to 75mm Hg; 35-100cm $H_2O$). Inspection of the Helin[107] data shows that some small and parallel changes in renal blood flow were observed in kidneys not previously exposed to VUR despite their failure to reach statistical significance.

Again the mechanisms underlying a renal blood flow response are unknown, but Thomsen, Talner and Higgins[101] in a study of 27 pigs found that decreased renal blood flow during exposure of kidneys to retrograde perfusion occurred only when there was intra-renal backflow of the perfusate. These results do not satisfactorily explain the changes in renal blood flow observed in dogs during voiding[106] since changes were also noted but to a lesser extent in the absence of VUR. Therefore, an independent action of VUR mediated through pressure effects on the renal parenchyma, pelvis and ureter cannot be discounted. Gottschalk[108] showed in rabbits and in dogs that renal interstitial pressures increase with applied intra-pelvic pressures and Gilmore[109] has suggested this causes compression of intra-renal blood vessels. Hix[110] reported that in patients with VUR, stimulation of the ureter with a catheter produced a fall in effective renal plasma flow and GFR. Although this latter study was unphysiological it implies that a reflex mechanism operates between the ureter and renal vasculature. This may be important in view of the observations that the frequency of ureteric peristalsis increases with urinary reflux[111,112].

Further support for a blood flow mediated mechanism of insult by VUR comes from histological studies. Heptinstall and Hodson[113] described fibrosis of arcuate and interlobular arteries in some pigs with renal scars resulting from high pressure sterile reflux. Similar features were also noted by Ransley and Risdon[7] in some of their unscarred kidneys from pigs with sterile VUR but with more moderate intra-vesical pressures.

The implications of the experimental results are substantiated by a recent clinical study which included a group of 20 children with unilateral VUR[114]. Effective renal plasma flow was measured with $^{131}$I-iodohippuran and showed a significant reduction in association with VUR, despite an apparent absence of pyelographic scarring. It is possible that a long term effect of VUR on renal blood flow during a period of growth and development could impair renal growth and prove detrimental to glomerular filtration rate. Renal blood flow changes may also affect salt and water homeostasis in the kidney through an action on chemically mediated autoregulatory systems (14.1).

# STERILE VESICOURETERIC REFLUX:

# PREVIOUS STUDIES OF RENAL GROWTH AND FUNCTION

## 3.1 Clinical studies

Most clinical studies concerned with the influence of VUR have considered the effects during a relatively short period in childhood. The most long term of these have considered only renal growth. Smellie and Edwards *et al*[115] in a prospective study of 111 kidneys with proven VUR for 2 to 22 years showed that impaired renal growth was associated independently with infection and renal scarring but not the severity of VUR. The same conclusions were made by Riebel and Kollermann *et al*[116] who followed 46 kidneys with VUR for 1 to 6 years. In a recent study where 58 girls were followed for a mean of 11.2 years Verrier Jones and Aggarwal *et al*[117] showed that renal size was not influenced by VUR even in the presence of covert bacteriuria but was strongly influenced by scarring.

Of the large number of clinical studies concerned with the influence of VUR there are few from which just conclusions can be made concerning the effects of VUR *per se* on renal function. In a prospective study of 48 schoolgirls with and without VUR, investigated as a result of asymptomatic bacteriuria, Verrier Jones and Asscher *et al*[93] found that individual kidney glomerular filtration rate (GFR) was not influenced by VUR alone and remained stable over a 4 year period. These results substantiate an earlier

study by Fritjoffsen and Sundin[118] who followed 9 adults with newly acquired (iatrogenic) VUR. In 6 patients who maintained a sterile urine, individual kidney inulin and para-amino hippuric acid (PAH) clearances remained intact for up to 11 years.

The results from these studies suggest that VUR does not adversely affect renal growth and function, since in all of them the presence of scarring and urinary infection were taken into account as factors which may independently influence renal growth and function. The failure to emphasize or appreciate the interaction between either of these factors and VUR *per se*, either by the investigators themselves or by others when reviewing the data, has contributed to a contrary premise that VUR is detrimental to renal performance.

Aperia and Broberger *et al*[85] measured individual kidney GFR and maximal tubular reabsorption of glucose in 22 children with either unilateral or bilateral reflux. The effects of VUR were determined by comparing function in kidneys with VUR to those without and analysing the GFR data in 3 groups defined by the patients age. The results showed that low grade VUR did not significantly impair GFR in any age group, although the mean value was slightly reduced in 'refluxing' kidneys of older children. In kidneys where there was high grade VUR, GFR was reduced in all age groups but was significant only in older children (8-12 years); the mean value also appeared lower than the expected individual kidney GFR for a normal population without VUR. Maximum tubular reabsorption of glucose was depressed only in proportion to GFR. It was concluded that with high grade

VUR there was a gradual deterioration in GFR which accelerated after the age of 6 years despite strict medical care for urinary infection. However, this was not a longitudinal follow up study. Furthermore, all children in this study had renal scars and the authors acknowledged that the association between VUR and reduced function could not be separated from an association with, or direct relation to, the reduced proportion of functioning renal parenchyma. Such a relation has been recognised and described by the same group[119]. The authors also recognised that the effects of VUR alone could not be deduced from this study since all children had a history of recurrent urinary infection.

Piepz and Hall *et al*[120] found that individual kidney GFR was less than pre-established normal values in half of 67 kidneys exposed to VUR in children who had received antibiotic therapy for 3 to 6 months previously. Tabulated results showed that the proportion with abnormally low values was upheld even in the absence of scarring. There was no discussion of this aspect, particularly in consideration of the sometimes short preceeding period of antibiotic therapy and the fact that many months or years may be required for the radiological detection of pyelographic scars[121]. These aspects together with the absence of any statistical analysis diminishes the value of these results.

The failure to take account of urinary infection and scarring in studies of Kass Ibsen, Uldall and Frokjaer[122] and of Orikasa and Takamura *et al*[123] confounds any consideration of the influence of VUR

on renal growth which was found to be normal in some kidneys, but in others significantly impaired.

The premise that VUR may be detrimental to kidney performance has been provoked by observations of improved GFR[124,125] and renal growth[126-129] after anti-reflux surgery, irrespective of the presence or absence of renal scarring. Such studies provide only extrapolated and indirect evidence concerning renal performance in the presence of VUR. Furthermore, improvement may be attributed to removing the influence of urinary infection on the kidney after correction of VUR. In the studies of Wilscher and Bauer *et al*[126]; Atwell and Vijay[127]; Atwell and Cox[128] results showing an improved renal growth after anti-reflux surgery can be explained by the analytical methods employed. In all these studies renal growth was assessed from the relationship between radiographic renal length and age. All included infants in whom renal length increases more with age than it does in older children. This was not accounted for when mean values were used in comparison with the normally slower change in renal length occurring in children after age 5 years. The analytical error is demonstrated by the results of Atwell and Vijay[127] and Atwell and Cox[128] since a greater or similar post-operative acceleration of growth was also observed in contralateral kidneys not previously subject to VUR. It is of interest, however, that in all these studies[126-128] of renal growth there were no significant differences in renal length between 'refluxing' and 'non-refluxing' kidneys pre-operatively. Subsequent studies where kidney length was related to body size and patients

were stratified for age failed to demonstrate accelerated growth after anti-reflux surgery[86,129].

All studies where the presence of urinary infection and renal scarring have been considered have shown no significant effect of VUR on either renal growth or function. A detrimental effect of VUR has been documented only in studies where these factors have not been fully acknowledged. There are, however, problems inherent in all clinical studies which means that they alone cannot satisfactorily resolve the issue of whether or not VUR *per se* affects the kidney.

### 3.1.1 Limitations of Clinical Studies

Clinical studies are of value in seeking a course of management which obviates or minimises the pathological potential of VUR in a population which has been exposed, almost invariably, to urinary infection at some time, and of whom many will have renal scarring (1.5). They do not accurately address the question of whether VUR alone affects renal growth and function. The ultimate answer to this question can be found only by following children with VUR and a sterile urine from birth to maturity. This is difficult because usually VUR cannot be identified in the absence of urinary infection although the advent of pre-natal ultrasound diagnosis may permit such studies in the future.

A further and inevitable limitation of clinical studies is that a formal study with the inclusion of controls without VUR is tantamount

to impossible. The clinical trials of management[82,86] are controlled but only for purposes of comparing treatment modalities. In these and other clinical studies any conclusions concerning the effects of VUR can be made only by comparison with previously established normal standards, or by considering the change with time of a particular parameter, the hazards of which have been described earlier (**3.1**) in relation to kidney growth. As well as this problem and the over-riding one of the cohesion between urinary infection and VUR there are others relating to measurements and to the population with VUR.

### 3.1.1.1 Renal Growth Measurements

Renal growth assessment in clinical practice is dependent upon kidney size measurements taken by convention, from the intra-venous pyelogram[130-134] although more recently from ultrasound images[135,136]. The pursuance of normal or abnormal renal growth is determined by relating specific measurements either to age or to to various aspects of body size. A number of nomograms are available for comparison[131-134] but are appropriate only for use with unscarred kidneys since the measurements are likely to be artefactual when scarred parenchyma, which cannot be expected to grow, is present. There are inherent errors associated with the measurement of a 3-dimensional kidney from a 2-dimensional image and these are discussed elsewhere (**15.1.1**). The problem of estimating renal growth in this way is exemplified in a study by Peratoner, Messi and Fonda[137] who included an evaluation of renal growth in the presence of VUR but in the absence of pyelographic scarring. The results

showed that whilst all (24) kidneys were of normal size when renal length measurements were used (after Lebowitz, Hopkins and Colodny[138]), 18 of the 24 were abnormally small when size was measured as a ratio of bipolar thickness to renal length (after Hodson[131]).

#### 3.1.1.2 Renal Function Measurements

Clinical investigations to detect effects of VUR on renal function are complicated by the difficulty of measuring individual kidney function. The longest follow up study where this latter was successfully achieved using radio-isotope renography was for only 4 years[93]. Besides radio-isotope renography the only other method of establishing individual kidney function requires ureteral compression applied during urine collection periods[139], which is not a practical method for the longitudinal study of relatively large numbers of children. The problem of obtaining functional data is apparent in the reports of the Birmingham Reflux Trial[82,87]. The results showed that glomerular filtration rate measured by a traditional clearance method was on the whole unnaffected by VUR. This was not unexpected because children with unilateral VUR were included in the study but not analysed separately. Since compensatory adaption by the contralateral kidney can occur when unilateral function is compromised[93,140,141] this may have obscured any deleterious effects of VUR on an individual kidney.

#### 3.1.1.3 Heterogeneity of VUR

A further limitation of clinical studies arises because of the

heterogenous nature of the VUR phenomenon and the diversity in age and symptomatology of children included for study. The anatomical heterogeneity of reflux is apparent in the schemes of cystographic grading (1.2) but the physiological diversity of the way in which reflux behaves is not. Neither the feature that urinary reflux can occur in some patients only with relatively high intra-vesical pressures during voiding whilst in others it occurs with low pressure during bladder filling[142,143], nor the associated urodynamics have received sufficient consideration with regard to the classification of VUR. A number of studies have shown that urodynamic abnormalities co-exist with VUR[144-150] and in one report[144] detrusor instability was noted in up to 50% of cases. In a recent study of 11 children with high grade VUR, reflux was revealed by radio-isotope cystography and combined urodynamics as a widely disparate phenomenon[145]. The disparity was observed both in the volumes of refluxing fluid and in the associated urodynamics. It has even been suggested that in a proportion of cases so called primary VUR is in fact secondary to a urodynamic abnormality[144,146,147]. This awaits confirmation and acceptance but there is evidence that detrusor instability in itself, as well as the high intra-vesical pressures resulting in some cases from voluntary sphincteric obstruction during instability, may initiate, increase or delay resolution of VUR[147-150].

### 3.2 Experimental Studies

Experimental studies can avoid some of the complexities which in the

clinical setting confound investigation of the effects of VUR on renal growth or function. The use of an animal model for VUR allows the question to be addressed both simplistically and in comparison with control animals without VUR. Renal growth can be assessed more directly from kidney weight and there is opportunity to estimate individual kidney function by orthodox methods when choosing a single kidney model. In addition, complicating factors of urinary infection and renal scarring can be avoided and any influence of bladder function tested.

Previous animal experiments have been few and not all have used the experimental situation to full advantage. With the exception of one study using pigs[107] the others employing various canine or monkey models have concluded that VUR does not affect either growth or function[53,72,151-158].

In the majority of experiments the effects of VUR have been examined in adult animals and earlier studies have almost exclusively used canine models with VUR being surgically induced by transvesical meatotomy. Most employed models with unilateral VUR and used methods involving ureteric catheterisation (split function technique) to estimate individual kidney function from clearances of para-aminohippuric acid (PAH) and of creatinine in anaesthetised animals. Using these methods King and Idriss[151] followed 8 dogs and after 17 to 20 months of sterile VUR, no consistent change in the clearance ratios between the 'refluxing' and 'non-refluxing' kidneys of pairs was demonstrated. The results showing no obvious effect of

VUR on clearance function were clearly tabulated for each animal but not analysed statistically. In 3 further animals followed for 14 to 20 months after a urinary infection supervened, PAH and creatinine clearances were markedly impaired.

Similar results showing no demonstrable effect of VUR on clearance function were reported by Lenaghan and Cass *et al*[152] for 8 dogs followed with sterile unilateral VUR for 4 to 72 months. In 3 of these dogs individual kidney function was measured by the split function technique and in the remainder by gamma camera renography with $^{131}$I-iodohippuran. An examination of the kidneys from these and one other animal showed that VUR had no effect on kidney weight or microscopic appearance.

James and Canham *et al*[153] employed a more elaborate canine model to examine the effects of both vesicoureteric and ileoureteric reflux in the same 5 animals. The split function method was used to measure inulin and free water clearances as well as urinary osmolality. The results showed that in comparison with control animals sterile VUR for 6 months did not impair any of these functional parameters. There was no obvious effect of VUR on kidney weight nor was any morphological damage observed.

In only one study has the solitary kidney model been used to simplify the practice and interpretation of individual kidney function tests. In an experiment to assess the pressure effects of VUR Danforth and Javadpour *et al*[154] surgically prepared an ingenious dog model with a

solitary kidney autotransplanted to the iliac fossa. Urine in the renal pelvis drained directly into the bladder through a pyelocystostomy. The control model consisted of the same autotransplanted solitary kidney but with urine drained into the bladder through the usual ureter. Only 3 test and 3 control animals were followed. After 1 year of sterile urinary reflux, causing distention of the renal pelvis and calyces, there was no obvious nor significant impairment of inulin clearance or maximal tubular reabsorption of PAH. No morphological differences were found between the kidneys of test and control animals and there was no apparent effect of VUR on kidney weight.

Two more recent studies, by Roberts and his group[53,72], used the monkey model with surgically induced unilateral VUR in the presence of urethral stenosis. In both studies the effects of VUR on individual kidney function were assessed during gamma camera renography by comparing the maximum uptake and excretion rate of $^{131}$I-iodohippuran by the 'refluxing' and 'non refluxing' kidneys. In the first study[53], 7 adolescent or adult monkeys were investigated for 3-17 months. The results showed no significant impairement of effective renal plasma flow (ERPF; isotope uptake) even in 3 animals in whom intra-renal reflux had been demonstrated during cystography. The second study[72] considered the effects of sterile VUR and bladder outflow obstruction present for 6 to 14 months during growth. Intra-vesical pressures, during voiding, at the beginning of the study period were a mean of 71cm $H_2O$ and after 6 months were a mean of 30cm $H_2O$. In 3 monkeys there was no change in ERPF by VUR but

in another 4 animals there was a deterioration of up to 30%. Functional loss with VUR was associated not with bladder voiding pressures but with functional obstruction of the kidney. This was indicated by delayed excretion of $^{131}$I-iodohippurate and the presence of elevated resting bladder pressures. Morphological studies showed diffuse interstitial scarring in affected kidneys but not in kidneys retaining normal clearance and excretory function in the presence of VUR.

Experiments employing either the dog or monkey may be of limited value in extrapolation to the human condition of VUR as neither of these species possess a multireniculate multipapillate kidney like that of the human. The pig and human kidney, however, are alike and in the one study where the pig model was used Helin[107] obtained quite different results. After 3 to 4 months of uninfected unilateral VUR at normal bladder pressures individual kidney $^{51}$Cr-EDTA glomerular filtration rate and tubular extraction of $^{125}$I-Hippuran were estimated in 6 pigs by methods involving catheterisation of both renal arteries and veins whilst the animals were anaesthetised. Both glomerular and tubular function were significantly reduced in the 'refluxing' kidneys compared with the 'non-refluxing' contralateral kidneys. These experimental results suggest that sterile VUR does indeed impair renal function; however, since there was no discussion of either morphology or size of both the kidneys and the ureters, the possibility of scarring or ureteric obstruction cannot be excluded from this study.

### 3.2.1 Limitations of Previous Experimental Studies

The limitation of the canine and monkey models in providing a model analogous to the human condition of VUR has already been mentioned but this is perhaps of lesser significance than other features of previous studies. The use of the 'split clearance' technique in the majority of studies meant that clearance tests had to be performed in anaesthetised or sedated animals. All studies were restricted to a small number of animals and most were of a design unsuitable for a statistical approach to determining the effects of VUR. Two aspects of the experimental models need further discussion; these are age and the bladder function conditions.

#### 3.2.1.1 Age of Animal

There have been no studies where the effects of VUR on renal growth have been adequately tested. In only one monkey study[72] has there been a satisfactory investigation of renal function in growing animals and this was restricted to an examination of effective renal plasma flow. King and Sellards[155] did use a growing canine model and concluded that VUR did not affect renal growth or developing renal function. The experimental results for kidney weight and renal clearance (creatinine and para-aminohippuric acid) were limited, however, in supporting these conclusions. Of 5 puppies who were followed for 18 to 28 months after induction of unilateral VUR 3 were found to have bilateral VUR. Although the PAH and creatinine clearances (per body surface area) showed, for 2 dogs in each case, an increase between the initial and final values, no control animals with bilateral VUR were available for comparison. Of the 2 dogs

with unilateral VUR the change in clearance ratios ('refluxing' to 'non refluxing' kidneys) between the initial and final studies were inconsistent. In only one dog was there any comment concerning kidney weight which was identical for the paired kidneys in spite of the presence of unilateral VUR.

#### 3.2.1.2 Effects of Bladder Function

There have been very few studies where bladder function, carefully assessed in conscious animals, has been considered in its effect on the outcome of VUR. In the few investigations of the effects of VUR in the presence of elevated bladder pressure there have been difficulties associated with renal scarring and infection[72,156]. In a further study, to compliment the first in dogs with low pressure bladders, Lenaghan and Cass *et al*[156] attempted to follow 6 dogs with sterile unilateral VUR in the presence of bladder outflow obstruction. Three animals developed urinary infections. Of the remaining 3, one died at 5 months, one developed a low grade urinary infection but was killed at 19 months and the third was killed at 26 months. Of these 3 dogs, 2 showed no obvious differences in paired kidney weights between the 'refluxing' and 'non-refluxing' kidneys but in one other, the kidney exposed to VUR weighed less than the contralateral control kidney. Individual kidney function was measured serially by $^{131}$I-iodohippuran in only one of the dogs and this showed no significant difference between the 'refluxing' and contralateral control kidney.

### 3.2.3 Conclusion

The failure of previous experimental studies to contribute significantly to an understanding of the effects of VUR upon the kidney, during a period of rapid growth and development, established the necessity for undertaking the experimental investigation described in this thesis.

CHAPTER 4

# OUTLINE OF EXPERIMENTAL INVESTIGATION

The experimental investigation described in Section IV and central to this thesis concerning the effects of sterile VUR on renal growth and function was carried out in young pigs followed from soon after birth through a period of rapid growth and development. A single kidney model with VUR was included (**Experiment I**) to facilitate evaluation of individual kidney clearance function and the effects of VUR tested by comparison with comparable control animals without reflux. The investigation also included a paired kidney model (**Experiment II**) and in common with previous experiments using this model, VUR was induced only unilaterally so that performance of the one kidney with VUR could be compared with the contralateral kidney; however, for comparison, the experiment also included control animals without any reflux.

The use of both the single and paired kidney models allows for examination of VUR effects in 2 situations of growth and development. The solitary kidney, following unilateral nephrectomy, is likely to be undergoing functional adaptation and accelerated growth in a renal compensatory response to the loss of the contralateral kidney whilst the paired kidney model reflects physiologically normal renal growth and development.

In both experiments the pathophysiological effects of VUR *per se* were examined by maintaining a proven sterile urine with chemoprophylaxis and by the avoidance of renal scarring. Since earlier studies have indicated that any blood flow mediated effects of VUR may vary with different retrograde pressures, both experiments used animals with abnormal bladder function and elevated intra-vesical pressures as well as those with normal bladder function and low voiding pressures. The relevent physiological parameters of the pig models were examined as part of the Section IV experiments and are discussed.

Renal clearance function was assessed by measuring glomerular filtration rate with $^{51}$Cr-ethylene-diamine-tetra-acetic acid. The urine concentrating capacity of the kidney was examined, as earlier studies had suggested this may be a sensitive indicator of renal functional disturbance. In addition, the renal uptake of $^{99m}$Tc-dimercaptosuccinic acid was included for evaluation since this may reflect a combination of various parameters which include renal blood flow and together determine overall renal performance. Its use meant that an index of individual kidney function could be examined in the paired kidney model. Renal growth was assessed from kidney weight at the end of the study.

# Section II

# The Pig Model

CHAPTER 5

# THE KIDNEY AND URINARY TRACT

The pig was chosen for the experimental investigation of VUR, as described in Section IV, since it is the only convenient laboratory animal to possess a kidney of similar architectonic form to that of the human. In addition to this, the pig resembles the human in both renal and urinary tract physiology and relevant aspects are reviewed.

## 5.1 Papillary Morphology

The distinctive multirenculate, multipapillate structure with an undivided cortex is relatively uncommon throughout the animal kingdom[157]. It is only in those species which possess a multirenculate kidney and where the renculi fuse during embryological development that compound papillae may be formed. The importance of the compound papillary variants in allowing intra-renal reflux (IRR) and the relation of this latter with the segmental scarring of *reflux nephropathy* has already been described (**1.6**). Similarly, papillary and calyceal morphology may be important determinants of the physiological renal response to VUR.

It was in studies employing the pig that Ransley and Risdon[66] made the important observation that IRR could occur only through ducts of Bellini present on flat or concave surfaces of the area cribosa and

that these non convex surfaces were exclusive to irregular shaped and complex (types II and III) compound papillae. A less complex variety of compound papillae (type I), was identified which had features more common to the simple papillae which are convex in shape and not associated with IRR. Ransley and Risdon[66] observed that ductal openings on non convex surfaces of the area cribosa were round or oval in shape and those on convex surfaces slit like. They also showed that all these morphological features of the papillae were common to the human kidney[67]. This provided a plausible explanation for the occurrence of IRR in both the porcine[7,65] and human[158] kidney since during an episode of VUR only the wide orifices on non convex surfaces would allow the free retrograde passage of fluid from the renal pelvis into the tubules. The essential detail of these observations has been corroborated by Tamminen and Kaprio[68] in that it is the combination of the shape of the ductal openings and the surface architecture which determines the potential for IRR although in this latter study of human kidneys and one other[159] of pig kidneys, wide orifices were found on convex papillae, albeit in preserved rather than fresh kidneys.

### 5.1.1 Papillary Variants in the Human

The number and proportions of the various papillary types may differ between paired kidneys of an individual[68]. An average of between 7 and 8 papillae per kidney has been reported[67,68,159] but in the absence of any fusion of the renculi the number may increase to a theoretical value of 14[160]. The majority of papillae present in an individual kidney are the simple or less complex variety which

normally prohibit IRR ('non-refluxing' papillae). About one third of human kidneys have none of the more complex compound papillary variants (refluxing papillae)[67,68]. In studies of between 14 and 40 kidneys the proportions of 'refluxing' papillary variants averaged between 12% and 33% of the total number of papillae found[67,68,159]. The higher value was made from an assessment of photomicrographs[159], and may not be representative of the proportions found by examination of specimens. In 2 studies where kidneys from young children were examined the occurrence of the 'refluxing' type of papillae in the mid-zone was rare[67,68].

### 5.1.2. Papillary Variants in the Pig

In the large domestic pig the number of papillae present in individual kidneys is similar or less to that found in the human and average values of between 5.8 and 9.9 have been reported for various breeds in studies comprising 6 to 34 kidneys[66,159,161]. As with the human the proportions of the papillary types is not the same in the individual kidneys of pairs and may vary with breed[159]. The pig kidney may differ markedly from that of the human in both the proportion and gross distribution of the various papillary types. Compound 'refluxing' papillary variants have been found to comprise average values of between 37% and 66% the total number of papillae in each kidney[66,159]. The complete absence of 'refluxing' papillae is rare and has never been encountered in Welsh[66] or Large White breeds (**9.3**). Whilst the compound 'refluxing' type of papillae are almost always found at the poles of the kidney their presence in the mid-zone of the kidney is common and occurs in the majority of

kidneys from Welsh[66] and Large White (9.3) pigs.

Papillary morphology in miniature pig varieties has not been documented and is considered in **Chapter 9**. However, there is circumstantial evidence that these swine also possess both 'refluxing' and 'non-refluxing' papillae since Hodson employed a miniature breed (Hormel) in his studies relating IRR with *reflux nephropathy*[65].

## 5.2 Aspects of Renal Function

There are subtle differences between the pig and man in both anatomical and functional aspects of renal physiology but on the whole renal processes are similar. Nephron and glomerular structure has been shown, in one variety of miniature pig, to closely resemble that in the human[162]. The glomerular number in the pig has been calculated as $11.93 \times 10^5$ and in the human as $10.95 \times 10^5$ with respective glomerular volumes (ml/g of kidney) of 37.3 and 29.4[163,164]. Like man the pig has a predominance of short looped nephrons; the long looped nephrons comprise 3% in the pig and 14% in man[157,165]. The relative medullary thickness (layer medulla thickness compared to whole kidney size) is 1.6 in the pig and 3.0 in man[157].

The most marked anatomical differences between the pig and human occur in the renal circulation. With the known exception of the Landrace pig, in other breeds the major divisions of the renal artery

are cranial and caudal in contrast to the anterior and posterior division in the human[166]. The medullary circulation of the pig is less organised than in man[157,167] and the muscular layer of arteries is reportedly greater in the pig than in human especially in the inter-lobular arteries[162]. These circulatory differences and the variation in the proportions of long looped nephrons may be of importance in the ability to concentrate urine and this is discussed further in **Chapter 14**. Nevertheless urinary concentration appears to be adequately achieved in the pig by the same or similar countercurrent mechanisms as operate in the human kidney[162,165,169]. During dehydration the maximal urine to plasma osmolality ratio is almost the same in pig (3.7) and in man (4.0) as is the maximal urine concentration (mosmol/kg $H_2O$), being about 1100 in the pig and 1200 in man[165,168-170].

Other notable differences between the pig and human include the excretion of creatinine which in the pig, unlike man, undergoes significant tubular reabsorption after glomerular filtration[162]. In the pig there is extensive acetylation of para-aminohippuric acid (PAH) in the liver and kidney, more so than occurs in the human, and in this form may go undetected in chemical assays[162,172]. This affects interpretation of renal function tests employing PAH in the pig. A further difference concerns the anti-diuretic hormone which in the pig is lysine vasopressin and not arginine vasopressin as in man[171]. Proteinuria has been shown to occur normally in one breed of miniature pig (Pitman-Moore)[162].

## 5.3 The Urinary Tract

### 5.3.1 Upper Tract

With the possible exception of the Landrace pig, in whom the renal pelvis is reportedly long and extra-renal[173], in general the porcine renal pelvis is more intra-renal than found in man[174]. In the young pig the upper ureter may exhibit some tortuosity, possibly exaggerated in the Landrace pig[173], otherwise the ureter is straight[17]. Despite some inter-breed variation the muscular arrangements and functional mechanisms of the ureters are similar in man and pig[175,176], as are intra-ureteric pressures (5-20mm Hg) and peristaltic activities during the transport of urine[16,176-178]. By virtue of the shared feature of a multicalyceal kidney, both the human and porcine kidney have multiple pacemakers sited at the attachment of each minor calyx[179,180]; their number depending upon the number of minor calyces in each kidney. The porcine calyx has a rhythmical contraction rate (of 12 per minute or less)[181-183], which in the absence of urinary tract pathology, is independent of urine flow and distention[183]. By contrast renal pelvic activity is erratic and increases with urine flow and distention[178,182,183].

### 5.3.2 Ureterovesical Junction

The porcine ureterovesical junction (UVJ) has the same apparent functional and anatomical attributes as its human counterpart (**1.1**). It may be distinguished from the human UVJ, however, by the thinner mucosal wall overlying the ureteral orifice[16] and by the length of the submucosal ureter, which is 10 to 15mm long in the young pig

weighing between 5kg and 30kg[184] compared with 3 to 5mm[18] found in children. The long submucosal ureter most likely contributes to the well documented absence of any naturally occurring VUR in pigs[7,15-17], although this has been found by one group[185] in about one third of young Landrace swine; again suggesting this breed of pig has some structural anomalies compared with other breeds.

In the Landrace pig, VUR has been established in experimental models by inducing bladder outflow obstruction[186]. In other pig varieties VUR has been successfully induced by surgically incising the intra-vesical wall anteriorly[7,65].

### 5.3.3 Lower Tract

The pig and human bladder have a similar cystographic appearance, both showing a smooth outline with no diverticuli and a funnel-shaped bladder neck during micturition[16,17]. Cystography can detect VUR in the pig[7,65] as it does in the human, although personal experience and that of others[16,17] indicates this is not normally observed even with bladder overfilling. In pigs the male and female urethras are different to the human forms; in the female the urethra opens some way back from the introitus and in the male there is a sharp deflection marking the division between the proximal (posterior) and the distal (anterior) portions. For these anatomical reasons suprapubic puncture of the bladder is necessary in males and preferred in females as an alternative to urethral catheterisation.

### 5.3.3.1 Normal Bladder Physiology

Intra-vesical pressures (Figure 1) recorded during bladder filling have shown that the pigs bladder is compliant with end fill pressures of 0 to 7cm $H_2O$[7,17,65,186]; the same as has been recorded in the human[187,188]. Micturition occurs with maximum intra-vesical pressures of 2 to 30 cm $H_2O$ and increase marginally with growth[7,17,65,186]. These last pressures are lower than often occurs in the human bladder; especially males in whom during voiding the normal detrusor pressure reaches a maximum of between 40 and 80 cm $H_2O$[187,188].

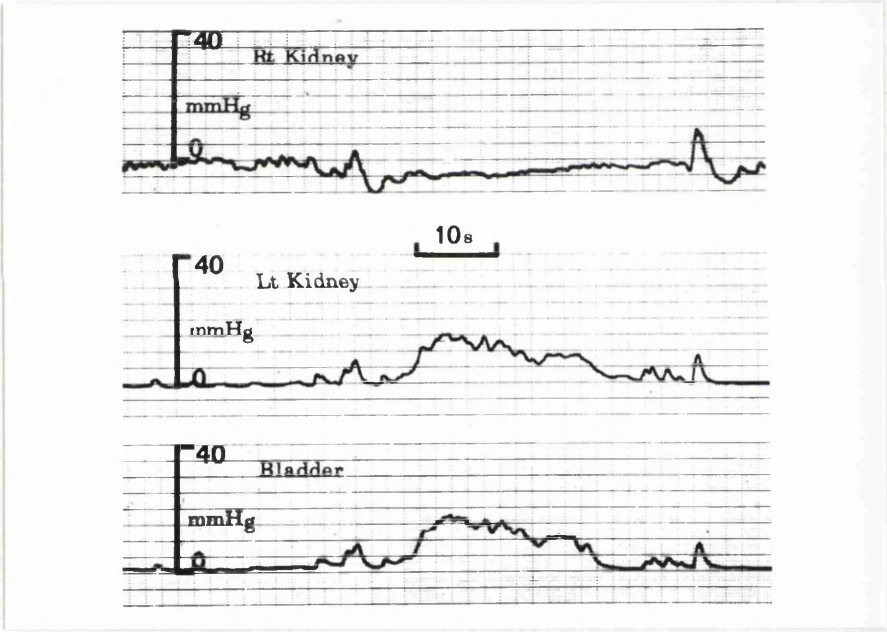



**Fig. 1 Cystometrogram From a Male Pig (Welsh) Without Bladder Outflow Obstruction.** Simultaneous intra-pelvic pressures were also recorded and demonstrate the transmission of intra-vesical pressures to the kidney in the presence of left VUR. (Previously published; Ransley[100]; reproduced with permission ).

## 5.4 Urinary Tract Function and the Section IV Experiments

Although definitive conclusions made from any animal study cannot be directly extrapolated to the human condition, the similarities between pig and human papillary morphology, and ureteric and calyceal physiology, mean that any effects of VUR in the pig are likely to be closely analagous to those occurring in the human. Since previous studies have proven the pig parallels the human in the histopathological effects of VUR, it is likely that it may also model any physiological effects of VUR.

The possible pathophysiological renal responses to VUR have been described (**Chapter 2**). Since these responses may be mediated through the ureter or renal pelvis, by effects on renal blood flow, on the papillary medulla or through intra-renal reflux, it is important that the experimental kidney and urinary tract are similar to the human. The resemblance of the pig to the human is not complete, however, particularly with regard to medullary vasculature which may influence the response to VUR. Plasma creatinine levels in the pig cannot be related to glomerular filtration function as they are in the human and protein excretion when used as a marker of renal dysfunction requires careful interpretation since this has been observed normally in one strain of pigs (**5.2**).

Of crucial importance are the low voiding pressures in the pig bladder. In order to assess, in the Section IV experiments, the effects of VUR at the extremes of hydrodynamic insult it was necessary therefore to elevate voiding pressure in some animals by

inducing bladder outflow obstruction.

## 5.5 Bladder Outflow Obstruction

Previous investigators have achieved bladder outflow obstruction in the pig by constricting the urethra with either a silk ligature, a teflon ring, or a loop of silver wire[7,17,65,186,189]. This last has the advantage that it may be preformed to an 'omega' shaped ring of known diameter providing some control over the degree of induced bladder outflow obstruction[65]. When implanted in the young animal to provide a close fit it avoids post-operative acute urinary retention, but during growth there is progressive urethral stenosis and bladder outflow obstruction[7,186].

### 5.5.1 Pathophysiological Effects

It is recognised[190] that bladder outflow obstruction in the human is characterised during voiding by abnormally high intra-vesical pressures, reduced urine flow rate and decreased voided volume. Accompanying abnormalities in bladder filling may be observed as unstable detrusor activity and/or a loss of detrusor compliance. Following implantation of a urethral stenosing device in pigs, Sibley[186] noted similar urodynamic abnormalities occurring both during bladder filling and emptying which were not observed in control pigs without bladder outflow obstruction. In pigs with bladder outflow obstruction from this latter[186] and other studies[7,17], intra-vesical pressures have been recorded in the range 15 to 72cm $H_2O$ in female pigs and 35 to 115cm $H_2O$ in males[7,17].

In addition, abnormal detrusor activity (detrusor instability; hyper-activity) occuring during the bladder filling phase has been noted[186,191] and on occasion a loss in bladder compliance has been recorded[186].

Morphological studies of the detrusor muscle have shown that bladder outflow obstruction in the pig and human causes an increase in muscle cell size and an increase in bladder wall thickness[189,192,193]. Pharmacological studies using isolated bladder strips from pigs with bladder outflow obstruction suggest these morphological changes are accompanied by partial denervation of the detrusor, affecting post-synaptic neurotransmitter supersensitivity[189,193].

In some circumstances when a critical degree of urethral stenosis results from pig growth, more severe bladder functional abnormalities develop which necessitate removal of the urethral constricting device or termination of the experiment. In profound contrast to normal bladder function (Figure 1), abnormalities present as frequent or dribbling micturition with especially high and fluctuating detrusor pressures (Figure 2a). In extreme cases, most often reported in studies employing female pigs[17,65,71,194], micturition is sometimes accompanied by abdominal straining and the bladder may become tense and palpable. Other associated pathologies have been reported as bladder diverticuli, dilatation of the upper urinary tracts and on occasion the presence of reflux in a previously 'non-refluxing' ureter[17]. When VUR occurs in the presence of such extreme urodynamic abnormalities renal scarring has been noted in the absence

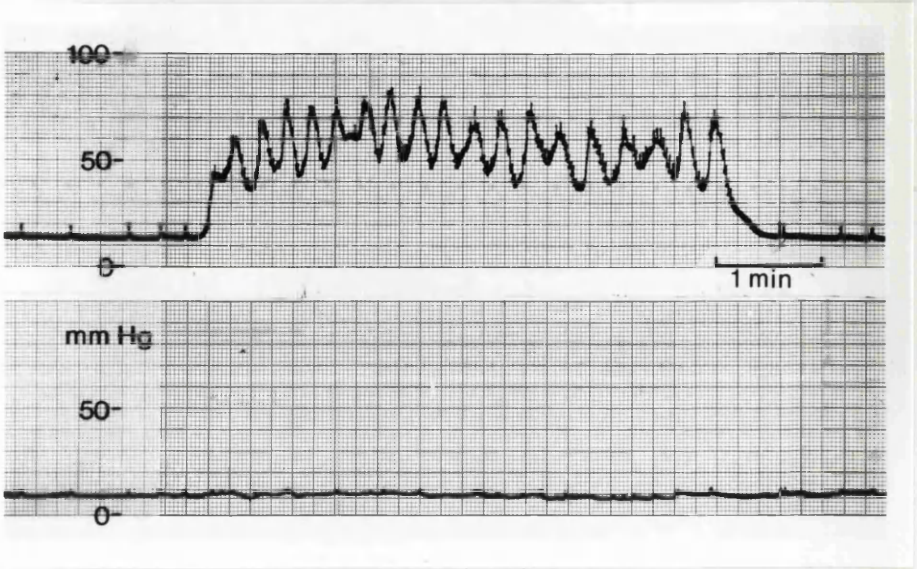



Fig. 2a Severe Urodynamic Abnormalities in a Male Pig (Large White) with Bladder Outflow Obstruction. One of a series of frequent, prolonged and fluctuating detrusor contractions taken from a cystometrogram, 13 weeks after implantation of a urethral ring. (Unpublished figure from a previous series[71]).

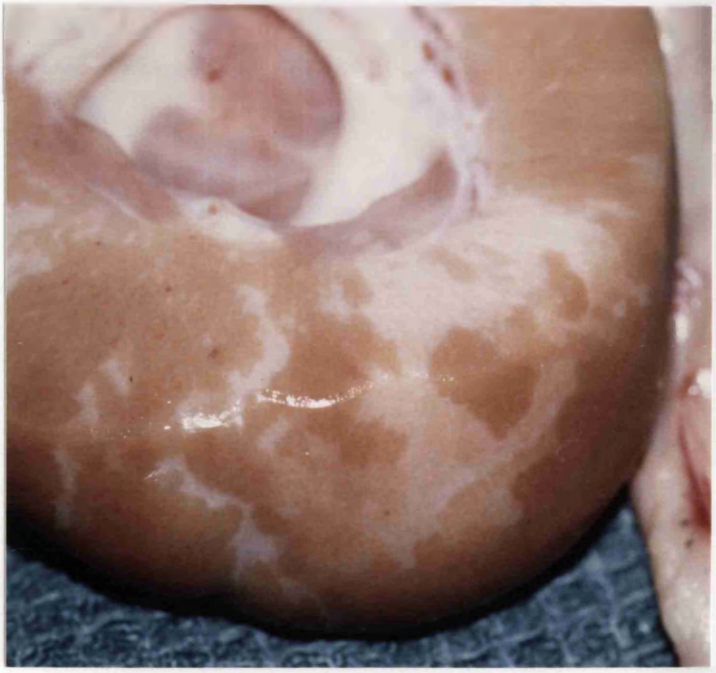


Fig. 2b Renal Scarring Associated with Severe Urodynamic Abnormalities. The upper pole of a kidney removed at *post mortem* from the same pig 2 weeks after the above cystometrogram when there was acute bladder outflow obstruction. The kidney had been exposed to VUR.

of urinary infection (Figure 2b)[65,71,73,194].

In the Section IV experiments, examining only the physiological effects of VUR it becomes important, therefore, to tailor the degree of bladder outflow obstruction so that the required conditions of abnormal bladder function and elevated intra-vesical pressures can be achieved but in the absence of renal scarring, and this is considered further in **Chapter 9**.

CHAPTER 6

# GROWTH

In assessing the effects of VUR on renal growth and function in a model to reflect as far as possible the clinical circumstances of VUR during infancy and childhood, it was important that a growing pig was used for the Section IV experiments and that animals were followed from soon after birth. In order to appreciate how growth and development of the pig compares with that of the human this chapter reviews somatic and kidney growth and is followed by a chapter considering the development of renal function.

## 6.1 Somatic Growth

The analysis of somatic growth is complex. Tanner[195] proposed a technique for measuring growth in children which requires 15 body measurements, representing 5 components of physical growth. Since the mathematical analysis of such multi-variables is complex, in the clinical setting growth is measured most often simply from height and/or weight[196]. In pigs, crown to rump length together with height might conceivably simulate anthropometric height measurement but it is rarely used and the preferred unit of growth is weight. Body weight, therefore, forms the common unit with which human and porcine growth can be compared, but has the disadvantages that, as a measure of growth, it may be affected by differences in fat deposition and other factors such as oedema and pregnancy.

### 6.1.1 Early Growth in the Pig and Human

At corresponding stages during intra-uterine development the pig and human are alike, although they differ in the time course of growth[197]. In the human a greater proportion of the gestation period is committed to the later stages of fetal development and accelerated growth than it is in the pig. In man, birth interrupts the steepest point of the sigmoidal growth curve whilst in the pig birth occurs before rapid growth is reached. The mean intra-uterine growth rate is 12.5g per day in the human compared with 4.2g per day in the pig. The neonatal pig weighs 1.5kg compared with 3.5kg for the newborn infant but during the suckling period the mean growth rate for the pig (295g/day) greatly exceeds that of the human (25g/day) and at the end of the natural weaning period the pig weighs about 18kg compared with the average human weight of 8kg at this stage[198,199].

### 6.1.2 The Pattern of Human Growth

In the human infant, growth is greatest during the first year of life and then declines to reach a minimum after the age of 2 years (Figure 3). Further growth is slow until the age of 10 years when body weight is about 30 to 40kg. A second phase of accelerated growth occurs with puberty between the ages of 12 and 15 years, after which growth slows dramatically and ceases at around 18 years. The average male body weight at this time is 64kg[196].

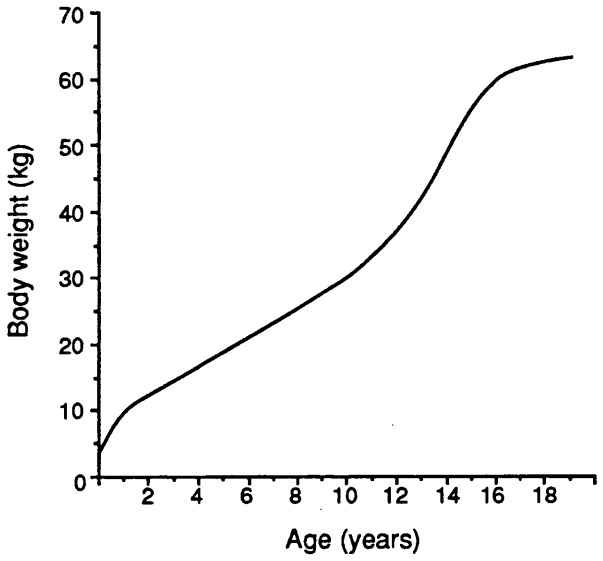



Fig.3 The Human Growth Curve for Weight.
(Adapted from Tanner, Whitehouse and Takaishi[196]).

### 6.1.3 The Pattern of Growth in the Pig

Compared with the nearly 2 decades taken for the human to reach full maturity and adult weight, the pig takes only 3 to 5 years. Besides the difference between the human and pig in the stage at which early growth rate is maximal, there is a further difference in that the porcine growth curve is not distinguished by a second phase of accelerated growth associated with puberty.

It should be noted that the growth rate and adult body weight of the pig are particularly susceptible to nutritional deprivation both before and after birth[200].

#### 6.1.3.1 The Large Domestic Pig

In the domestic pig the greatest velocity of growth is attained by

the age of 6 to 8 months (Figure 4), and continues to the age of one year when body weight is about 160kg. Thereafter, growth rate declines but is nevertheless substantial and at the age of 3 years the body weight can reach 370kg[200]. Sexual maturity occurs around the age of 6 months[201].

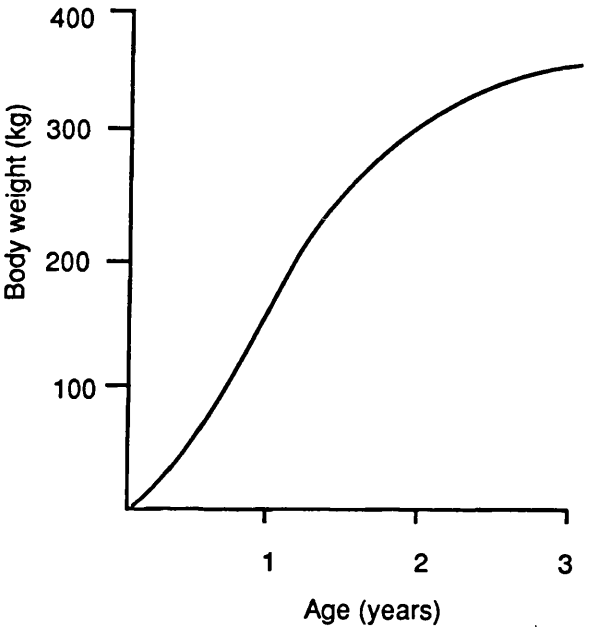



Fig.4 Weight Growth Curve for the Domestic Pig.
(Large White; adapted from Widdowsen[200])

### 6.1.3.2 The Miniature Pig

Since the pig provides such a useful model for the human, selective breeding has made available miniature swine of more convenient size[202-205]. There may be some variation between specific breeds of miniature pigs but in general their adult weight, when aged 2-3 years, rarely exceeds 160kg, and in general is considerably less at around 70-80kg[203,205]. Whilst the mean growth rate of these animals

from a birth weight of about 0.8kg is inevitably less than that of large domestic pigs, their pattern of growth is similar (Figure 5). Maximum growth velocity is attained by the age of 6 to 8 months and continues to the age of one year when body weight is about 50kg. Compared with large pig varieties, a more abrupt decline in growth rate occurs after the age of 18 months when body weight is about 70kg.

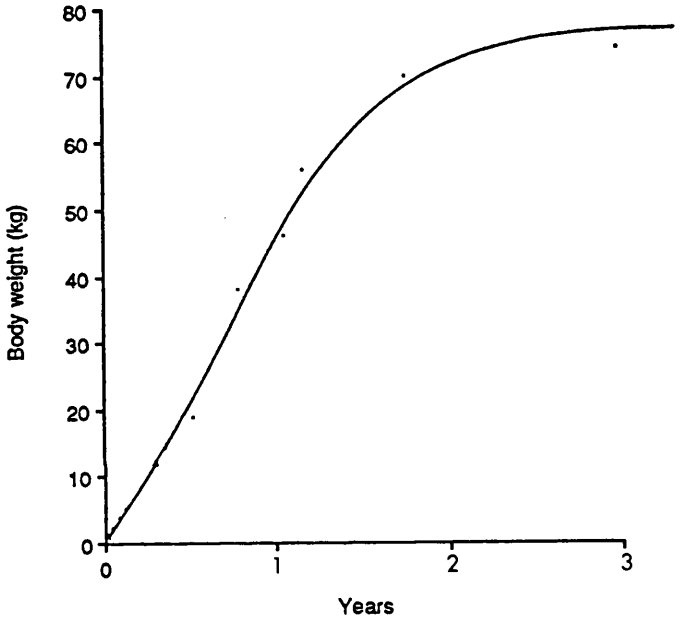



Fig. 5 Weight Growth Curve for Miniature Swine.
(Pitman-Moore variety; adapted from Thomas and Beamer[205])

## 6.2 Kidney Growth

From comparative studies[206] in a number of mammals, ranging in size from that of the kangaroo rat to the elephant, it has been shown that a similitude exists in the relationship between body and kidney mass which fits the equation; kidney weight(g) = 8.22 × body weight(kg)$^{0.85}$. Although this relationship is only an approximation, it implies that

with increasing inter-species body weight the relative kidney weight decreases. Also, it suggests that in animals of different species, but of the same body weight, kidney weight may be expected to be similar. It is presumptuous to assume that such an inter-species similitude is retained for immature mammals, nevertheless a similarity in the relationship of body and kidney weight is apparent between the domestic pig and the human at their various body weights during growth (Figure 6). In terms of weight, kidney growth is slower than body growth as a whole and therefore exhibits negative allometric growth (Figure 6).

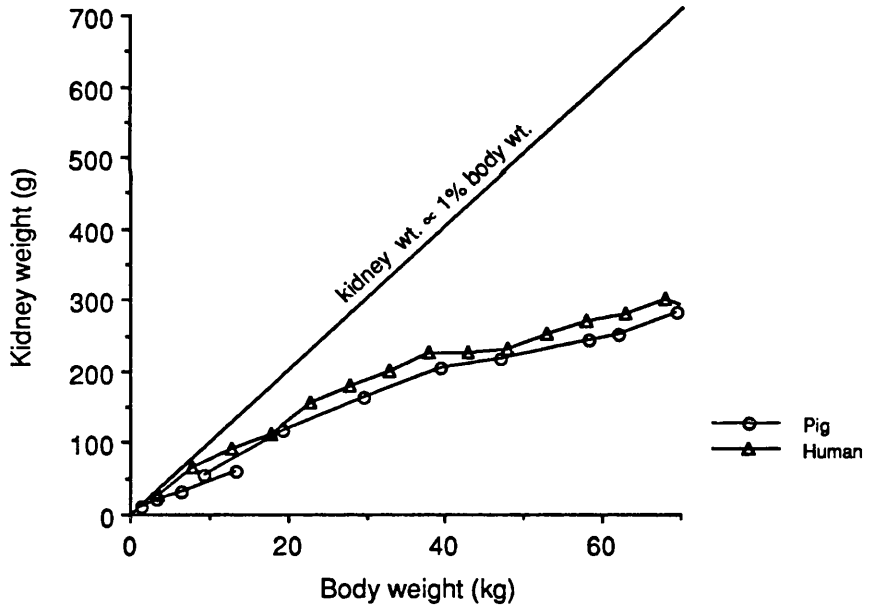



**Fig. 6 The Relationship between Renal and Body Mass in the Human, and Large Domestic Pig. (Human data from De Jesus, De Leon and Ramos[210]; Pig data from Sarker, Lodge and Friend[215]; Doornenbal and Tong[206]).**

### 6.2.1 Kidney Growth in the Human

In the full term human neonate combined kidney weights are normally between 24 and 30g[207,208] and during growth increase 10-fold to reach a combined weight of about 300g in the adult male[209,210]. In general the pattern of human kidney growth is described from the relationship between renal mass and body surface area when a constant proportionality appears to exist after early infancy[211,212]. Analysis of published data from Copelletta and Wolbach[207] and from Oliver and Rubenstein *et al*[213] shows that whilst kidney growth is slower than somatic growth its pattern, with age, in children up to 12 years, is similar to that for body growth as a whole. The greatest velocity of renal growth is achieved by the age of 1 year, whereupon it declines. From age 2 to 3 years it is slow until the age of 10 to 12 years when the combined kidney weight is about 170g. Combined kidney weight relative to body weight derived from published data[196,207,210,213] is about 0.8% in the new born, and remains at this value for about 6 months. Subsequently it decreases to 0.6% at 10 years and to 0.35% in the adult male.

### 6.2.2 Kidney Growth in the Domestic Pig

In new born pigs, individual kidney weights of about 5g, or combined kidney weights of about 10g, are common in a number of domestic breeds[198,214,215]. During 3 years of growth, kidney weight may increase 50-fold and a combined kidney weight of 502g has been recorded in a Large White pig weighing 370kg[200]. Kidney weight has been observed to double within the first 10 days of life in unison with a 2-fold increase in body weight[214,215]. Consistent with this

observation, combined kidney weight relative to body weight values, calculated from published data[215], are found to remain at the new born level of about 0.7% (also corroborated by other studies[198,214]) for the first 10 days and to fall precipitously in the following 10 days, thereafter, to decline gradually[216]. At 3 years relative kidney weight may be as low as 0.14% of body weight.

### 6.2.3 Kidney Growth in the Miniature Pig

In one strain of miniature swine (Pitman-Moore) a combined mean kidney weight value of 6.5g has been reported for new born animals compared with a mean value of 240g recorded for animals aged 3 years and weighing a mean of 74kg[205]. This is equivalent to a 37-fold increase in kidney weight during growth. As in the large domestic pig, kidney weight doubles concurrently with body weight gain within the first 10 days of life. Combined kidney weight relative to body weight is calculated as 1.0% in the new born and 0.3% in the adult.

### 6.2.4 Comparative Aspects of Kidney Growth.

Although renal growth in humans and the large and miniature pig is allometric over the span of the growing period, it is relatively more proportional to body growth during the post-natal period than it is in later life. In the pig the rapid and isometric kidney growth in the first days of life contrasts with, and may reflect, the absence of any significant renal growth during the final 16 days of intra-uterine life, despite body growth during this period[214]. It is apparent (Figure 6) that although there is undoubtedly rapid renal growth in the pig this merely reflects rapid body growth consistent

with the achievement of a large adult size, relative to that at birth, in a short growing period. In respect to body size, pig and human kidney growth is similar (Figure 6).

CHAPTER 7

# RENAL FUNCTION DEVELOPMENT

## 7.1 Development of Renal Function

In utero the kidney has a passive role secondary to the placenta in maintaining salt and water homeostasis. In both the human and the pig, glomerular filtration rate (GFR) has been observed to increase abruptly in the days after birth, with further less dramatic increases during growth (7.2; 7.3). The development of renal function, observed in early life, depends largely upon maturation of physiological processes (see below), although growth-related changes affecting the size and length of the nephrons and the surface area of the glomerular basement membrane have a contributory role[217]. In the porcine kidney these latter changes will also include nephrogenesis which has been shown to persist for up to 3 weeks of extra-uterine life with continued glomerular differentiation up to age 3 months[218]. This is unlike the human in whom nephrogenesis is completed during fetal development, at about 35 weeks of gestation[219].

Studies in various experimental animals have established the likely factors determining the developmental rise in GFR. This latter cannot be attributed solely to an increase in renal mass with growth since GFR relative to kidney weight is low in the new-born compared with that which occurs during later growth and in the adult[220-222]. Of the factors associated with the *post natal* increase in GFR,

increased glomerular transcapillary hydraulic pressure is of considerable importance and is effected in part by an increase in systemic blood pressure[223,224]. In the later maturation of GFR greater significance is ascribed to increased renal and glomerular plasma flow rate accountable by a decrease in intrinsic vascular resistance[225-228] as well as a fall in haematocrit[226]. The rise in renal blood flow which occurs in early life has been found to favour the renal cortex in dogs[229] but not lambs[226]. In dogs the consequent increase in glomerular perfusion may be associated more with superficial nephrons since it has been shown that during development intra-renal blood flow distribution changes to favour the outer cortex[230]. Consistent with these observations are those showing a developmental increase in the superficial individual nephron GFR in both dogs and rats[222,227].

Further experimental studies have also indicated that both morphological and physiological changes are required for the achievement of full urine concentrating capacity by the kidney irrespective of its dependence upon glomerular filtration. In common with observations in some animals[222,231,232] maximum concentrating capacity has been shown to be lower in the human neonate and infant than in the adult[231,233-235]. Concentrating capacity in rats has been shown to correlate with the corticopapillary distance and elongation of the superficial loops of Henle; changes likely to influence establishment of high medullary osmolality by an effect on urea recycling[236,237]. In addition, a decreased ability of vasopressin to stimulate adenyle cyclase in

isolated distal tubules from the neonatal rabbit has been demonstrated in comparison with results obtained from the adult rabbit preparation[238].

## 7.2 Glomerular Filtration Rate During Human Growth

By convention, in clinical practice, glomerular filtration rate (GFR) is expressed as a unit of body size; taken as the average body surface area (BSA;1.73m$^2$) of the adult male, and this is discussed further in Section III (10.2). When GFR in children and adults is expressed in this manner it is moderately independent of age or body weight and in general is between 90 and 140ml/min/1.73m$^2$BSA when measured by inulin clearance[239]. Nevertheless, it is recognised that after birth it takes 2 to 3 years for GFR to reach these levels (Figure 7).

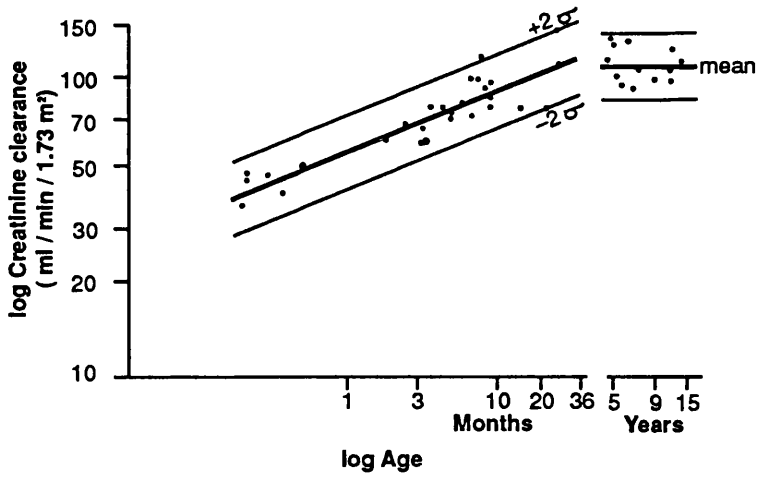



Fig.7 Endogenous Creatinine Clearance GFR ml/min/1.73m$^2$ and Age in the Human.
(Adapted from Winberg[240])

Early work by Oh, Oh and Lind[241], investigating 43 normal neonates, suggests that in the first day of life inulin clearance GFR is a mean of 24ml/min/1.73m$^2$BSA. Lower values have been reported in a more recent study but this included premature and growth retarded neonates, only 4 of whom were aged 1 day[242]. In the first few months of life GFR (per unit fraction BSA) increases rapidly and has been found to more than double during the first few weeks of life[242,243]. In infants aged between 6 months and one year a mannitol clearance GFR of about 77 ml/min/1.73m$^2$ appears to be normal and adult values have been found in infants aged between 2 and 3 years[244]. However, mannitol clearance is lower than inulin clearance by a ratio of 0.88[245,246].

There is a paucity of data documenting the development of GFR in absolute values of ml per minute. A graph (Figure 8) constructed by McCrory[247], using published data, suggests that the increase in GFR (ml per minute) is greatest in the first year of life, with a further gradual increase up to 14 years, when adult levels are approached but are yet to be finally attained. This graph indicates that the velocity of developing GFR is in keeping with that for body weight growth as a whole[196] and for kidney weight (**6.2.1**). There is some indirect evidence, however, that the early increase in GFR exceeds renal growth[244], in common with observations in experimental animals[220-222].

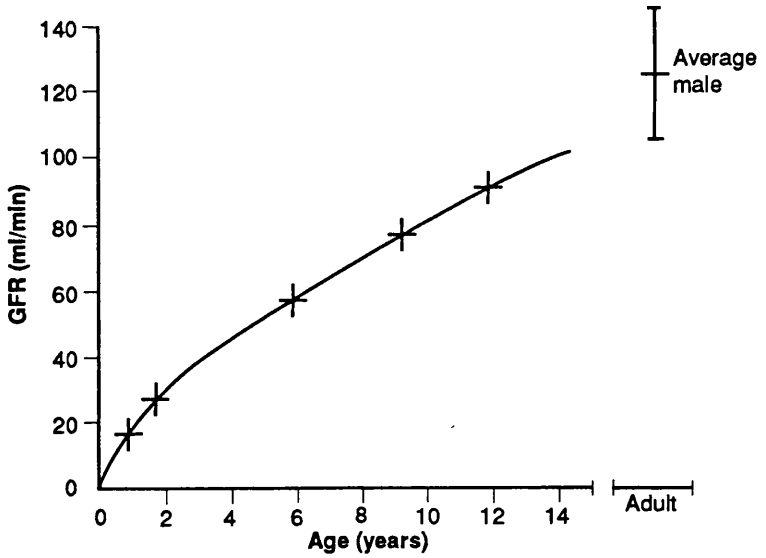



Fig.8 Mean Values for Inulin Clearance GFR ml/min with Age in the Human.
(Adapted from McCrory[247]).

### 7.2.1 The Association With Renal Blood Flow

There is only indirect evidence that in the human the developmental increase in GFR is associated with haemodynamic events. Of these, a contributory factor may be an increase in mean systemic arterial blood pressure, since this has been shown to progressively rise from about 60 to 70mm Hg in the neonate to 100mm Hg at puberty[248-250]. In addition to these observations there is a well documented 5- to 6-fold increase in para-aminohippuric acid clearance ($C_{PAH}$) during the first year of life[251-252]. Although renal plasma flow calculated from $C_{PAH}$ is underestimated by up to 50% in infants up to the age of 5 months[244], there remains at least a 3-fold increase, relative to body surface area, accounted for by an increasing renal plasma flow. Gruskin, Edelmann and Yuan[228] have calculated that the fractional cardiac output to the kidneys is likely to be 5-6% in the human neonate compared with 15-25% in the adult[253]. They have

suggested that these changes in renal blood flow can be largely explained by their observations in experimental animals of a fall in intrinsic renal vascular resistance after birth and during early life. That a similar change, in intrinsic renal vascular resistance, also occurs in the human is supported by morphological studies showing that the ratio of human renal arteriolar wall thickness to lumen diameter decreases from birth through early childhood[254].

An immaturity of the renal circulation, acting both independently and as a factor in affecting GFR, is consistent with observations made in infants of poor renal uptake of dimercaptosuccinic acid (DMSA)[255]. The renal uptake of DMSA has been found to be related to GFR[256,257]; to renal blood flow[258] and to effective renal plasma flow [259-261]. In addition, since both PAH and DMSA are organic acids, factors which effect the poor renal extraction of PAH may also affect the renal uptake of DMSA.

### 7.3 Glomerular Filtration Rate During Pig Growth

In experimental practice there is no common unit for defining GFR, which may be expressed as ratio values with kidney weight or, as is most usual, with body weight. The dangers of using this latter for comparison of GFR in animals of different breeds or ages is discussed later (**10.2**). Although the body surface area of the pig has been estimated[262] its application in relation to GFR is infrequent. It is recognised that contradictory estimates of porcine GFR preclude

its definition in pigs[263]. Published data is presented here only from studies where GFR was estimated from inulin clearance and preference is given to those using conscious animals. It was necessary to include studies in animals anaesthetised with sodium pentobarbitone, but this has been shown to have little or no effect on renal blood flow and GFR[264]. Apparently, anaesthesia induced by a variety of agents does not affect renal function in neonatal pigs[265], but the same may not be true for older animals.

### 7.3.1 Glomerular Filtration Rate: The Large Domestic Pig

In new born Landrace pigs mean values for GFR of 4 and 5ml/min have been estimated from conscious and anaesthetised pigs weighing between 1.5 and 2.5kg[266,267]. These values have been shown to more than double in the first 3 weeks to reach an estimated mean of 12ml/min, and by the age of 8 weeks are a mean of 46ml/min[266]. An increase in GFR with later growth has been demonstrated in individual Landrace pigs as a progressive rise with increasing body weight[268] (Table 1).

Table 1 Inulin clearance GFR in large domestic pigs.

| Age (Weeks) | Body weight (kg) | GFR (ml/min) | Reference |
|---|---|---|---|
| >1 | 1.5 - 2.0 | 4 - 5 | 266;267 |
| 3 | 5.5 | 12 | 266 |
| 8 | 15 | 46 | 266 |
| | 28 | 64 | 268 |
| | 39 | 80 | " |
| | 49 | 122 | " |
| | 58 | 122 | " |
| | 69 | 145 | " |
| | 90 | 162 | " |
| | 123 | 234 | " |

The rise in GFR during early life has been shown to exceed that of kidney growth in a study using Landrace swine[266]. The mean values for GFR ml/min/g kidney weight increased from 0.27 in newborn pigs to 0.39 in pigs aged 3 weeks and to 0.59 in pigs aged 8 weeks. This last value is similar to the mean of 0.56 found in other Landrace pigs ranging in weight from 28 to 123kg body weight[268].

### 7.3.2 Glomerular Filtration Rate: The Miniature Pig

There is less information available concerning the development of GFR in miniature swine. In pigs aged 1 week GFR has been estimated as a mean of about 5ml/min and shown to double to an estimated 10 ml/min by the age of 2 weeks[269]. A rise in GFR with body weight gain has been noted[162] in individual Pitman-Moore pigs (Table 2). In both these latter studies, pigs were anaesthetised with sodium pentobarbitone. In a further study of mature Sinclair pigs aged between 2 and 5 years and weighing between 115 and 158kg there was a small positive association between GFR and increasing body weight, irrespective of obesity. The mean GFR value for the group was 129ml/min[203].

Table 2 Inulin clearance GFR in miniature pigs.

| Age Weeks) | Body weight (kg) | GFR (ml/min) | Reference |
|---|---|---|---|
| 1 | 2 | 5 | 269 |
| 2 | 3 | 10 | " |
| | 13 | 36 | 162 |
| | 15 | 54 | " |
| | 18 | 62 | 269 |
| | 24 | 106 | 162 |
| | 27 | 83 | " |

There is evidence, but only from studies with animals anaesthetised with sodium pentobarbitol, that the increase in GFR during early growth also exceeds kidney growth in the miniature pig. Results in the Sinclair pig have shown that with an increase in body weight from a mean of 2kg at age 1 week, to a mean of 18kg at an unspecified age, the mean GFR ml/min/g kidney weight value doubles from 0.34 to 0.67[269]. This last value is greater than has been reported in domestic pigs of equivalent or greater degree of maturity[268]. There are indications, however, that variation occurs in the ratio of GFR to kidney weight since lower values have been noted in individual pigs of the Pitman-Moore variety weighing between 12 and 27kg and in this last case was a mean of 0.52[162].

## 7.4 Renal Blood Flow During Growth in the Pig

The use of more invasive techniques in the pig have allowed measurement of renal blood flow, with a flow transducer, or by microsphere trapping methods, as well as from the clearance to extraction ratio of para-aminohippuric acid (PAH), *i.e.* $C_{(PAH)}/[E_{(PAH)} \times 1\text{-haematocrit}]$; however, these measures necessitated the use of anaesthesia. In the young pig poor tubular transport of PAH has been established[270] and as in the human the renal extraction is low in the neonatal pig but reaches the 87% efficiency of the adult by age 3 weeks[266].

### 7.4.1 Renal Blood Flow: The Large Domestic Pig

In a study using the PAH method, Fris[266] demonstrated that as units per kidney weight an approximate 3-fold increase in renal blood flow accompanied the 2-fold increase in GFR occurring in the Landrace pig between birth and 8 weeks of age. These results were substantiated and further elaborated by microsphere trapping measurements of renal blood flow undertaken by Gruskin, Edelmann and Yuan[228], in pigs of unspecified breed and weight. They showed that an increase in renal blood flow (per unit body surface area) occurring in the first 6 weeks after birth was accompanied by an increase in the proportion of the cardiac output perfusing the kidneys. This was effected during the first 3 weeks of post-natal life by increases in both systemic blood pressure and cardiac output, but the increased renal perfusion occurring in the following 3 weeks could be attributed only to a decrease in the intrinsic renal vascular resistance.

By the age of 8 weeks renal blood flow determined by Fris[266] was found to be between 3 - 4ml/min/g kidney weight and to be similar to values reported for older animals with body weights up to 123kg[266,268]. It would appear, therefore, that after 8 weeks of age the increase in renal blood flow results from accumulating renal mass and in older animals this is likely to be the primary determinant of GFR. This is further supported by renoscintigraphy studies, using $^{131}$I-iodohippuran and $^{99m}$Tc-DTPA showing effective renal plasma flow rate (ml/min) rises in parallel with the increase in GFR in Large White pigs between ages 4 and 12 months[271].

### 7.4.2 Renal Blood Flow: The Miniature Pig

A decrease in renal vascular resistance and a rise in renal blood flow has been found to accompany the developmental rise in GFR in miniature swine (Sinclair variety)[269]. Renal blood flow was measured with a flow transducer and noted as a mean of 2ml/min/g kidney weight in pigs aged 2 weeks and weighing 3.0kg compared with a mean of 4 ml/min/g kidney weight in pigs weighing a mean of 18kg This last value for renal blood flow is the same as has been reported for large Landrace swine of equivalent or greater degree of maturity[266,268].

## 7.5 Summary: Comparative Developmental Function

Despite an apparent immaturity of the porcine kidney at birth compared with the human the absolute values for GFR, approaching 5ml/min, are similar for the human[272], large[266,267] and miniature pigs[269] and the fraction of cardiac output reaching the kidney is 5-6% in humans and 4-5% in pigs[228]. In both the human and porcine kidney the most dramatic changes in renal function occur in the period immediately following birth. There are further rapid but less dramatic increases which extend up to 6 months to 1 year in the human and up to 8 weeks in the large pig. In the miniature pig this period is unknown but may well be longer than for large breeds of pig. In the pig it has been shown that these early changes exceed the increase in kidney weight and that they are related to haemodynamic events. It is recognised that this probably also occurs in the human.

A direct comparison of GFR between the pig and man during growth is complicated by the use of different derived units for expressing GFR, but an inulin clearance GFR of 140 ml/min recorded in a large pig weighing 70kg compares favourably with the normal adult human GFR. In the human adult, GFR is about 0.4ml/min/g of renal weight[239]; in the large pig the corresponding value is about 0.56[268] and in the miniature pig this may be about 0.5[162]. Renal blood flow is approximately the same at 4ml/min/g kidney weight in adult man[273] and mature large[266,268] and miniature pigs[269]. Since an approximate similarity in renal weight with respect to body mass has been demonstrated for the pig and human (**6.2**) it may be reasoned that a similarity in the development of GFR with body weight gain may also exist.

CHAPTER 8

# THE SOLITARY KIDNEY MODEL:

# THE RENAL RESPONSE TO UNILATERAL NEPHRECTOMY

Since the investigation of VUR described in Section IV required the use of pigs retaining solitary kidneys following unilateral nephrectomy it is important to consider how contralateral nephrectomy might affect growth and function of the remaining kidney. There is a scarcity of reports concerning this aspect in the pig but an impression of the likely events can be gained from a brief review of the published literature. The subject has been extensively investigated and recently reviewed in some considerable depth by Wesson[274].

## 8.1 A Review

It is now well established that following unilateral nephrectomy the remaining kidney functionally adapts and enlarges to compensate for the loss of renal mass[275].

In the initial period following unilateral nephrectomy there is a rapid functional response. The majority of studies in rats and dogs show there is an immediate increase in urine flow and in cation excretion[274,276-278]. In human kidney donors creatinine and para-aminohippuric acid clearances reach about 70% of the pre-operative values in 7 days following surgery[279,280]. Similar findings have been documented in dogs and individual kidney clearance

values increased by between 20% and 30% within 24h of unilateral nephrectomy[281]. This functional adaption has been attributed in dogs to an immediate increase (30%) in blood flow to the remaining kidney[282]. However, an absence of any immediate increment in glomerular filtration rate (GFR) and renal blood flow in dogs has been observed in other studies[283]. In rats, an early (within 24h) rise in GFR has been noted in some studies[284-286] but not in others[276,278]; however, morphological studies using fluorescent staining techniques have indicated that remaining kidney glomerular perfusion increases within minutes of contralateral nephrectomy[287]. In the first weeks following unilateral nephrectomy in rats there is a substantial increase in GFR which although concomitant with accumulating renal mass is associated with, and apparently governed by, glomerular plasma flow[288-290]. Although studies of the early response have to be interpreted cautiously, in view of the stressful effects of surgery and in many cases the absence of sham-operated controls, it does appear that the early adaptive response to nephron loss involves heamodynamic and humoral factors and may not necessarily be dependent upon early compensatory growth. This latter may be important, however, in restoration of tubular function. Fractional proximal tubular reabsorption of water is decreased in rats and dogs immediately after unilateral nephrectomy[276,277]. Urinary concentrating ability, reduced after unilateral nephrectomy in dogs, returns to normal levels only 8 weeks after surgery[291]. Bugge-Asperheim and Kiil[292] concluded from their studies in dogs that increased clearance function was not dependent upon growth but that tubular transport of glucose and para-aminohippuric acid was.

There is evidence from studies with mice that contralateral kidney growth is initiated within minutes of unilateral nephrectomy[293]. After removal of one kidney in rats both the wet and dry weight of the contralateral organ increase by the second day[294]. After 2 to 3 weeks, single kidney weight in uninephrectomised rats is on average about 35% more than that of one of a normal kidney pair[274]. In reviewing the literature Wesson[274] found, in general, that a similar degree of increase in remaining kidney weight occurred in dogs during the weeks following unilateral nephrectomy. In the human, radiographic measurements suggests compensatory renal enlargement also occurs[295-299] but only over a period of years[299].

Both the rate of compensatory renal growth and the maximum mass attained may vary with species. Compared with the years required for compensatory enlargement in the human, in the rat this is complete by 40 days[300]. However, greater significance has been attached to age at the time of nephrectomy, since the capacity for a compensatory response appears to be enhanced in the young and attenuated by age[297,299-305]. Nevertheless, irrespective of age, the renal mass achieved by compensatory growth is unlikely to match that of combined paired kidneys. Studies in rats suggest that it is only in the very young that compensatory growth can exceed a 50% increase over the expected weight of an equivalent but normal single kidney (i.e. the solitary kidney achieves 75% or less of the expected renal mass)[274]. A similar limit to the degree of compensatory renal growth following unilateral nephrectomy also appears to exist in the dog[291] and in humans[298,299].

The increased cortical mass resulting from compensatory growth reflects an enlargement of nephrons. Glomerular diameters increase in the rat but not in the dog; however, in both, the lengths and widths of the convoluted tubules enlarge[306-310]. There has been some question as to whether or not new nephrons are formed in response to renal loss in the neonate; however, cumulative evidence has formulated the opinion that they are not; even in the rat in whom a nephrogenic zone is present for about one week following birth[274,304,311-313]. An increase in the mean single nephron perfusion accounts for the functional adaption in GFR and has been demonstrated in rats[288,289]. In the neonatal guinea pig there is an acceleration of the normal redistribution of renal blood flow which occurs during development[314]. It is unknown whether this precocious perfusion of immature nephrons enhances the compensatory response in the very young.

Studies in rodents have shown that compensatory growth is initiated by an immediate increase in cell size followed by a burst of mitotic activity which returns to normal levels within days[315-320]. This cellular response has been shown to occur in weanling, mature, and old rats[320,321]. These studies suggest that after an early phase, which includes cell division, subsequent growth is accomplished predominantly by cell enlargement, and the mechanism may well be largely independent of age, despite reports to the contrary[302,303]. Observations of an increased level of cellular hyperplasia and enhanced compensatory growth in the very young may be explained by

considering the effect of a burst in mitotic activity occurring during the usual processes of renal growth, which include both cell enlargement and cell proliferation[322,323]. Thus, daughter cells produced during the brief episode of compensatory cellular hyperplasia are, in the growing animal, subjected to further hyperplasia as well as hypertrophy in the normal course of growth, unlike those in the mature animal where cell division is normally minimal[321]. In any event it would appear from Wessons review[274] that growth terminates with a normal ratio of cytoplasm to nucleus and near to normal proportions of various cell types and ratio of cortex to medulla.

There is no published documentation concerning the cellular mechanisms of compensatory renal enlargement in the pig but normal renal growth is characterised by continued cell multiplication, certainly for the first 50 days of extra-uterine life[215]. It is likely, therefore, that unilateral nephrectomy performed during this period will induce an enhanced compensatory response similar to that which occurs in the young rat. Unpublished results from a personal investigation in the young pig support this view[324]. The results showed, in a comparison with sham operated control pigs, that following unilateral nephrectomy at either the age of 2 or 6 weeks compensatory growth is achieved by an augmentation of normal growth involving both cell multiplication and enlargement.

Following compensatory renal growth clearance function may improve slightly from the earlier, post-operative, levels attained but it

only rarely compensates fully for the loss of the contralateral kidney. This limit to the compensatory increase in function has been observed in rats where clearance values reach 80-92% the expected value of bi-nephric animals[284,307] as well as in dogs[291] and in humans[296,298,299,325], when, for both these, the levels are usually 80-85%. In exception to these observations 2 independent groups; Simon and Zamora *et al*[326] and Wikstad and Celsi *et al*[327]; have described inulin and para-aminohippuric acid clearances of 100% the expected values in patients who were born with unilateral renal agenesis or unilaterally nephrectomised in childhood. In one of these studies[327] renal function appeared to exceed the compensatory increase in renal size and showed a deterioration with time.

In the pig with unilateral nephrectomy performed in early life the solitary kidney may conceivably be defined as one which is performing strenuously. It is probable that growth will proceed rapidly by an initial enhancement of cell proliferation followed by the normal processes of renal growth and that individual nephron perfusion will greatly exceed that of an individual normally paired kidney. The solitary kidney pig models used in the Section IV investigation and prepared by unilateral nephrectomy performed in the first weeks of life, may, therefore, have a reduced threshold above which the influence of VUR can be detected and so allow the disclosure of any potential for VUR to affect renal growth and function.

CHAPTER 9

# THE GOTTINGEN PIG MODEL

The experiments described in Section IV employed the Gottingen miniature pig. A miniature variety was preferred because smaller adult size confers practical advantages in husbandry and permits the housing and investigation of a larger number at any time. Because it was necessary to recruit animals for study over the long term the Gottingen pig was favoured as an established breed[328] which could be obtained reliably from a closed colony (**Appendix I**).

Following the general description of the pig model it is necessary to review certain aspects with particular reference to the Gottingen pig.

## 9.1 Growth

Published data[328] indicated that the Gottingen pig like other miniature varieties was especially valuable as as a model in which growth forms an essential component.

### 9.1.1 Somatic Growth

The average size or weight achieved by the adult Gottingen pig may vary between colonies since this depends upon the gene pool of the herd and the selective breeding techniques employed[328]. In an unselected sample of Gottingen miniature pigs (n = 204) generated from the nucleus herd but housed in experimental laboratories the

mean birth weight was 0.5kg. Their mean body weight is plotted against age for the first 6 months from birth in Figure 9.

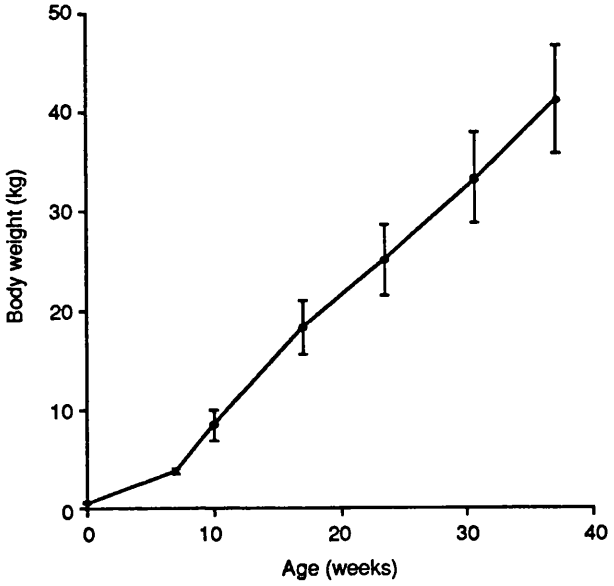



**Fig.9 Growth of the Gottingen Pig.**
(Mean (and SEM) values from Glodek[328])

Analysis of this data shows that during the suckling period (up to age 5 to 7 weeks) the mean growth rate of 64g/day is considerably less than for the large domestic pig and about twice that for the human (**6.1.1**). Body weight on weaning is about 4kg, and by the age of 6 months after more rapid growth (175g/day) is a mean of 41kg. The growth of the Gottingen pig appears similar to the one other variety of miniature pig considered (**6.1.3.2**) in whom the maximum velocity of growth occurred between ages 4 and 6 months.

### 9.1.2 Renal Growth

It was assumed that in the Gottingen pig, renal growth is similar in respect to body growth as that reported (**6.2.3**) for one other

miniature breed. This assumption was made because it is recognised that for many mammals there exists an approximate similitude in the relationship between body and renal mass, and because this has been demonstrated in a comparison of the human with a large pig variety (6.2). The assumption is supported by Figure 10 which shows combined kidney weights from 5 Gottingen pigs superimposed on the graph of data for the human and the large domestic pig.

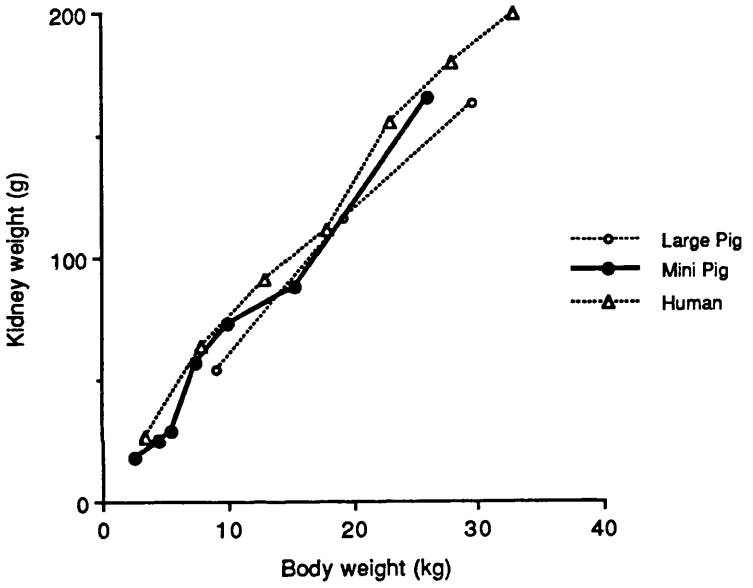



**Fig.10 Kidney Weight and Body Weight in the Young Gottingen Pig. Comparison with the Large Domestic Pig* and the Human*. (*Data as before: Fig.4).**

## 9.2 Sterile VUR and Bladder Outflow Obstruction

Previous use of the Gottingen pig had shown that the urinary tract resembled that of Large White and Welsh pigs used in earlier studies of VUR[7,69,71]. None of the anomalies associated with Landrace swine were revealed. The division of the renal arteries and the appearances of the bladder and upper tracts were similar to those

described earlier for other swine (**Chapter 5**). An earlier experiment[71] using 14 Gottingen pigs, following induction of bladder outflow obstruction with an urethral ring, had indicated that the essential conditions of sterile VUR and abnormal bladder function could be attained.

### 9.2.1 VUR

Naturally occurring VUR was not found in any of the 14 pigs examined. In all pigs the ureterovesical junction displayed the same long intra-vesical tunnel as occurs in most large breeds of pig and which appears to prohibit VUR. In all cases VUR was induced using the same surgical techniques as used previously for large pigs (5.3.2) and its presence confirmed by cystography (Figure 11).

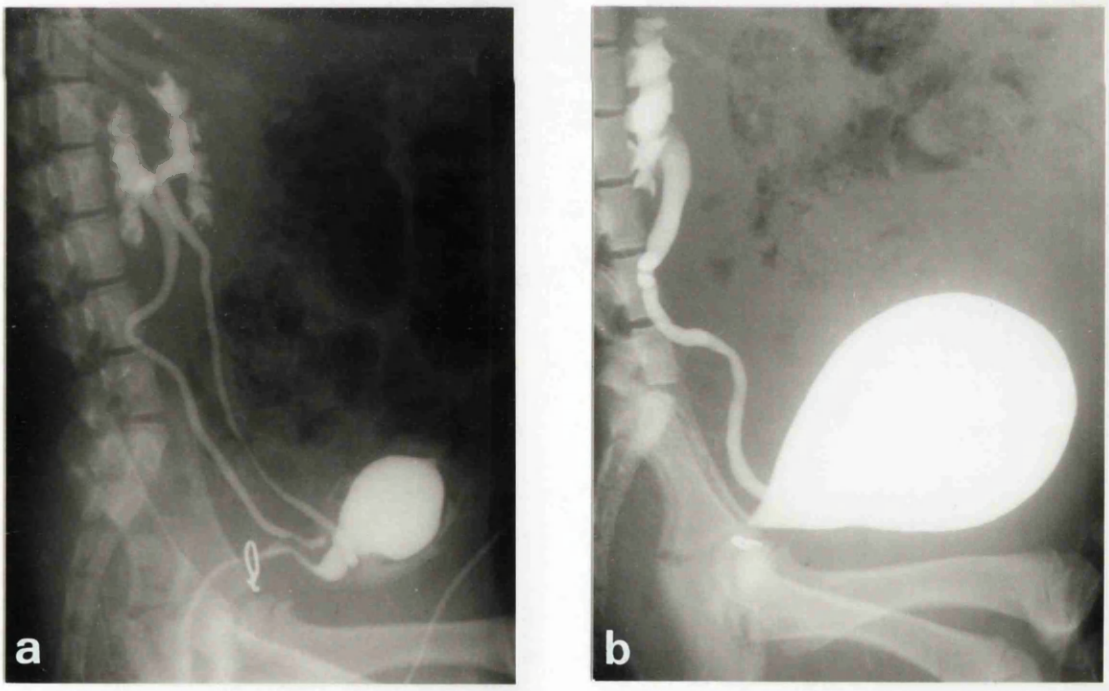



**Fig.11 Cystographic Appearance of VUR in Gottingen Pigs.**
Cystography perfomed 2 weeks after surgical induction of VUR, either bilaterally in a male (a) or unilaterally in a female (b). The implanted urethral ring is apparent in both and was 6-7mm in the male and 3mm in the female.

### 9.2.2 Sterile VUR

During study periods extending up to 6 months regular examination and culture of urine samples demonstrated that, in the majority of cases, in the presence of both VUR and bladder outflow obstruction, urine can be kept sterile with continuous chemoprophylaxis. In 2 of the 14 pigs an urinary infection was detected which reinforces the necessity of repeated urine examination in the experiments of Section IV, so that infected animals can be excluded and the essential condition of sterile VUR maintained.

### 9.2.3 Abnormal Bladder Function.

The urodynamic characteristics of 12 pigs (5 females, 7 males) maintained with sterile VUR were reviewed. In about half the male and female animals some transient acute post-operative bladder outflow obstruction occurred, but was resolved either of its own accord or by suprapubic aspiration of urine. There was a difference, however, between the males and females in the achievement of chronic bladder outflow obstruction with growth, which was related to differences in urethral anatomy and its response to stricture by an urethral ring.

#### 9.2.3.1 In the Female

Following a short period of a stable degree of bladder outflow obstruction there was a rapid and often abrupt transition from a relatively normal voiding pattern to abnormally prolonged and frequent voids with a poor stream. Micturition was achieved only by abdominal effort and this was often superimposed on prolonged and

elevated intra-vesical pressures (Figure 12) leading to gross retention of urine within 3 and 6 weeks.

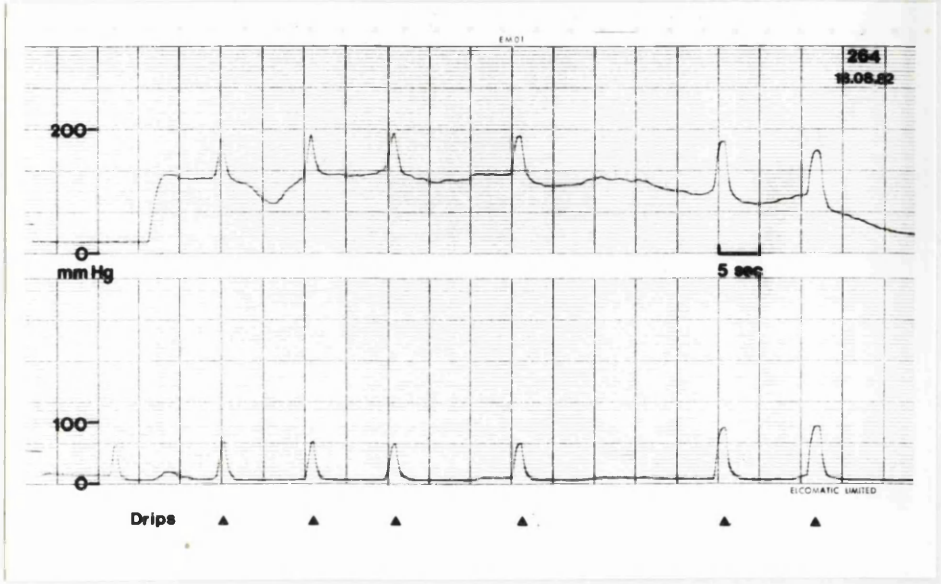



Fig.12 Cystometrogram From a Female Pig with Bladder Outflow Obstruction.
The upper trace shows intra-vesical pessure and the lower abdominal pressure.

### 9.2.3.2 In the Male

In the 7 male pigs there was, over a period of several months, a gradual progression in prolongation of micturition, deterioration of the urine stream and incomplete bladder emptying. By the end of the study periods (12 to 26 weeks) voiding pressures of between 60 and 80cm $H_2O$ were recorded compared with those of 30 to 50cm $H_2O$ observed after 4 to 8 weeks of study. In 6 of the 7 pigs unstable detrusor contractions were apparent during the filling phase and additionally, in some cases, these were superimposed on the pre-micturition rise in pressure (Figure 13). An abrupt progression to acute urinary retention occurred in only 2 of the 7 pigs.

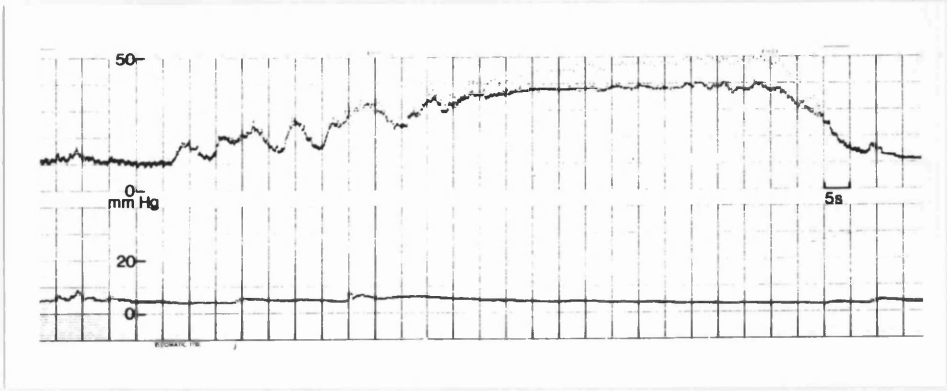



Fig. 13 Cystometrogram from a Male Pig with Bladder Outflow Obstruction.
The upper trace shows intra-vesical pressure and the lower abdominal pressure.

## 9.3 Papillary Morphology: An Experimental Evaluation

Papillary morphology has not been previously documented in the Gottingen pig. Because this may be an important determinant of the physiological effects of VUR it was evaluated in a separate study.

The evaluation was made using 59 kidneys from 30 Gottingen pigs in comparison with 16 kidneys from Froxfied miniature pigs (n=8) and 34 kidneys from Large White pigs (n=20). The calyces of each fresh kidney were dissected after careful opening from the hilum. In each one third zone (upper pole, mid-zone, lower pole) the papillae were counted after being classified according to the criteria of Ransley and Risdon[66,67], as those not normally associated with intra-renal reflux ('non-refluxing'; simple or compound types I), or those which can be associated with intra-renal reflux, ('refluxing' compound types II, or III). Types II and III papillae were distinguished by

the number and degree of fusion of the component pyramids. The numbers of the various papillary types found in individual kidneys are shown in **Appendix II** for each of the 3 pig varieties examined .

### 9.3.1 The Number of Papillae and the Proportions of Papillary Variants

The average number of papillae in individual kidneys and the percentage proportions of the papillary variants are presented in Table 3 for each pig variety. Also included are the data from Ransley and Risdon for kidneys from Welsh pigs[66] and from children[67].

Table 3. Average papillary number and percentage distribution of papillary types in kidneys from large and miniature pigs and children

| | Large Pigs | | Miniature Pigs | | |
|---|---|---|---|---|---|
| | Welsh[1] | L.whites | Froxfield | Gottingen | Children[1] |
| No. of Kidneys | 25 | 34 | 16 | 54 | 33 |
| Average No. of Papillae | 7.7 | 8.9 | 8.3 | 12.4 | 7.7 |
| Distribution | | | | | |
| Simple & Compound Type I (non-refluxing) % | 46 | 34 | 51 | 87 | 88 |
| Compound Type II & III (refluxing) % | 54 | 66 | 49 | 13 | 12 |

[1]Previously published data from Ransley and Risdon[66,67].

In the Gottingen pig the average number of papillae per kidney was greater than found for other breeds of pig examined and for the human

material presented here and elsewhere[68]. In contrast with other breeds of pig there was a predominance of 'non-refluxing' papillae, similar to the human. The proportion of 'refluxing' papillae was similar in the Gottingen pig and the human (13% and 12% respectively).

### 9.3.2 Distribution of the Papillary Variants

The percentage of kidneys in which the compound types II and III papillary variants were found is shown in Table 4 for each pig breed and for the human. Each area of the kidney; upper pole, lower pole, mid-zone; was considered separately.

Table 4. Number of kidneys (percentage) from large and miniature pigs and children in relation to the pattern of distribution of compound types II and III papillae.

| | Large Pigs | | Miniature Pigs | | |
|---|---|---|---|---|---|
| | Welsh[1] | L.whites | Froxfield | Gottingen | Children[1] |
| Poles | | | | | |
| Both upper and lower | 96 | 100 | 94 | 51 | 18 |
| Upper and not lower | 4 | 0 | 6.3 | 31 | 42 |
| Lower and not upper | 0 | 0 | 0 | 8.4 | 4.0 |
| Mid-zone | 88 | 97 | 63 | 8.5 | 3.0 |
| Absent | 0 | 0 | 0 | 10 | 27 |

[1]from data of Ransley and Risdon[66,67]

In 6 Gottingen pig kidneys (10%) there were no papillae of the 'refluxing', types. An absence of these papillae is more common in human kidneys, since almost 30% of these have been found to have all

'non refluxing' papillae[67,68]. By contrast, in kidneys from other breeds of pig, examined both here and by others[66,159], 'refluxing' papillae were always present.

The Gottingen pig, in contrast to other pig breeds, has a predominantly polar distribution of compound type II and III papillae like the human. In about half the Gottingen pig material the type II or III papillary variants were present at both poles compared with the human where they are found more commonly at only the upper pole.

### 9.4 Vulnerability to Renal Scar Formation

An earlier experiment[71] (9.2) had confirmed that segmental scar formation occurred in the Gottingen pig as in other pig models once the essential conditions of VUR and urinary infection or VUR and severe bladder outflow obstruction occurred.

### 9.5 Discussion of the Gottingen Pig Model

These preliminary considerations have shown that as a model for determining the physiological effects of VUR the Gottingen pig is possibly superior to other pig breeds. Papillary and hence calyceal morphology in the Gottingen pig more closely resembles that of the human than it does in other breeds of pig. This was apparent in the proportion of 'refluxing' to 'non refluxing' papillae as well as the almost exclusively polar distribution of the 'refluxing' papillary variants. That *reflux nephropathy* occurred as a

consequence of VUR in certain circumstances indicates that the Gottingen pig can model the human pathological response to VUR as do other varieties of large and miniature swine[7,65]. These features may be important determinants of the influence of VUR on the kidney; with or without mediation through the mechanism of intra-renal reflux.

Female pigs, however, do not provide good models for chronic bladder outflow obstruction so they are necessarily confined to models with normal bladder function. In males, however, the degree of urethral stenosis can be tailored to induce bladder outflow obstruction, without causing renal scarring, which means that VUR effects can be confidently tested in the presence of elevated bladder pressures and abnormalities in bladder function. The appropriate degree of bladder outflow obstruction can be maintained for several months but subtle variations with time make the regular assessment of bladder function necessary. The demonstration[71], like those of others[72,73], that renal scarring can occur as a consequence of sterile VUR in the presence of severe bladder function abnormalities underlines the importance of avoiding the influence of any acute obstructive episode on the kidney immediately following surgical preparation of the model.

When fully mature both body and kidney weight of the Gottingen pig are likely to be similar to the human adult. At birth, its body and kidney mass are comparable to the human at 24 weeks gestation. By the age of 6 months renal growth in weight is likely to be equivalent

to that in the human at age 10-12 years. Previous studies have shown that neonatal pigs can be reared successfully if kept with the sow for the natural suckling period and that VUR can be successfully induced in new borns. However, surgery is best performed only after the first 2-3 weeks of life because new born piglets are particularly susceptible to stress induced diarrhoea. The adoption of these procedures and precautions in the experiments of Section IV allows the best practicable model for congenital VUR in the human.

# Section III

# The Measurement of Renal Growth and Function

CHAPTER 10

# GLOMERULAR FILTRATION RATE

## 10.1 Quantitation

The glomerular filtration rate (GFR) is a measure of the rate at which a filtrate of plasma is formed in the glomerulus, but can also be regarded as a mathematical concept. It is derived from renal clearance, defined as the volume of plasma from which a specified substance is removed, by the kidney, per unit time. Renal clearance itself is determined by the excretory properties of that substance. When the substance is freely filtered in the glomerulus and not subject to tubular secretion or reabsorption, then its renal clearance may be equated with GFR[329]. The standard measure of clearance is obtained from the rate of urinary excretion of the substance (UV/P method). In alternative methods clearance is derived from the rate of disappearance of the substance from plasma, assuming it is not metabolised and is excreted only by the kidney (C-slope methods). More complex refinements of these methods have evolved with the introduction of gamma camera scintigraphy.

### 10.1.1 UV/P Method

In the most simple arithmetical derivation, GFR is equated with renal clearance of an appropriate substance measured by the rate of its excretion in urine with reference to its concentration in plasma. This is defined by the expression UV/P; where U = concentration in urine, V = urine volume/unit time, P = concentration in plasma[329].

The method requires that during the clearance procedure the reference substance is sustained at a fairly constant concentration in plasma, and in general this is achieved by intra-venous infusion[330]. Since 1935, following the work of Shannon and Smith[331], the UV/P clearance of inulin has formed the standard measure of GFR in the human.

### 10.1.2 C-slope Method

The necessity for urine collection and intra-venous infusion of the reference substance are obviated in a simplified procedure which measures the rate at which the marker disappears from plasma (plasma decay) after a single, 'shot', injection[332,333] (C-slope method; Figure 14). Clearance is represented as the inverse function of plasma decay, assuming this is attributed solely to glomerular filtration. Because clearance has the dimension volume as well as time the initial fluid volume in which the marker is distributed needs to be determined. The method involves a more complex mathematical derivation which in its simplest form depends upon the assumption that the marker is rapidly equilibrated in a single compartment and that its disappearance from plasma can be represented as a single exponential function (Figure 14). The oversimplification can be reduced by defining 2 compartments analysed by double exponential analysis[334-336]. Both models allow estimation of the apparent volume of distribution ($V_D$) of marker, which in the single compartment model is found by extrapolation of the logarithmic curve back to zero time (Figure 14).

The validity of the C-slope method for measuring clearance, and hence

GFR, depends upon the freedom of diffusion of the reference substance between plasma and interstitial fluid compartments because equilibration needs to be rapidly established and maintained throughout the clearance period. The substance must be accurately measurable at low concentrations, and therefore highly ionisable radio-labelled compounds are suitable, whereas inulin is not. Of these compounds, $^{51}$Cr-ethylene-diamine-tetra-acetic acid ($^{51}$Cr-EDTA) has an established role, following its introduction by Stacey and Thorburn[337] in 1966. Its clearance by the C-slope method was chosen for the experiments in Section IV after a separate evaluation in pigs (**Chapter 11**).

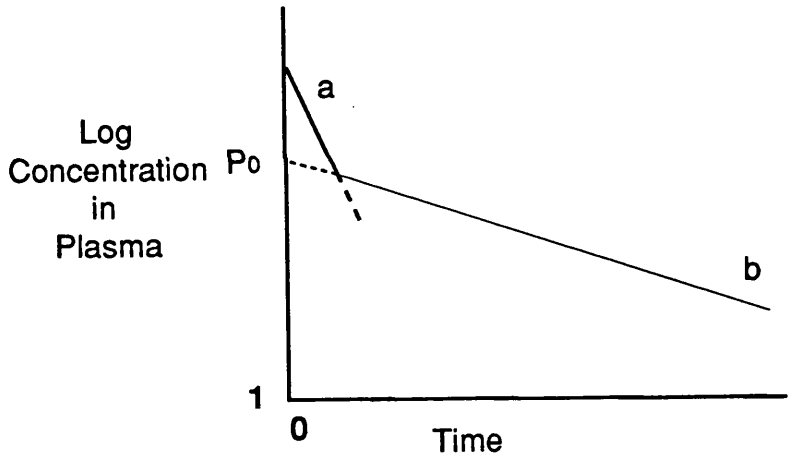



**Fig.14** C-slope Clearance. Exponential plasma decay with time. Slope 'a' represents decay attributed to equilibration throughout plasma and interstitial fluid compartments. Slope 'b' represents decay due to renal clearance. In the 2 compartment model 'a' and 'b' are separately established. In the single compartment model only 'b' is established, after an assumed period of equilibration. $P_o$ is the apparent concentration of marker at time zero and is used to establish an apparent volume of distribution ($V_D$; see 11.3). Clearance = slope 'b' × $V_D$ in ml/min.

### 10.1.3 Gamma Camera Scintigraphy Methods

More mathematically complex techniques for estimating clearance after a single injection of a radio-labelled marker (DTPA; diethylene-triamine-penta-acetic acid) were developed with the introduction into clinical practice of gamma camera scintigraphy[338]. These methods require computer assisted data acquisition and analysis of the passage of radio-activity through plasma and renal compartments. They could not be considered for the Section IV experiments because the essential computer on-line with the gamma camera was unavailable. Furthermore, at that time, the methods had not been fully evaluated.

## 10.2 Expression of Glomerular Filtration Rate

The convention in clinical practice is to express GFR as a ratio to body surface area (BSA), to provide a common unit or standard with which members of a population of different ages and sizes can be compared. Its use followed the suggestions of McIntosh, Moller and van Slyke[339] and Holten[340] because both kidney weight and basal metabolic rate (BMR) are closely proportional to BSA. However, direct proportionality between any 2 parameters can be said to occur only if the regression line depicting the relationship of those parameters passes through zero. The ratio standard does not describe the true relationship if the regression line intercept deviates from zero. Tanner[341] showed that even when this latter is apparently small, as is the case with BMR versus BSA, the difference between the ratio standard and the true relationship will be of increasing significance as the range (e.g. body size) extends from

the population mean, such as occurs in children and unusually large adults. It is apparent from the plot of kidney weight versus BSA (Figure 15) that the same limitations apply for this relationship even though a regression equation has not been calculated. The fallacies in the use of such ratio standards are comprehensively described by Tanner[341] together with an example of how their application leads to erroneous assumptions concerning the 'norm' for a physiological index.

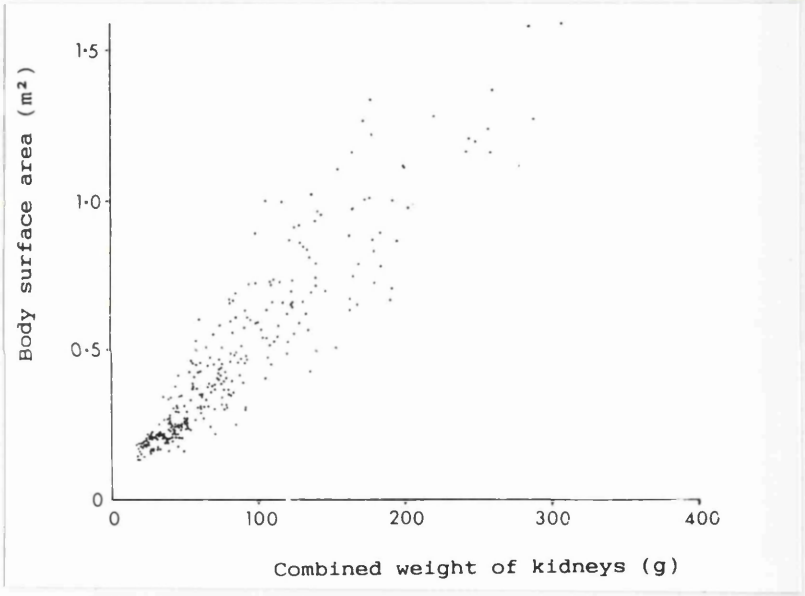



**Fig. 15. Kidney Weight and Body Surface Area.**
(From Risdon[212]; reproduced with permission).

The practice of standardising GFR in children to 1.73m² BSA in reference to the surface area of the adult male may influence interpretation of the time scale with which GFR develops to full efficiency after birth. McCance and Widdowson[342] suggested that

total body water (which forms a greater percentage of body weight in neonates and children than in adults) provides a more correct basis for expression and comparison of GFR. By this means GFR reaches adult levels within the first month of life and exceeds them subsequently during growth and development. In pigs GFR is described most often as a per body weight ratio but there is no arithmetical support for this index; GFR is rarely described as a ratio with BSA, although this latter has been formulated for large domestic pigs[262]. An aim, therefore, in the design of experiments in Section IV was to ensure that comparisons of GFR were made only in animals of comparable ages and body weight so that 'the spurious correlations and pitfalls of derived indices'[341] might be avoided.

CHAPTER 11

# QUANTITATION OF GLOMERULAR FILTRATION RATE IN PIGS

## 11.1 A Comparison Between UV/P and C-slope Clearance of $^{51}$Cr-EDTA: An Experimental Evaluation.

Previous clinical and experimental studies have shown that $^{51}$Cr-EDTA clearance, measured by the UV/P method, is similar but less (5 - 15%) than the corresponding UV/P clearance for inulin[334,337,343-347]. The major differences[345] have been attributed to difficulties in the chemical assay of inulin[347].

The C-slope clearance of $^{51}$Cr-EDTA measured using the single compartmental model, which requires a minimum of only 2 blood samples, has been shown in adults to predict a corresponding UV/P clearance with an accuracy of +/- 9% (95% confidence limit)[348]. However, in both adults and children the single exponential C-slope clearance exceeds UV/P clearance. A correction factor which accounts for this discrepancy and the innate difference between inulin and $^{51}$Cr-EDTA clearances is included in order that $^{51}$Cr-EDTA C-slope clearance can be equated with glomerular filtration rate (GFR)[334,348,349].

It is assumed from a number of studies in humans and experimental animals that the UV/P clearance of $^{51}$Cr-EDTA can be acceptably substituted for inulin UV/P clearance as determinant of GFR in pigs. This is supported by the results of Farhaeus and Lorentzen[350] who

showed that for 6 pigs UV/P $^{51}$Cr-EDTA clearance values were similar to UV/P inulin values although lower by a mean of 7%. This disparity approximates that found in most other studies[334,337,343,344,346-348].

In view of the assumptions required for the determination of C-slope clearance it was important to know this method allowed an acceptable measure of GFR in pigs. This was established for the single compartment model in comparison with the UV/P method in an earlier experiment designed to test the effects of early and late unilateral nephrectomy on compensatory renal adaption[324].

### 11.1.1 Experiment Design

The study used a total of 18 Large White pigs, of whom 14 were successfully investigated for about 13 weeks, either from age 2 weeks (**early group**) or from age 7 weeks (**late group**). The $^{51}$Cr-EDTA clearance was measured by the UV/P and C-slope methods on successive days, providing a paired data set for each animal. The paired studies were performed on alternate weeks, except for pigs in the **early** group when they were performed weekly for the first 3 weeks. One further pig was investigated only at the end of the study period. Renal weights obtained at the end of the study periods were related to the immediately preceeding estimates of GFR.

The rapid growth of Large White pigs allowed comparison of the clearance methods over a wide range of body weights. In both the early and late groups pigs with a solitary kidney following

unilateral nephrectomy were included as well as those with normally paired kidneys. This allowed clearance evaluation in both the pig model types used in the Section IV experiments and also permitted a statement concerning GFR in the solitary kidney model.

### 11.1.2 Procedural Considerations

All clearance studies were performed in conscious animals, because previous personal observations had shown that gaseous anaesthesia with Halothane and $N_2O$ inhibits urine production. In addition, experience had shown that alternative anaesthesia with sodium pentobarbitone, which in appropriate doses is considered not to affect GFR or urine production, is difficult to control in pigs. The use of conscious pigs necessitated the implantation of 2 central venous lines with one placed more centrally than the other, for injection and blood sampling respectively. Additionally, all animals had vesicostomies to facilitate urine collection. Both these practical manoeuvres caused problems with infection. All animals had positive urine cultures of mixed organisms at some time during the study period. These were often recurrent but there was no associated pyuria. Blood cultures of samples collected from venous lines were positive in all cases. Four animals were pyrexial during the course of the study and microbiological cultures of peripheral blood samples were positive. One animal was successfully treated with antibiotics but the remaining 3 were killed and their data excluded from analysis. Additional problems were that with growth the central venous lines had to be replaced at least once in each pig and, despite anticoagulant instillation into the lines

daily, they became occluded on occasions.

### 11.1.3 Methods for GFR Determination

For the duration (about 1.5h) of each UV/P clearance study animals were placed in a suitable restraining cage. For each C-slope study (duration 4h) animals were allowed to remain in their usual pens, being restrained if necessary only for injection and sampling. All UV/P or C-slope studies were performed during the same hours of the morning. Animals were fed prior to the investigations and had free access to water at all times as well as being encouraged to drink milk (40ml/kg) during the clearance procedures. The central venous lines were used in all cases for intra-venous injections and blood sampling as described (**11.1.2**).

#### 11.1.3.1 UV/P Method

In each animal, on all occasions, a Foley balloon catheter (8F - 14F) was inserted into the vesicostomy for bladder drainage. Once a free flow of urine was established a loading dose of $^{51}$Cr-EDTA (2$\mu$Ci;0.074MBq/kg body weight) contained in saline (approximately 0.25 - 0.5ml/kg body weight), was injected intravenously. The remaining dose was diluted in a 60 - 120ml volume of 2% dextrose and infused intra-venously using a syringe pump. An infusion rate of 0.5 - 1.3ml/min (0.02 - 0.1ml/kg body weight/min) was used to provide a dose of $^{51}$Cr-EDTA of 2$\mu$Ci;0.074MBq/kg body weight/hour.

After a 45min equilibration period, urine was collected during 3 sequential 10min periods. In each case the volume of urine was

determined by weight assuming a urine specific gravity not significantly different from zero. At the half time of each of the 3 urine collections the infusion was stopped for a period not exceeding 30s whilst a 5ml volume of venous blood was collected into a bottle containing anti-coagulant (EDTA). Plasma was separated after centrifugation ($2 \times 10^3$g for 10min). Additional blood and urine samples were obtained before injection of the loading dose, in order to establish a plasma and urine blank.

Equal volumes (2ml) from each of the urine and plasma collections (one blank and 3 clearance samples) were assayed for gamma activity by placing in a well counter for 25min or acquisition of $10^4$ counts, whichever came first. The recorded activity in each of the clearance samples was obtained after subtracting the activity (counts/min) in corresponding urine and plasma blanks. Urine samples were assayed in duplicates. Positive displacement automatic pipettes were used for dispensing volumes.

The UV/P clearance of $^{51}$Cr EDTA was expressed as the mean of the 3 clearances (urine collection periods) each calculated as:

$$\text{Clearance} = \frac{\text{urine concentration (cpm/ml)} \times \text{urine volume (ml/min)}}{\text{plasma concentration (cpm/ml)}}$$

### 11.1.3.2 C-slope Method

For each pig, on all occasions, a dose of $^{51}$Cr-EDTA, 4$\mu$Ci;0.15MBq/0.3ml saline/kg body weight, was prepared and a sample reserved in a separate vial as standard. The dose syringe was

weighed before and after the dose injection to determine the precise volume administered. At accurately timed intervals 2 and 4 hours after the dose injection, 5ml venous blood samples were collected. An additional venous sample was obtained prior to the dose injection for use as a blank. Plasma was separated and assayed as previously (**11.1.3.1**), together with duplicate standards prepared from the reserved dose by making a 1:500 dilution.

The total isotope activity injected was calculated from the dose volume and the activity of the standard:

$$\text{Dose (cpm)} = \text{volume injected (ml)} \times \text{standard activity (cpm/ml)} \times 500$$

The logarithm of the isotope activity in each of the 2 plasma samples (cpm/ml less cpm/ml in plasma blank) was plotted as a function of time and the apparent activity in plasma at the time of injection ($P_o$) determined by extrapolation (Fig.14). The apparent volume of distribution ($V_D$) was then derived:

$$V_D(\text{ml}) = \frac{\text{Total activity injected (cpm)}}{P_o(\text{cpm/ml})}$$

Clearance was determined from the apparent volume of distribution and the disappearance rate of radioactivity from plasma.

$$\text{Clearance} = \frac{\log_e 2 \times V_D\text{ml}}{T^{1/2}(\text{min})}$$

where:

$T^{1/2}$ is the time taken for the activity (cpm/ml) in plasma to fall to half that at $P_o$.
$\log_e 2 / T^{1/2}$ is the inverse function of the negative exponential slope (plasma decay rate:cpm/ml).

### 11.1.4 Results

A total of 105 paired clearance studies from 14 pigs were available for analysis. The individual values for each pig are included in **Appendix III.**

The number of paired clearance studies included in the analysis and the range of results for both the UV/P and C-slope methods, together with the associated body weight range, are shown in Table 5 for individual pigs of each group. The number of studies in each group was similar.

Table 5 Ranges for body weights and values for UV/P and C-slope clearances for pigs included in the study with solitary or paired kidneys

| | Pig | Studies (n) | Range Body Weight (kg) | Range GFR (ml/min) UV/P | C-slope |
|---|---|---|---|---|---|
| Early | 1 | 6 | 6 - 42 | 19 - 116 | 22 - 122 |
| Solitary | 2 | 9 | 3 - 43 | 12 - 104 | 12 - 140 |
| | 3 | 9 | 3 - 34 | 4 - 65 | 6 - 80 |
| | 4 | 8 | 3 - 30 | 8 - 103 | 9 - 125 |
| Paired | 5 | 9 | 3 - 39 | 11 - 85 | 9 - 100 |
| | 6 | 9 | 3 - 41 | 10 - 89 | 8 - 100 |
| Late | 7 | 7 | 14 - 57 | 40 - 93 | 41 - 112 |
| Solitary | 8 | 7 | 16 - 54 | 48 - 111 | 48 - 130 |
| | 9 | 7 | 22 - 68 | 55 - 123 | 66 - 160 |
| | 10 | 7 | 25 - 76 | 47 - 146 | 70 - 160 |
| | 11 | 7 | 15 - 57 | 52 - 121 | 61 - 134 |
| Paired | 12 | 6 | 13 - 58 | 42 - 94 | 46 - 123 |
| | 13 | 7 | 25 - 73 | 72 - 150 | 95 - 176 |
| | 14 | 7 | 22 - 67 | 79 - 176 | 92 - 208 |

### 11.1.4.1 Individual Clearance Values and Body Weight

The individual values for each UV/P and C-slope clearance study are shown plotted against pig body weight in Figure 16 for pigs with solitary kidneys and in Figure 17 for pigs with paired kidneys. In both pigs with solitary and paired kidneys there was a curvilinear increase in both UV/P and C-slope clearance with body weight.

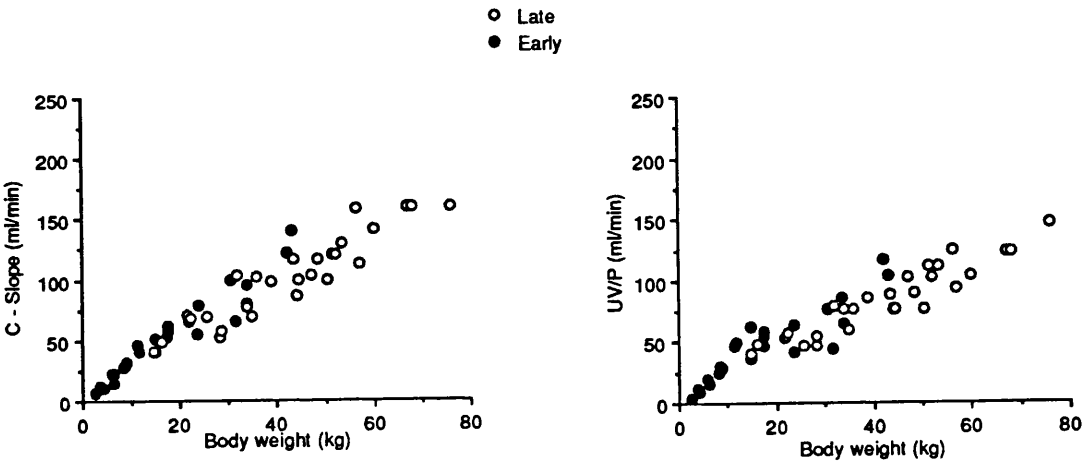



Fig.16 C-slope and UV/P $^{51}$Cr-EDTA Clearance Values for Pigs with Solitary Kidneys. Individual values plotted against body weight for pigs in Early and Late groups.

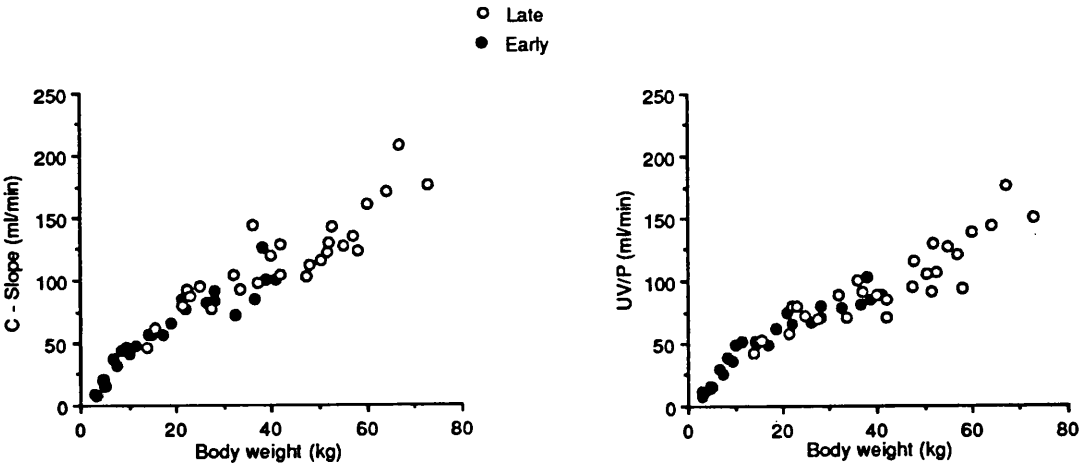



Fig.17 C-slope and UV/P $^{51}$Cr-EDTA Clearance Values for Pigs with Paired Kidneys. Individual values plotted against body weight for pigs in Early and Late groups.

### 11.1.4.2 Individual Clearance Values and Kidney Weight

Data were available from 15 pigs. The values for UV/P and C-slope clearances obtained within the week before the animals were killed are shown in Table 6 both as absolute values and expressed per gram of kidney weight.

Table 6. Relationship between kidney weight and $^{51}$Cr-EDTA clearance values in large white pigs. Total weight (right + left or right solitary) of kidneys removed at the end of the experiment and corresponding final clearance values (within one week).

| Group | Pig | Kidney wt. (g) | Clearance (ml/min) UV/P | C-slope | (ml/min/g kidney wt.) UV/P | Mean | C-slope | Mean |
|---|---|---|---|---|---|---|---|---|
| Early | 1 | 180 | [1]- | 116 | [1]- | | 0.64 | |
| Solitary | 2 | 263 | [1]- | 130 | [1]- | | 0.49 | |
| | 3 | 99 | 65 | 80 | 0.66 | | 0.81 | |
| | [2]15 | 111 | 84 | 117 | 0.76 | | 0.95 | |
| | | | | | | 0.71 | | 0.72 |
| | 4 | 282 | 103 | 125 | 0.37 | | 0.44 | |
| Paired | 5 | 161 | 85 | 100 | 0.53 | | 0.62 | |
| | 6 | 135 | 89 | 100 | 0.66 | | 0.72 | |
| | | | | | | 0.52 | | 0.60 |
| Late | 7 | 179 | 93 | 112 | 0.52 | | 0.63 | |
| Solitary | 8 | 173 | 111 | 130 | 0.64 | | 0.75 | |
| | 9 | 205 | 123 | 160 | 0.60 | | 0.78 | |
| | 10 | 259 | 146 | 160 | 0.56 | | 0.62 | |
| | | | | | | 0.58 | | 0.70 |
| | 11 | 237 | 121 | 134 | 0.51 | | 0.57 | |
| Paired | 12 | 202 | 94 | 123 | 0.46 | | 0.61 | |
| | 13 | 311 | 150 | 176 | 0.48 | | 0.57 | |
| | 14 | 291 | 176 | 208 | 0.60 | | 0.71 | |
| | | | | | | 0.51 | | 0.62 |

[1]Missing values due to problems with catheters.
[2]Additional pig, investigated only at the end of the study period and not during growth.

With increasing total renal weight both C-slope and UV/P clearance values increased (Figure 18); however when the clearance values were determined per gram of total renal weight a systematic decrease was observed for both paired and solitary kidneys (Figure 19).

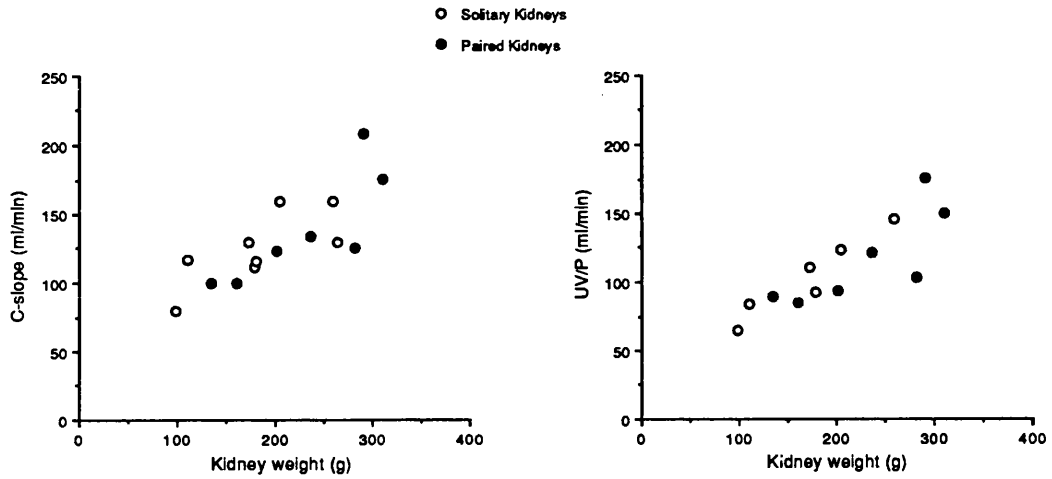



Fig.18 C-slope and UV/P $^{51}$Cr-EDTA Clearance Values Plotted Against Kidney Weight. Individual values for pigs with solitary and paired kidneys.

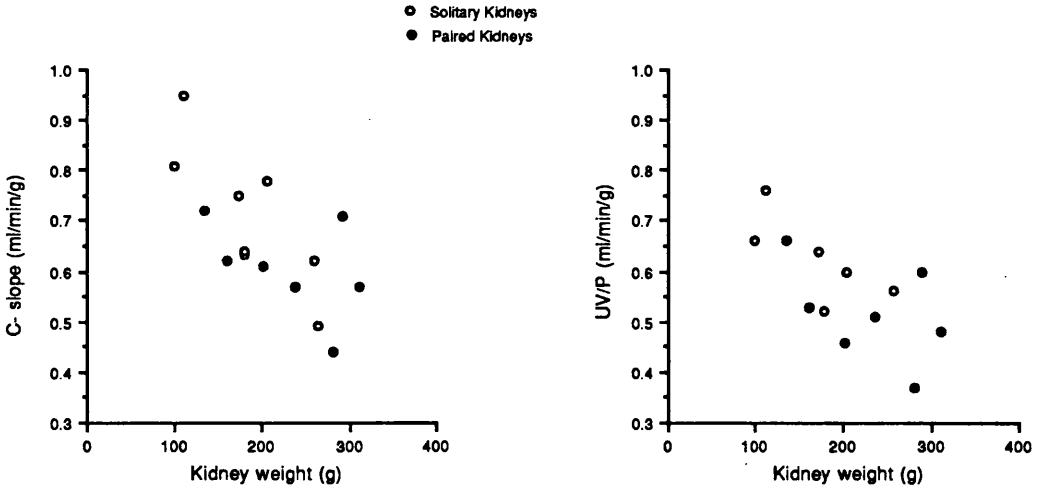



Fig.19 C-slope and UV/P $^{51}$Cr-EDTA Clearance per gram Kidney Weight Plotted Against Kidney Weight. Individual values for pigs with solitary and paired Kidneys.

### 11.1.4.3 Comparison of C-slope and UV/P Clearances

The relationship between the C-slope and corresponding paired UV/P clearance is shown in Figure 20 for the solitary and paired kidney pigs in both early and late groups. After log transformation the regression equation for C-slope on UV/P was Y = 0.0367 + 1.0268x. There was a good correlation (r = 0.98) between the C-slope and UV/P methods but the individual results for C-slope were greater than those for UV/P in 96 of the 105 paired clearance studies.

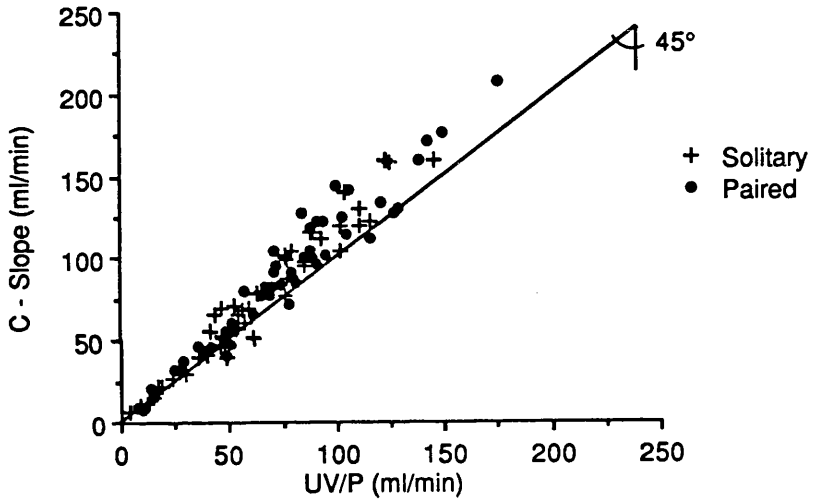



**Fig.20 C-slope Versus UV/P $^{51}$Cr-EDTA Clearance. Individual values for pigs with solitary and paired kidneys in Early and Late groups.**

There was a small but significant tendency for the log of the ratio (C-slope:UV/P) to increase with the log of the mean of paired clearance values ($r=0.23$, $F_{14,90}=1.87$, $p=0.04$). Thus the difference between the 2 methods became greater as clearance values increased.

The mean ratios (C-slope:UV/P), together with the 95% confidence

limits (mean +/- 2 × SEM) for solitary and paired kidney groups are shown in Table 7 for both the early and the late groups. The confidence limits when expressed conversely and as the percentage of UV/P clearance to C-slope were 87-93% for the early group and 81-86% for the late group.

Table 7 The mean ratio values (C-slope:UV/P) for pigs with solitary and paired kidneys in the early and late groups.

| | Solitary | Paired | Total | 95% CL |
|---|---|---|---|---|
| Early | 1.13 | 1.10 | 1.11 | 1.07 - 1.15 |
| Late | 1.19 | 1.19 | 1.19 | 1.16 - 1.23 |
| Total | 1.16 | 1.15 | 1.16 | 1.12 - 1.18 |
| 95% CL | 1.12 - 1.21 | 1.11 - 1.19 | | |

There was no difference in the ratio (C-slope:UV/P) between the solitary and paired groups ($F_{12,1} = 6.2$, $p = 0.6$) but a significant difference between the early and late groups ($F_{1,12}=7.09$, $p<0.05$). This difference was not significant after accounting for pig body weight ($F_{1,12}=1.64$, $p>0.05$) nor was there any significant effect of individual pigs on the ratio (C-slope:UV/P; $F_{12,90}=1.2$).

### 11.1.5 Discussion of Results

In pigs with both paired and solitary kidneys the clearance of $^{51}$Cr-EDTA measured by both the UV/P and C-slope methods showed the expected increase with growth. This was apparent when either body weight or kidney weight was used as an index of growth. For pigs

with 2 kidneys in place the initial UV/P and C-slope clearance values were similar to inulin clearance values reported by Fris[266] for animals of comparable age (2 weeks), and they demonstrated the same early rapid increase with a gain in body weight from about 3kg to 20kg at the age of 8 weeks. This was more pronounced in the present study, even by the UV/P method, than that reported by Fris[266] but may be explained by differences between the 2 studies in methodology or pig breed.

The C-slope clearance overestimated that of UV/P by slightly greater amounts as clearance increased with body weight. The difference between the UV/P and C-slope methods was slightly less in the pigs of the present study than that found in a clinical study by Chantler and Barratt[348], where UV/P was a mean of 77% the C-slope values. The 95% confidence interval for the ratio of C-slope to UV/P indicated that the difference between the 2 methods was consistent **within** the early and late groups. The disparity in the ratio (C-slope:UV/P) **between** the early and late groups was attributed to the different body weight range of animals in these groups.

The systematic discrepancy between the UV/P and C-slope methods, which occurs with increasing clearance values, has been documented in studies with humans, where GFR was expressed as a ratio with body surface area[348], and in a comparison of absolute values for $^{51}$Cr-EDTA C-slope and UV/P inulin clearances in rabbits[336]. It is attributed in part to errors in the extrapolation of $P_o$ (and thus the volume of distribution) from the slope of plasma decay. This arises from

false assumptions of the single exponential disappearance of activity from plasma. It is clear that the UV/P and C-slope methods do not provide directly comparable results over a range of clearance values and that the 2 methods cannot be used inter-changeably as a measure of GFR. Furthermore, it is inappropriate to apply a single simple ratio or percentage factor to the C-slope values in order to establish their equivalence with the UV/P clearance as a measure of GFR, although this procedure is adopted in clinical practice.

Despite the disparity between the UV/P and C-slope methods in providing an absolute measure of GFR the least squares regression analysis indicates that there is a common association between the results achieved by the 2 methods. However, further statistical analysis[351] is required to confirm the impression that GFR may be soundly predicted from the C-slope clearance and calculated by incorporation of an appropriate correction factor derived from the regression statistic. This procedure was not adopted because such a factor derived from large pigs with normal renal function of this study may not necessarily be appropriate for use with miniature pigs used in the Section IV investigation, and in whom renal function may possibly be impaired by the imposed experimental conditions.

The results from this present study also demonstrate that expression of GFR as a ratio with either body weight or kidney weight, in order to establish a uniform value for GFR, is fallacious. The relationship between GFR and body weight is curvilinear, therefore, direct proportionality between these 2 parameters cannot possibly

occur (10.2). Even if the relationship (GFR with body weight) is described by 2 linear components separated by body weight (*i.e.* within ranges 0-15kg or 16-80kg) it is apparent that although the intercept for a regression line fitted to the early rise in GFR may pass through a point about zero, that fitted to the later rise in GFR with body weight does not. Similarly, direct proportionality does not occur between GFR and kidney weight, and results showed that clearance values per g kidney weight decreased with increasing kidney weight. These observations confirm the impression that ratio values with body and kidney weight should be used only in restricted circumstances *i.e.* when actual body weight is defined, or for purposes of comparison, when it is controlled.

### 11.1.6 Significance of the Present Study for Section IV Experiments

#### 11.1.6.1 C-slope versus UV/P

In the Section IV experiments it was necessary only to measure an index of glomerular function and the results from this study indicate that the C-slope clearance can provide such an index. The results showed that body weight, as a determinant of absolute GFR, is a prime factor in affecting the disparity between C-slope clearance and 'true' GFR. The influence of this disparity in the interpretation of clearance values can be minimised by making comparisons only in animals of similar body weight. In an experimental study incorporating control animals such an approach to investigating GFR is more acceptable than one which depends upon equating C-slope clearance with GFR by use of a derived correction factor.

### 11.1.6.2 Clearance in Pigs with a Solitary Kidney

This present study also allowed a statement concerning renal clearance in pigs with a solitary kidney following unilateral nephrectomy. The infection problem and the body weight variation within the early and late groups precluded a definitive conclusion regarding this aspect but data inspection indicated that as expected (8.1), renal clearance in pigs with a solitary kidney approached, but did not fully equate with, the clearance in corresponding pigs with normally paired kidneys. The disparity between the C-slope and UV/P clearance values was similar in pigs with solitary and paired kidneys as was the relationship between clearance values and total kidney weight. An impression that for any given renal mass clearance per g total kidney weight was greater in pigs with solitary kidneys cannot be confirmed from this experiment.

### 11.1.6.3 Practical Considerations

There were problems associated with both luminal occlusion and systemic infection in the use of indwelling catheters. It was clear that their use was not practical in a long term study particularly when sterility is an essential feature of the experimental model. In future experiments both injection and sampling procedures would have to be carried out by direct venous puncture whilst the pigs are anaesthetised briefly. The results of C-slope clearances measured in Gottingen miniature pigs by this method are presented to support its use.

## 11.2 The C-slope Clearance in Gottingen pigs

The $^{51}$Cr-EDTA C-slope clearance was examined in 9 Gottingen pigs aged between 2 and 30 weeks. The animals were followed for varying periods (1-8 weeks). Clearance studies were performed in pigs with 2 kidneys in place (9 pigs) and following unilateral nephrectomy (6 pigs). All pigs were otherwise normal, although they formed part of other studies concerning the renal uptake of DMSA[374].

The method used for estimating C-slope clearance was the same as described previously except that pigs were anaesthetised for 3 periods (for injection and blood sampling), each not exceeding 10 minutes from induction of anaesthesia to complete arousal. Veins from opposite ears were used for injection and sampling. The previous study had shown that there was no residue of radio-activity in the plasma, even the day following a clearance study, therefore only ambient background activity was measured and plasma samples for blanks were not obtained.

The values for C-slope clearance are shown plotted against pig body weight in Figure 21 and the majority from pigs with either paired or solitary kidneys are similar to those observed (**11.1.4.1**) for corresponding Large White pigs of an equivalent body weight. Thus, for Gottingen pigs, in whom clearance studies were performed with brief anaesthesia during sampling and injection, and for Large White pigs, in whom clearance was measured in the fully conscious state, the paired kidney C-slope clearance was about 40ml/min at 10kg body weight, 80ml/min at 20kg, and 90-100ml/min at 25kg. These values

are similar, but (as would be expected) slightly higher, than values quoted earlier (7.3.2) for inulin clearance in other miniature pig varieties.

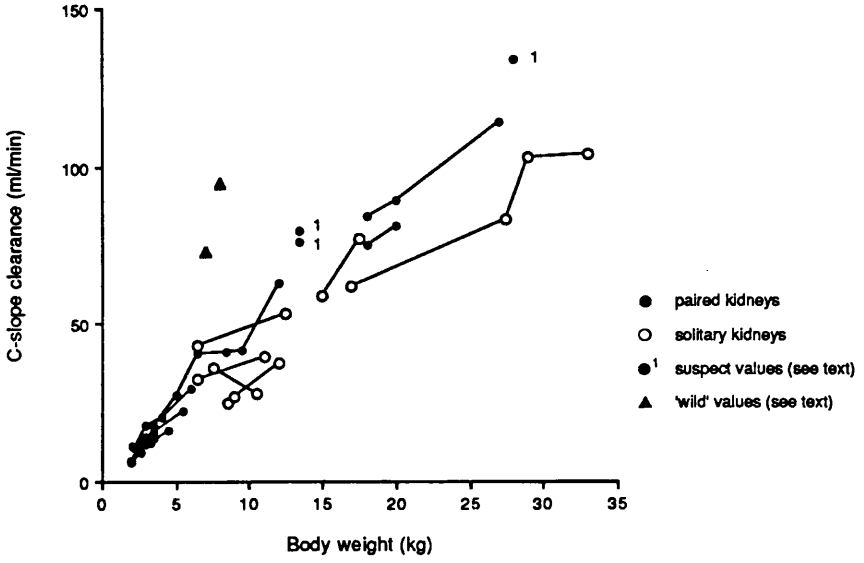



Fig. 21 C-slope $^{51}$Cr-EDTA GFR in Gottingen Miniature Pigs. The pigs were anaesthetised briefly for injection and sampling. Lines connect individual pigs.

The results in pigs with solitary kidneys (recorded 2 weeks or more after recovery from unilateral nephrectomy) showed, on the whole, that as with large pigs clearance values approached but did not fully equate with those for pigs of an equivalent body weight but with normally paired kidneys.

There were some problems, however, in that 2 of the 47 values obtained were wildly disparate and a further 3 were higher than might be expected. This was thought to be related to the frequent observation that activity in the second plasma sample was low (only about 4 times background or less). This was particularly noticeable

in very young pigs (6-15kg) with paired kidneys. In order to counteract this problem in subsequent experiments it was proposed that plasma samples would be collected at intervals of 100 mins; representing periods of 1h 40min and 3h 20min following dose injection, and that the dose of $^{51}$Cr-EDTA would be increased by 20%.

CHAPTER 12

## RENAL UPTAKE OF $^{99m}$Tc-DMSA

$^{99m}$Technetium-dimercaptosuccinic acid (DMSA) has an established use in defining functional renal cortex during imaging by gamma camera scintigraphy[256,352,353,354]. After its intra-venous injection there is progressive renal accumulation which, in normal circumstances in adults and children, reaches a stable maximum at 6h when approximately 50% of the dose is distributed equally between paired kidneys[355,356]. The fraction of the dose accumulating in kidneys is independent of the amount of DMSA administered, although saturation of uptake has been demonstrated in rats at doses (100$\mu$g/kg body weight) which far exceed those used in clinical practice[357]. During the period of renal uptake 20 to 30% of the dose is excreted in urine[353] and animal studies have shown that most of the remaining extra-renal distribution is in liver, blood and bone marrow[358,359,360]. The relative specificity of DMSA for renal parenchyma provides a high ratio of kidney to background radio-activity. This together with the characteristic of a consistent level of uptake by normal kidneys has promoted the use of methods to measure the proportional accumulation of $^{99m}$Tc-DMSA by separate kidneys of pairs (relative uptake)[257,258] and/or the capacity of an individual kidney to accumulate DMSA (absolute uptake or % uptake/dose)[355,361]. These parameters are used to describe the overall functional capacity of the kidney and calculation of relative uptake has become an acceptable method for establishing the

contribution of each kidney to total renal performance[37,257,258].

**12.1 Uptake of $^{99m}$Tc-DMSA and Renal Function.**

Of the DMSA accumulating in renal parenchyma most is sited within proximal tubular cells with some additional localization in cells of the distal tubule and the upper loop of Henle[357]. The processes involved in cellular uptake of DMSA and its subsequent metabolism and excretion are unclear. Animal studies have shown that binding occurs mostly to soluble cytoplasmic proteins and mitochondria although, additionally, there is some binding to microsomes and DNA[362]. The intra-cellular biological half-life may be around 6 days[363]. Although DMSA has molecular properties consistent with its free filtration at the glomerulus it has a strong affinity for plasma proteins and 90 - 96% of the injected dose may be protein bound[357,364]. For this reason it has been assumed that renal extraction occurs mostly from the post-glomerular peri-tubular capillaries through uptake of protein bound DMSA[355,364,365]. Although this concept has been questioned it has persisted in the absence of any formal kinetic studies to determine the plasma dissociation constant for DMSA. It is in fact likely that an equilibrium is maintained between protein bound and 'free' DMSA, thus providing a sustained pool of DMSA available for glomerular filtration. Recently, Peters and Jones *et al*[366] presented a theoretical analysis, supported by clinical data, showing that glomerular filtration of 'free' DMSA, is likely to form the major route of extraction and that cellular localisation occurs from the tubular lumen. Other recent studies[367,368] were unable to

corroborate this entirely but did conclude that reabsorption of the glomerular filtrate as well as peri-tubular uptake is important for renal localisation.

It is apparent that quantitation of DMSA uptake by the kidney cannot be equated with any single aspect of renal function because it has been found to correlate with a number of different parameters. These include functional cortical mass[355] renal blood flow[258], effective renal plasma flow[259,260,261], creatinine clearance[256,369], $^{99m}$Tc-DTPA glomerular filtration rate[256,257] and tubular function[370-372]. Additionally, it has been shown, both in humans[373] and in pigs[374], that uptake of DMSA by the solitary remaining kidney following unilateral nephrectomy is about double that which occurs in an individual but paired kidney. The animal study[374] indicated that increased uptake was independent of any compensatory renal growth but could be explained by the rapid functional adaptation known to occur in response to lost renal mass (**8.1**). It appears, therefore, that the renal facility for DMSA uptake reflects a combination of interrelated parameters which together determine overall renal performance. It was in view of this that renal uptake of DMSA was included as a test parameter in the Section IV investigation of VUR and in particular because in controlled experimental conditions it may prove a more sensitive indicator of the effects of VUR on the kidney than independent parameters of renal blood flow, glomerular filtration rate or kidney growth. Thus, determination of **relative** uptake in the 2 kidney model (**Experiment II**) may in comparison with control animals detect any abnormal distribution of renal performance

mediated by unilateral VUR. Furthermore, in pigs with a solitary kidney following unilateral nephrectomy (**Experiment I**) any detrimental effect of VUR on the kidney may be resolved by comparison of an enhanced level of **absolute** uptake of DMSA between animals with and without VUR.

## 12.2 The Measurement of $^{99m}$Tc-DMSA Renal Uptake

The sensitivity of uptake quantitation for the detection of renal impairment will be limited by errors which can arise from a number of sources and these are considered below. The methods used to minimise their effect in quantifying DMSA in the pig are included in **Chapter 13**.

### 12.2.1 Preparation of $^{99m}$Tc-DMSA

Dimercaptosuccinic acid is a dicarboxylic acid with sulphahydryl groups in the 2 and 3 positions. It has a low biological toxicity and forms a strong affinity with heavy metal ions making it suitable as an *in vivo* radio-tracer compound. The radio-ligand $^{99m}$Tc, favoured for its short half-life (6.03h), is generated as sodium pertechnetate by elution of $^{99}$Molybdenum with physiological saline. The formation of the $^{99m}$Tc-DMSA chelate from DMSA and sodium pertechnetate requires stannous chloride for reduction of the pertechnetate ($TcO_4^-$). The precise molecular form of the chelate depends upon the proportions of the reactants and the prevailing pH of the medium. Whilst at least 4 types of $^{99m}$Tc-DMSA complex may be formed, animal studies have shown that only one, defined by Ikeda[359] as Complex II, has the appropriate affinity for the renal

cortex[260,358,359]. The optimal conditions for the formation of Complex II have been well established and commercial kits are available which contain the appropriately formulated and lyophilised reactants to which the pertechnetate is added. At maximal efficiency about 90% of complex II is formed from complex I, in a rate limiting reaction which requires about 15 min[359].

Despite the use of commercial kits there are operator dependant variables which may affect the yield of Complex II, and thus the efficiency of renal uptake. The maximum yield from the lyophilised reactants is achieved only when a fixed reconstitution volume provides optimal molar concentrations of DMSA and $SnCl_2$ (1.35mM and 0.45mM respectively). Because Complex II is readily oxidised[260,359] the introduction of air, both during preparation and during withdrawal of successive doses, constitutes a significant source of error despite inclusion of ascorbic acid in the commercial kits and the use of air evacuated or nitrogen filled vials.

The presence of the breakdown product $^{99}Tc$ in the $^{99}Mo$ eluate has been shown to decrease the yield of Complex II[359] but is not considered now of real significance at the concentrations of $^{99}Mo$ used in the pertechnetate generators supplied for clinical use[375].

### 12.2.2 Instrumentation

For convenience a large field of view gamma camera is best used for acquisition of images. Source gamma emission is detected as photons after passage through a crystal (sodium iodide) and

amplification in one or more of a composite and parallel series of photo-multiplier tubes. One of a variety of collimators, usually composed of lead and each of a specific lattice or honeycomb structure, is attached to the gamma camera detector face. Each specific collimator is a fixed but interchangeable determinant of the sensitivity and spatial resolution of the system.

The energy spectrum of $^{99m}$Tc (Figure 22) has a dominant peak at 140 keV with a second smaller peak at 100 keV. A gamma camera set with a 20% window allows detection of radiation between 126 and 154 keV and excludes low energy radiation produced by scatter. Confirmation of the optimal alignment of the window and peak activity is necessary since this may shift with small fluctuations in internal voltages and produce errors in recording the level of radio-activity.

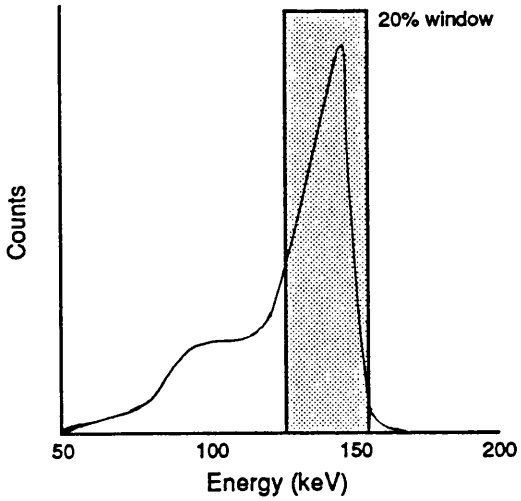



Fig.22 The Energy Spectrum of $^{99m}$Tc.

The performance of the crystal and of the photo-multiplier tubes both contribute to the uniformity of sensitivity across the gamma camera field of view. The efficiency of the crystal deteriorates with age and may be affected by environmental factors. Small changes in sensitivity of individual photo-multiplier tubes can have a considerable effect on uniformity and hence reproducibility, since recorded counts from a single source should be independent of the position of the source on the gamma camera face. Even under optimal conditions counts from a single source, recorded over different areas of the field of view, rarely agree by less than 5% and 12% is a more usual figure. These errors may be accounted for during on-line computer assisted acquisition of images, if this facility is available.

The efficiency of the photo-multiplier detection system decreases with increasing rates of gamma emission. The deviation between true count rate and recorded count rate must be corrected by incorporation of a pre-determined factor, or eliminated by attenuation of the activity at source. This latter procedure itself, requires a factor to account for the attenuation of activity (see below).

### 12.2.3 Recording Radio-activity in the Kidney

#### 12.2.3.1 Extra-renal activity

When an external detector is used to acquire images of the kidneys in place in the body (*in-situ*) it is inevitable that other organs and tissues are included. In order to record radio-activity in individual kidneys whilst minimising that arising from other tissues,

counts are acquired from selected areas (regions of interest - ROIs) which define each of the renal images. Any extra-renal activity (background) remaining in the renal ROI is subtracted (as counts per unit area of ROI) after its assessment from a further separately defined area. There is no ideal site for this background ROI, as all present some inaccuracy, but that favoured is situated around the margin of the renal ROI but avoiding the mid-line between the 2 kidneys[356,375]. This excludes activity in the spine and includes a small fraction of the liver or spleen which are included respectively, in the right and left renal ROIs.

**12.2.3.2 Intra-renal Activity**

The spatial characteristics of the kidney *in-situ* can affect the activity recorded by an external detector. Both size and shape of the kidney determine the distribution of radio-activity but their effects on recorded counts are small. They account for errors of only a few per cent even when comparing the renal activity in extreme situations of size and shape[375].

Of considerable importance, however, is the depth of the kidney below the skin surface. Although quantitation of renal activity is applied only to images acquired in the posterior projection, where tissue thickness is minimal, the absorption of radiation from $^{99m}$Tc represents at 1cm an attenuation to recorded counts of 13%[376,377]. A correction to account for this attenuation is necessary in order to obtain true renal counts from those recorded. The tissue thickness between kidney and detector is measured from calibrated images

obtained either from ultrasound or from a lateral gamma camera view. Both methods are subject to errors resulting from a wrong axis of measurement since tissue thickness must be measured in precisely the same plane as the emission path from the kidney to the gamma camera detector. A lateral gamma camera view has the objection that the 2 kidneys are superimposed and thus only an average depth for the paired kidneys can be obtained. For this reason the ultrasound method was preferred for the Section IV experiments. However, there is a problem with this method in that a depth estimate obtained with the pig prone is unlikely to be the same as when the pig is supine which means the former position has to be adopted for the imaging procedures.

CHAPTER 13

# QUANTITATION OF DMSA RENAL UPTAKE IN THE PIG

An earlier study established the methods for quantitatation of $^{99m}$Tc-DMSA renal uptake in pigs and showed that in the pig this is similar to that in the human. The experiment formed part of a study to determine the relationship between renal uptake of DMSA and renal mass[374]. This study is reviewed with the emphasis on methodology and variability control pertinent to the Section IV experiments. All pigs underwent unilateral nephrectomy at some stage, so providing additional information concerning DMSA uptake by the solitary kidney.

## 13.1 Instrumentation

Renal images were acquired using a 10.5" field of view gamma camera fitted with a collimator suitable for high sensitivity detection at 140 keV. Images were displayed on a long persistence X,Y oscilloscope. A 20% window, recording activity within the band 126-154 keV, was set from the console. The alignment of the window and the peak of the energy spectrum for $^{99m}$Tc was checked using an external voltmeter connected to the appropriate pulse height analyser within the gamma camera. The uniformity of sensitivity across the field of view, estimated by recording count rate from a source placed in 9 marked areas of the gamma camera face varied between 5% and 11%. To eliminate this variable a defined area of the camera face was used for acquisition of count rates both for calibration tests and for the animal studies. Within the defined area the sensitivity of the

gamma camera to 37MBq (1mCi) $^{99m}$Tc pertechnetate was within the range 11,000 - 12,000cps and was checked daily.

The efficiency of the gamma camera was determined (Figure 23a) by recording the count rate (corrected for background activity and decay) from samples of different known activity ($^{99m}$Tc) in a total volume of 4ml. The attenuation to recorded counts by absorption of activity through tissue was determined (Figure 23b) from a source of known activity and a perspex (tissue equivalent) phantom. Count rates acquired through increasing depths of perspex were corrected for background activity and decay.

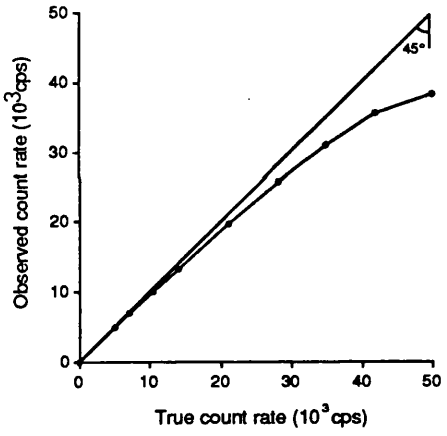



Fig.23a Camera Efficiency

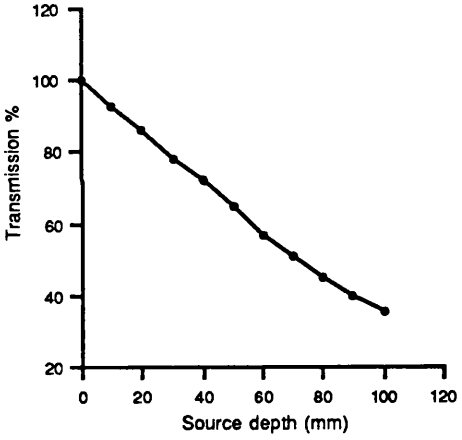



Fig.23b Attenuation of Counts by Tissue Equivalent Media

## 13.2 Preparation of $^{99m}$Tc-DMSA

The DMSA was obtained in kit form in an air evacuated vial and stored at 4°C. $^{99m}$Technetium was obtained as the eluate from a $^{99}$Mo column. In order to reduce $^{99}$Tc-pertechnetate in the final solution

the $^{99}$Mo column was eluted each evening and the eluate discarded. This was repeated the following morning before collecting the eluate for preparation of the $^{99m}$Tc-DMSA chelate. The lyophilised DMSA formulation was reconstituted in a 5ml volume to provide in solution with physiological saline $^{99m}$Tc-pertechnetate, 740MBq (20mCi); DMSA, 1.10mM (200$\mu$g/ml) and stannous chloride, 0.32mM. The $^{99m}$Tc-DMSA solution was kept at room temperature without agitation. Between 30 and 60 min after preparation each required dose was withdrawn into a separate syringe, for immediate injection.

### 13.3 Experiment Design

The experiment design is detailed in Table 8.

Table 8 Experiment design: Quantitation of $^{99m}$Tc-DMSA uptake.
The renal uptake of $^{99m}$Tc-DMSA was measured at weekly intervals in pigs with paired kidneys (++) or solitary kidneys (+) following right nephrectomy. Renal accumulation with time (plateau study - P) was performed immediately before and 1 and 5 weeks after nephrectomy.

| | | Pig No. | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Week | 1 | ++$^{P}$ | ++ | ++ | ++ | ++ |
| | 2 | +$^{P}$ | ++$^{P}$ | ++ | ++ | ++ |
| | 3 | + | +$^{P}$ | ++$^{P}$ | ++ | ++ |
| | 4 | + | + | +$^{P}$ | ++$^{P}$ | ++ |
| | 5 | + | + | + | +$^{P}$ | ++$^{P}$ |
| | 6 | +$^{P}$ | + | + | + | +$^{P}$ |
| | 7 | | +$^{P}$ | + | + | + |
| | 8 | | | +$^{P}$ | + | + |
| | 9 | | | | +$^{P}$ | + |
| | 10 | | | | | +$^{P}$ |

Five Gottingen miniature pigs (3 males and 2 females) were investigated from the age of 4 weeks when they weighed 2.6 - 3.0kg. The pigs were followed for 6 to 10 weeks.

In order to determine when DMSA accumulation reached a stable and maximum level in both paired and solitary kidneys, plateau studies were performed on 3 occasions in each pig (see Table 8). Variability in both absolute and relative uptake was considered from the data at weeks 1 and 2. Information concerning uptake by solitary kidneys was provided after unilateral nephrectomy was performed on each animal in turn on successive weeks 1 to 5.

In addition, an examination was made of the accuracy of deriving true renal counts from external counting of kidneys in place (*in situ*). The true renal counts (*in situ*) obtained in the final uptake study for each kidney were compared with those recorded from the same kidney after its isolation from the body (*ex situ*). In all cases *in situ* count rate was recorded immediately before the kidneys were removed, either by right nephrectomy or *post mortem*. The *ex situ* kidneys were counted in air on full field at a fixed distance of 10cm from the gamma camera face. These procedures followed a dose injection (5-11MBq; 150-300$\mu$Ci/kg) by 3 to 4 hours except on 2 occasions (pigs 1, 3) when they followed the usual dose injection by 24 hours.

**13.4 Method for Measurement of $^{99m}$Tc-DMSA Renal Uptake**

The animals were lightly anaesthetised (Halothane/$O_2$/$N_2O$) for dose injection and for imaging procedures. The dose was injected into an ear vein using a 'butterfly' needle (25 guage). The activity of the injected dose (5$\mu$g DMSA and 3-4MBq; ≈100$\mu$Ci/kg body weight) was determined after counting the dose syringe both before and after

injection (13.4.2). Counts were acquired for 100s at a fixed distance of 10cm from the gamma camera face. No efficiency correction to recorded counts was necessary as count rates were less than 20 × $10^3$counts per s on all occasions.

### 13.4.1 Quantitation

Renal images for the plateau studies (Table 8) were acquired at 1-3h intervals for up to 9h following injection and again at approximately 24h. On other occasions imaging took place between 5h and 7h after injection and again after intervals of 1 - 2h until no further increase (<5%) in renal count rate was recorded. In both situations uptake was calculated from the mean of 2 recordings obtained on the confirmed plateau.

Renal counts were recorded for a preset time of 100s during imaging in the posterior projection, with the pig lying prone and with the aim of maintaining the dorsal surface in contact with the gamma camera face. In practice, in the region of the kidneys the gamma camera face was up to 10cm distant from the dorsal surface. A dual area selection facility on the gamma camera console was used for recording counts. This permitted 2 superimposed regions of interest of different sizes ($ROI_1$,$ROI_2$) to be positioned over each kidney image in turn. The 2 areas were placed eccentrically to minimise the inclusion of activity in the spine (Figure 24). The area size of each ROI and the recorded counts within the ROIs were stored in scaler memories and noted.

Mean depth of each kidney below the skin surface was measured from a calibrated ultrasound image acquired, using a linear array transducer, at the time of renal imaging and with the pig undisturbed so that a correction factor (F) for attenuation of counts by tissue could be made.

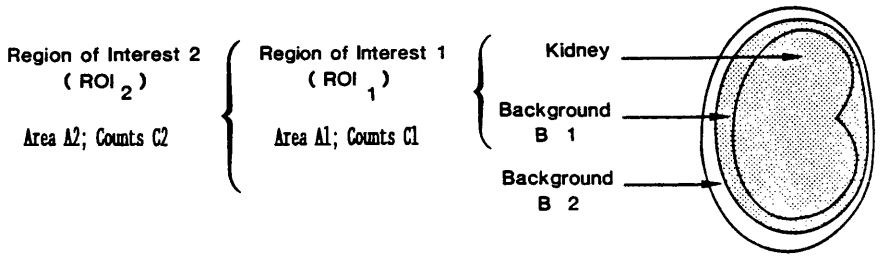



**Fig.24** Acquisition of Renal Counts from Regions of Interest. Counts ($C_1$) from the smaller inner area ($ROI_1$;$A_1$) were derived from the kidney and immediately adjacent background ($B_1$). Counts ($C_2$) from the larger superimposed area ($ROI_2$;$A_2$) were derived from the kidney, immediately adjacent background ($B_1$) and perirenal background ($B_2$). It is assumed background $B_1$ = background $B_2$. (Reprinted[374] with permission; Chapman and Hall, London.)

### 13.4.1.1 True Renal Counts

To obtain true renal counts of an individual kidney background subtraction was performed as follows (Figure 24):

the background ($B_2$) area = $A_2 - A_1$ units

the backgound ($B_2$) count = $C_2 - C_1$ counts

mean background ($B_2$) count = $\frac{[C_2 - C_1]}{[A_2 - A_1]}$ counts/unit area

background ($B_1$) in $ROI_1$ = $A_1 \times \frac{[C_2 - C_1]}{[A_2 - A_1]}$ counts

true renal counts $Rc_1$ = $C_1 - A_1 \times \frac{[C_2 - C_1]}{[A_2 - A_1]}$ counts

True renal counts were corrected for decay occurring during the time interval (hours T) between injection of the dose and imaging of each kidney:

Decay corrected renal counts $Rc_2 = Rc_1 / \exp(-0.1149 \times T)$

where:

0.1149 = the decay constant (per hour) for $^{99m}Tc$ ($\log_e 2 / 6.03h$).

T = time interval (hours decimilised) betwen injection and acquisition of renal counts.

A further correction was made for attenuation of renal counts by tissue :

Depth corrected renal counts $Rc_3 = Rc_2 / F$

where:

F was obtained by applying the measured kidney depth to a graph (Figure 23b) obtained independently using a perspex phantom.

### 13.4.1.2 Absolute Uptake

The activity of the effective dose injected was calculated:

$D = D_1 - D_2$

where:

$D_1$ = dose counts $\times \exp[-0.1149 \times t_1]$

$D_2$ = residue counts / $\exp[-0.1149 \times t_2]$

and:

$t_1$ = time interval (hours decimilised) between counting dose and injection.

$t_2$ = time interval (hours decimilised) between injection and counting the residue.

The uptake of $^{99m}$Tc-DMSA by individual kidneys was calculated as a percentage fraction of the dose:

Absolute Uptake % = $Rc_3$ / D × 100

The calculation of absolute percentage uptake by individual kidneys may be expressed as the algorithm:

$$100 \left(C_1-(C_2-C_1)\times\frac{A_1}{A_2-A_1}\right) \Big/ \exp(-0.1149\times T)\times F \times (D_1-D_2)$$

### 13.4.1.3 Relative Uptake

The uptake of the left kidney relative to the right was calculated:

100 × Absolute uptake left / [Absolute uptake right + left]

## 13.5 Results

The body weights of the pigs at the times of nephrectomy and *post mortem* are shown in Table 9 together with the weights of the right and left kidney obtained on these occasions.

Table 9 Pig body weights and kidney weights at times of nephrectomy and Post Mortem. (Previously published data[374]).

| | | Pig | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Pig weight (kg) | | | | | | |
| | at nephrectomy | 2.5 | 4.5 | 5.5 | 7.5 | 10.5 |
| | post mortem | 11.0 | 10.5 | 12.0 | 12.5 | 20.5 |
| Kidney weight (g) | | | | | | |
| Right | at nephrectomy | 9.0 | 12.3 | 14.5 | 28.3 | 36.6 |
| Left | post mortem | 53.9 | 46.4 | 58.7 | 69.8 | 79.1 |

## 13.5.1 Plateau Studies

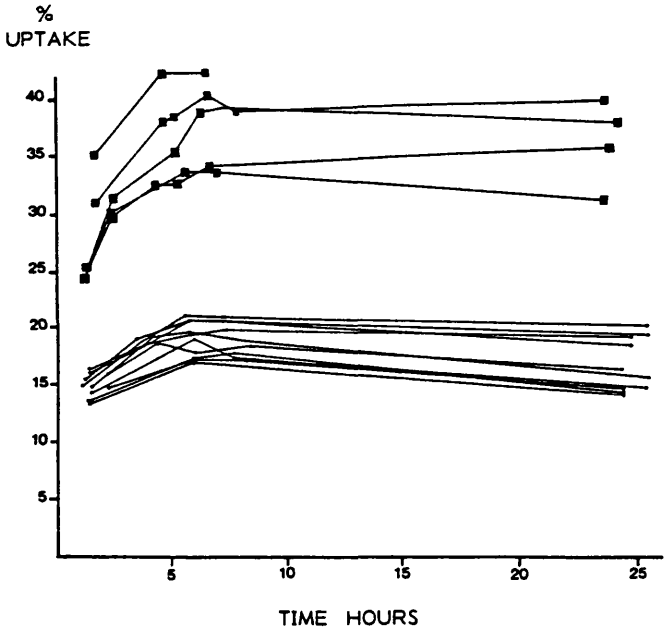



Fig.25 Renal Uptake of $^{99m}$Tc-DMSA as a Percentage of the Injected Dose Against Time After Injection. Values for individual kidneys (right and left) •——• or for solitary kidneys 5 weeks after nephrectomy ■——■ . (Reprinted[374] with permission; Chapman and Hall, London.)

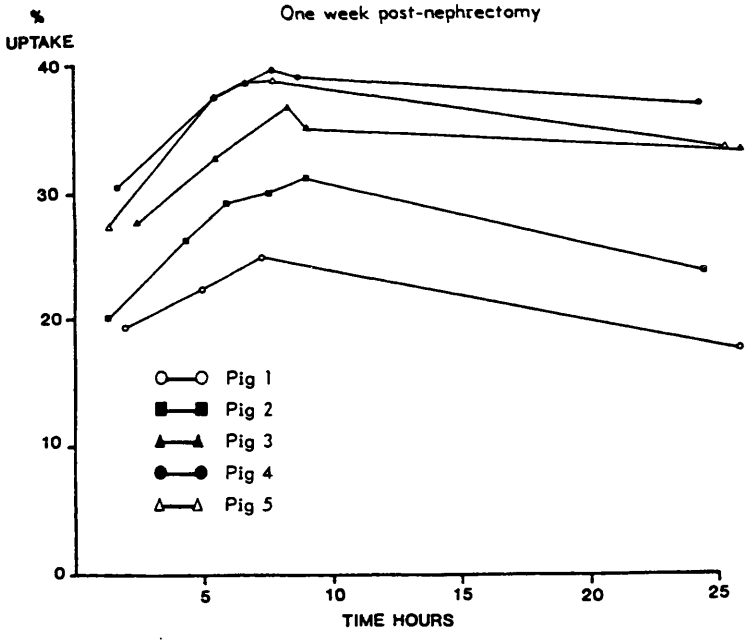



Fig.26 Renal Uptake of $^{99m}$Tc-DMSA as a Percentage of the Injected Dose Against Time After Injection for Solitary Kidneys 1 week After Right Nephrectomy: this performed at successive ages 4 - 8 weeks in pigs 1 - 5 respectively. (Reprinted[374] with permission; Chapman and Hall, London.)

The uptake of $^{99m}$Tc-DMSA by individual kidneys of pairs and by solitary kidneys, 5 weeks after nephrectomy, reached a maximal level between 5 and 7 hours after the dose injection (Figure 25). The uptake recorded at 24h was slightly less than that obtained at 6h for both the paired kidneys (mean 6h = 18.8%, mean 24h = 16.5%) and for the solitary kidneys (mean 6h = 37.6%, mean 24h = 35.7%).

In solitary kidneys 1 week after nephrectomy a satisfactory plateau of uptake, was obtained only in the 3 older pigs (Figure 26).

### 13.5.2 Absolute Uptake by Paired Kidneys

The results are shown below.

Table 10 Absolute uptake of $^{99m}$Tc-DMSA by paired kidneys. Confidence limits (CL) at week 1; mean +/- SE × 2.57, at week 2; mean +/- SE × 2.78. (Includes previously published data[374]).

| | | Pig | | | | | Mean | CL |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | | (Mean +/- SE×$t_{df}$) |
| Week | Kidney | | | | | | | |
| 1 | Left | 17.2 | 17.2 | 18.7 | 18.1 | 19.6 | 18.2 | 17.0 - 19.3 |
| | Right | 18.2 | 16.6 | 19.0 | 18.8 | 18.0 | 18.1 | 17.0 - 19.2 |
| 2 | Left | | 19.2 | 18.2 | 18.1 | 18.0 | 18.4 | 17.6 - 19.2 |
| | Right | | 17.7 | 17.1 | 18.2 | 18.1 | 17.8 | 17.1 - 18.5 |
| 3 | Left | | | 16.8 | 20.1 | 17.0 | 18.0 | |
| | Right | | | 17.6 | 20.3 | 17.7 | 18.5 | |
| 4 | Left | | | | 19.6 | 20.5 | 20.6 | |
| | Right | | | | 20.5 | 21.0 | 20.8 | |
| 5 | Left | | | | | 20.7 | | |
| | Right | | | | | 20.5 | | |
| Mean | Left | | 18.2 | 17.9 | 19.0 | 19.4 | | |
| | Right | | 17.2 | 17.9 | 19.5 | 19.1 | | |

There was little variability in the values for absolute uptake by individual kidneys of pairs (Table 10) although values increased marginally in older pigs (after week 3 when the pigs were aged 6 weeks and weighed 6.0kg.).

The total uptake (left + right) was within the range 33.8% (pig 2 at week 1) and 42.5% (pig 5 at week 4). There was no difference in uptake between right and left kidneys of pairs.

### 13.5.3 Relative Uptake

There was little variability in the values for left relative uptake (Table 11).

Table 11 Left relative uptake (100×L/[L+R]) of $^{99m}$Tc-DMSA by paired kidneys. Confidence limits (CL) at week 1; mean +/- SE×2.57. At week 2; mean +/- SE × 2.78.

| | Pig | | | | | Mean | CL |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | (Mean +/- SE×$t_{df}$) |
| Week | | | | | | | |
| 1 | 48.5 | 50.8 | 49.6 | 49.1 | 52.1 | 50.5 | 48.5 - 50.5 |
| 2 | | 52.0 | 51.6 | 49.9 | 49.5 | 50.8 | 49.2 - 52.7 |
| 3 | | | 48.8 | 49.8 | 49.0 | 49.2 | |
| 4 | | | | 48.9 | 50.6 | 49.8 | |
| 5 | | | | | 50.2 | | |
| Mean | | 51.4 | 50.0 | 49.4 | 50.3 | | |

### 13.5.4 Absolute Uptake by Solitary Kidneys

The individual values for absolute uptake by the remaining kidney are shown in Table 12 for each successive week following right nephrectomy. By 5 weeks after unilateral nephrectomy absolute uptake by the solitary kidney was approximately twice that of the pre-nephrectomy values in individual kidneys of pairs. This was also apparent the first week after nephrectomy in the 3 older pigs (pigs 3,4 and 5; nephrectomy at ages 6,7 and 8 weeks). In the younger animals (nephrectomy at ages 4 and 5 weeks) absolute uptake increased during the weeks following unilateral nephrectomy. In individual pigs the greatest difference between uptake values (recorded during a confirmed plateau) on successive weeks was 5.4% of the dose (mean 4.2%).

Table 12 Absolute uptake of $^{99m}$Tc-DMSA by solitary kidneys in the 5 weeks following unilateral nephrectomy; this latter performed at various ages.

| | Pig 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Age (weeks) at right nephrectomy | 4 | 5 | 6 | 7 | 8 |
| Pig weight (kg) at right nephrectomy | 2.5 | 4.5 | 5.5 | 7.5 | 10.0 |
| Absolute uptake % | | | | | |
| Post-nephrectomy week 1 | 25.6[1] | 31.2[1] | 36.7 | 39.6 | 38.6 |
| 2 | 31.1 | 35.2 | 36.2 | 36.5 | 38.3 |
| 3 | - [2] | 34.4 | 41.6 | 39.0 | 40.4 |
| 4 | 27.6 | 35.2 | 37.5 | 36.9 | 41.4 |
| 5 | 33.5 | 38.8 | 39.1 | 34.7 | 42.1 |
| Mean | 30.7 | 35.9 | 38.2 | 37.3 | 40.1 |

[1] Not recorded from plateau, see text and Fig.26 (13.5.1).
[2] Data unavailable due to equipment failure.

(Adapted from previously published table[374]; Reprinted with permission, Chapman & Hall, London.)

### 13.5.5 Renal Counts (*in situ* and *ex situ*)

The 'true' fully corrected count rate recorded from the isolated kidney (*ex-situ*) was greater than that recorded from the kidney in the body (*in-situ*), on 8 of 9 occasions (Table 13). The difference between the paired results was greatest when the count rate was low (pig 1,right). On all other occasions the increase (*ex-situ/in-situ* ×100) did not exceed 11% and was a mean of 5%.

Table 13 Comparison between 'true' renal counts recorded from the kidneys within the animal (*in situ*) and those recorded from the same kidneys isolated after *Post Mortem* (*ex situ*).

| Pig | Kidney | Counts/s *in situ* | Counts/s *ex situ* | Ratio (*ex situ:in situ*) |
|---|---|---|---|---|
| 1 | Right[1] | 365 | 552 | 1.34 |
| | Left | 14330 | 14310 | 1.00 |
| 2 | Right[2] | - | - | - |
| | Left | 7134 | 7540 | 1.06 |
| 3 | Right[1] | 1006 | 1080 | 1.07 |
| | Left | 5796 | 6410 | 1.10 |
| 4 | Right | 4326 | 4460 | 1.03 |
| | Left | 6635 | 7381 | 1.11 |
| 5 | Right | 5812 | 6148 | 1.06 |
| | Left | 7763 | 8052 | 1.04 |

[1]Counts recorded 24 hours after dose injection(see 13.3).
[2]Data lost due to equipment failure.

## 13.6 Discussion

This study has shown that in pigs with normal paired kidneys renal uptake of $^{99m}$Tc-DMSA is similar to that which occurs in the human[356,361] and reaches a maximum and relatively stable level at about 6 hours after the dose injection. The decline in renal activity observed at 24 hours may be mostly explained by errors

inherent in the low count rates recorded at this time particularly in the smallest pigs. This is implied by the results of pig 1 in a comparison of the count rate recorded from the kidney *in-situ* with that after its isolation from the attenuating effects of body tissue (Table 13). It is therefore important, when implementing these methods in the Section IV experiments, that a dose of $^{99m}$Tc-DMSA is used which is sufficient to provide an externally recorded 'true' and fully corrected renal count rate of at least 1000c/s, or raw renal counts of about 200c/s or more at 24h.

There was no difference in absolute uptake between right and left kidneys of pairs and the total (left + right) was within the range reported for the human but lower than has been encountered in clinical practice[355,361]. The disparity may result from subtle differences in both the equipment and the practice between institutions or by the preparation of $^{99m}$Tc-DMSA. The reconstituted DMSA used in this experiment contained DMSA and $SnCl_2$ in lower molar concentrations and in a higher ratio of DMSA to $SnCl_2$ (3.44 compared to that of 3.0) than that providing optimal affinity for the renal cortex[359]. The uptake values were similar to those observed in children using the same preparation of $^{99m}$Tc-DMSA as used in this study[356].

For individual kidneys of pairs the variability in both absolute and relative uptake values appeared to be small although the experimental design was not suited to a formal statistical analysis of these aspects. The 95% confidence limits calculated from the data at

weeks 1 and 2 suggest that if the same level of variability is maintained in the Section IV investigation then unilateral VUR effects can be detected, in comparison with control pigs, if they produce a change in either absolute or relative uptake of +/- 2% and +/- 3% respectively.

A comment concerning variability of uptake in solitary kidneys cannot be made directly from the post-nephrectomy data since uptake appeared to be affected by the age at which unilateral nephrectomy was performed and the interval between nephrectomy and the uptake determination. However, if in the solitary kidney model in the Section IV investigation, absolute uptake values in a homogenous series of pigs have a consistency similar to that achieved here by the paired kidneys, then it is likely that VUR effects can be detected, in comparison with control pigs, if they produce a change of about +/- 4% absolute uptake.

The observation that in solitary kidneys the adaptation in uptake to unilateral nephrectomy is affected by age has important consequences for the Section IV investigation. This is because in **Experiment I** it is essential to perform unilateral nephrectomy in the weeks shortly after birth and it may not be possible to operate on all pigs at precisely the same age and weight. As a precaution, when analysing the uptake data for **Experiment I**, it will be necessary to establish whether or not there is an association between uptake and age or body weight at the time of unilateral nephrectomy and if so to account for this.

The apparent underestimation of counts recorded from kidneys within the animal in comparison with those recorded from isolated kidneys was, with the exception of the one with especially low counts, fairly consistent, producing an effective mean error of -1.2% absolute uptake in individual kidneys of pairs or twice this in solitary kidneys. The sources of this discrepancy could be associated with estimation of *in situ* background activity and/or the depth of the kidney below the skin surface. Of these, the estimation of kidney depth was considered to be of major significance. This means it is important to measure kidney depth, by a carefully executed and consistent technique, as part of every uptake study. Recording renal count rate in the posterior projection with the pig lying prone allows ultrasound depth estimation of individual kidneys whilst the pig is in the same undisturbed position. It was found that depth estimates could vary quite considerably with the position of the ultrasound probe on the dorsal skin surface. A position was chosen where the recording surface of the probe was in the same horizontal plane as the gamma camera face. This position often gave a poor renal image but was sufficient to determine the mean depth (skin to renal pelvis). Despite applying these technical considerations it is important that errors associated with kidney depth are assessed in the Section IV experiments by testing the agreement between *in situ* and *ex situ* recordings at the end of the study period.

CHAPTER 14

## MAXIMAL URINARY CONCENTRATION

Urine concentration refers to the number of dissolved particles in solution, irrespective of whether or not the particles are in the form of molecules or are dissociated ions. In either case, although the latter is assumed, the particles are equivalent to moles whose concentration (osmoles) is measured by freezing point depression relative to the cryoscopic constant for water *i.e* the molal freezing point depression constant (1.86)[378]. In determining the concentration of urine it is now customary to measure the quantity of dissociated solute in 1 kg of water (osmolality) in preference to the amount in 1 litre of water (osmolarity). This is because large amounts of solute significantly expand 1kg of water to more than a one litre volume. In normal physiological conditions the urine specific gravity parallels urine osmolality but refers only to the weight of solutes in solution not their number[379].

The highest urine osmolality which can be achieved during conditions of anti-diuresis, when there is a homeostatic need for water conservation, is a measure of the kidney's capacity to form a urine more concentrated than that of plasma. The formation and control of a hypertonic urine requires the institution and moderation of the medullary concentration gradient and the regulatory action of anti-diuretic hormone (ADH) on the collecting duct.

## 14.1 The Urinary Concentrating Process

The formation of a hyperosmolar medulla depends upon countercurrent exchange in the the long corticomedullary loops of Henle. The process is driven in the thick ascending limb of the outer medulla by active $Cl^-$ secretion which allows passive diffusion of $Na^+$ from the tubule to the interstitium. The vasa recta are critical in establishing a medullary osmotic gradient because they act as countercurrent multipliers allowing solutes, of which the presence of urea is essential, to become trapped within the interstitium whilst water bypasses it[380,381]. The countercurrent system thereby works to produce a hypertonic interstitium and a marginally hypotonic distal tubular fluid. Under the control of ADH, which increases the water permeability of the collecting duct by stimulating cell production of cyclic AMP[382], a maximum modification to urine concentration is permitted during its subsequent flow through the collecting duct in the outer and inner medulla.

Unresolved questions remain concerning the countercurrent operation and its control. Furthermore, this brief account of the control of urine osmolality is inevitably simplified. It is known, for example, that prostaglandin $E_2$ antagonises the action of ADH on the collecting duct and is involved in both moderating and influencing water reabsorption[383,384]. Additionally, it has been theorised that any decrease in blood flow through the vasa recta may increase the efficiency of the countercurrent exchange and in this way may mediate in an increase in the medullary concentration gradient[380]. Recently, Chou, Porush and Faubert[385] reviewed the evidence for, and

importance of, an independent regulation of medullary blood flow. As well as the renal nerves a number of autocoids are implicated of which angiotensin II and the prostaglandins are likely to be of special importance as both specifically and uniquely affect the inner medullary microvascular circulation. This means that any influence on the kidney which affects medullary blood flow or its regulation may, as a consequence, disturb the medullary concentration gradient.

In addition to a regulatory role in the concentrating process by the more established autocoids there is also current interest in endothelin, a potent vasoconstrictor peptide[386]. Evidence from a recent experiment has indicated that it can decrease urine concentration by inhibition of the anti-diuretic action of vasopressin[387]. Furthermore, in a topical review, Campbell and Heinrich[388] noted the possibility that endothelin may be involved in modulation of the renin-angiotensin system, and therefore perhaps may influence regional medullary blood flow, because it has a potent renin inhibitory effect whilst its release from endothelial cells is itself stimulated by vasopressin and angiotensin. Endothelin is of especial interest in relation to any effects of VUR because it is released in response to a number of stimuli, some of which are associated with endothelial cell stress or damage[389,390]. It may be significant, in this context, that on occasions fibrosis of the arcuate arteries has been noted in otherwise macroscopically and histologically normal kidneys taken from pigs with VUR and elevated intra-vesical pressures[7]. Far more information is required, however, before speculation concerning the role of endothelin can be soundly placed.

## 14.2 The Maximal Urinary Concentration Test as a Sensitive Indicator of Renal Disorder.

It is apparent that formation and control of a maximally concentrated urine is a complex operation dependent upon hydrostatic pressures within the tubules and the control of intra-renal blood flow, as well as tubular cell metabolic integrity and sensitivity to hormonal influences. The complexity may ensure a high degree of homeostatic control but the obverse may apply, meaning that production of a maximally concentrated urine is particularly vulnerable. In addition, in the process of urinary concentration, there is also interplay with other homeostatic concerns specifically directed towards excretion of toxic metabolites, natriuresis and the conservation of other essential electrolytes besides sodium. A loss of maximal concentrating ability may, therefore, represent a final common response to various adverse stimuli affecting a number of cellular, regulatory and/or homeostatic mechanisms.

There is evidence suggesting that the urinary concentrating test may be more sensitive than other renal function tests for detecting renal involvement in disease. This has been noted, for example, in hypercalcaemia[391] and in some instances as the late sequelae after recovery from acute haemolytic uraemic syndrome[392] as well as in children with VUR in the absence of renal scarring[89].

Impaired concentrating ability is one of the classical characteristics of chronic partial ureteric obstruction[393]. In an experimental study of this, using dogs, urinary concentrating ability

was affected more profoundly and with greater consistency than clearance function[394]. Other studies in dogs have shown that a concentrating defect follows raised ureteric pressures (100mm Hg or more) sustained only for a matter of minutes[395]. The post-obstructive concentrating defect was not due to 'washout' of urine trapped in the renal pelvis during the obstructive period, nor was it secondary to any effect on glomerular filtration rate and in some cases it persisted for up to 4 hours. That concentrating ability did eventually return indicates that a concentrating defect does not necessarily represent irreversible renal impairment. Similarly, the concentrating defect which can accompany acute urinary infection, and most likely represents upper tract or renal involvement[396-398], can be reversed by treatment[105,398,399] although complete reversal may take as long as 12 weeks[105].

### 14.3 Urinary Concentration in the Pig

Because the length of the corticomedullary loops of Henle are important determinants of the medullary concentration gradient it follows that inter-species differences in relative medullary thickness affect the urinary osmotic ceiling. Thus, animal species such as the desert rat which have relatively high medulla-to-cortex ratios and long loops of Henle, can produce urine of considerably greater hypertonicity than, for example, the beaver with a small medullary thickness and no long loops of Henle[165]. The pig has only half the relative medullary thickness of the human kidney and a smaller percentage of long loops of Henle[157]. Despite this, the maximum achievable urinary concentration is only slightly less in the

pig than in man[169].

However, there may be differences in the operation of the concentrating mechanism between the human and pig. In the human[400] and some experimental animals[165,168,401-403] concentrating capacity and the excretion of non-urea electrolyte can be enhanced by increasing urea excretion. In contrast, evidence from one report suggests the pig may be one of several mammals in which the excretion of urea and non-urea electrolytes is inversely related[168]. Plaake and Pfeiffer[167] have related this to the absence of a distinct zonation of the medullary capillary plexus in the pig and suggested that because of this the vasa recta are less able to trap urea.

### 14.4 The Maximal Urinary Concentration Test

Maximal urinary concentrating ability is tested either after a period of water deprivation or after the administration of diamino-8-d-arginine vasopressin (Desmopressin;DDAVP), a synthetic analogue of human vasopressin producing only minimal pressor effects. In clinical practice the DDAVP test is preferred because, with the water deprivation test absolute maximum urinary concentration may not be achieved for between 24 and 36 hours[379]. Urine collections are made 5 to 9 hours after DDAVP administration, during which period water intake is limited but not withdrawn completely. By either method a minimum of 2 urine collections are required in order to establish that maximal concentration is attained. It is recognised that with the DDAVP test the maximum urinary concentration attained is marginally less than with water deprivation[379].

Both tests have limitations when applied to pigs. As mentioned before (5.2), pig vasopressin contains lysine and not arginine, therefore the DDAVP test may not provide a true measure of the maximal urinary concentration achievable. With the water deprivation test there is an ethical objection to the withholding of fluid from experimental animals for periods much exceeding 20 hours. A pilot study involving 3 Gottingen pigs showed that the DDAVP test gave lower maximal urinary concentrations (700-900 mosmol/kg $H_2O$) than water deprivation for 19 to 21 hours (>1000 mosmol/kg $H_2O$). In addition, adverse effects to DDAVP such as shivering and pilo-erection were noted. The water deprivation test showed, from 3 urine collections obtained after 17, 19 and 21 hours of water deprivation, that maximal urinary concentration was attained by 19 hours. Animals were anaesthetised for the urine collections which were obtained by suprapubic puncture of the bladder using ultrasound assistance. On each occasion the bladder was emptied. Food was not withdrawn since it consists of dry pellets and this may have contributed to the achievement of maximal urinary concentration in less time than occurs in the human.

**14.5 Conclusions**

Water deprivation for 17-20h induces maximal urinary concentration in the pig and therefore is the method of choice for the Section IV investigation. The urinary concentrating ability test is likely to be a sensitive indicator of any effects of VUR on the kidney, however the mechanisms of any such effects cannot be deduced and indeed could

be multifactorial. As there may be subtle differences in the concentrating process between the pig and the human the results concerning the effects of VUR on maximal concentrating ability should be interpreted with caution in relation to the human condition of VUR.

CHAPTER 15

# RENAL GROWTH

Biological growth is represented by an increase in unit size, or shape with time. As a quantitative rather than qualitative aspect of development it is distinct from associated cellular differentiation or the renewal activity which balances the rate of cell synthesis with that of cell death.

During development of an individual or an organ, enlargement occurs in concurrence with quantitative, organised changes in component structures. By this reasoning the size of the nephron may be considered the appropriate index of renal growth since it is the primary functional unit of the kidney. The complexities of accurately measuring tubular mass are such that more traditional methods are favoured although perhaps less expressive of true renal growth.

## 15.1 Methods of Renal Growth Assessment

Direct methods of measurement require the removal of all or part of the kidney. Because they are limited to a single discrete measure they provide information only concerning the results of growth. In order to examine the progress or rate of growth repeated but indirect measures are necessary. In the Section IV experiments renal growth was assessed from kidney weight at the end of the study period. No attempt was made to assess the progress of renal growth using

indirect methods. These aspects are discussed.

### 15.1.1 Indirect Methods

Indirect methods depend upon measuring kidney size from an image provided either by ultrasound or intra-venous urography. As well as the difficulty of assessing the size of a 3-dimensional kidney from a 2-dimensional image there is debate concerning the choice of the most appropriate dimensions from those available[404]. Of these, length, width, parenchymal thickness, parenchymal area and cross sectional area have been used either independently or in combinations [119,130-134,137].

The major source of error in measuring renal size arises from the adverse effects of magnification on apparent dimensions. Even when magnification of the radiograph is accounted for, either from the magnification ratio recorded at the time of imaging or by relating renal dimensions to an internal reference of body size (such as the distance between lumbar segments[132]), a further uncontrolable error remains. This occurs because the normal rotation of the kidney from the horizontal plane causes distortion of the magnification ratio across the image. Griffiths, Cartwright and McLachlan[404] found from a study of cadavers that kidney rotation may vary between 0 and 24° affecting reductions in renal length of the supine image between 0 and 14mm. The degree of rotation was not necessarily the same for the left and right kidneys of pairs, being usually greater for the right kidney than for the left. Griffiths and co-workers[404] suggest this may account for the radiological observation that the right

kidney is shorter than the left[405] by more than would be expected from *post mortem* studies[406].

Notwithstanding limitations, radiographic renal length provides a reasonable index of overall kidney size[404] but problems remain in relating size changes with growth. An increase in renal length, width or cross sectional area is not truly representative of the growth of renal substance because these measurements take no account of the proportion of the kidney occupied by the renal sinus which has been shown to decrease between birth and 30 years of age[131]. When there is pathological enlargement of the renal sinus, as may occur in the presence of VUR, length measurements may even conceal a reduction in parenchymal growth. To overcome this it has been suggested[131] that bipolar parenchymal thickness is also taken into account, but this dimension is in itself subject to artefactual enhancement by rotation of the kidneys[404] and large individual variations have been reported[407,408]. Planimetric assessment of parenchymal area[133] may offer an improved technique but has not been validated against actual renal size.

Ultrasound imaging provides for the measurement of kidney size in 3 dimensions which allows estimation of renal volume by applying the formula for an elipsoid. In a study of 325 children Dinkel and Ertel *et al*[135] found that volume and length parameters correlated well with height or age but the best were obtained with log transfomed kidney volume versus body weight or body surface area. A significant discrepacy, which may be 20% or more, between

sonographic and radiographic size measurements has been noted[135,409]. However, as yet there are no studies which define the agreement between either the sonographic or radiographic measurement and the true dimension of the kidney.

### 15.1.2 Direct Methods

The most commonly used growth index is kidney weight, however the weight of the fresh wet kidney will be determined largely by its fluid content. The method may be refined by prior dessication of the kidney to eliminate any contribution by artefactual water content, but is more suited to studies with small animals.

After water, protein is the next largest component of kidney weight. Total protein weight will approximate closely to dry kidney weight and in normal rat kidneys has been shown to be directly proportional to wet kidney weight[323]. Further observations in the growing rat have shown that total DNA content, which is related directly to cell number since the weight of DNA in each mammalian cell is a constant[322], increases in almost direct proportion to wet kidney weight[323]. Therefore, in normal circumstances proportional water content does not vary significantly and wet kidney weight gives a reasonable index of the extent of growth of renal substance.

Calculation of relative kidney weight, as the percentage ratio of wet kidney weight to body weight, is an alternative refinement, of value in minimising the effect of whole body growth on kidney weight when examining the influence of some factor, such as VUR, on renal growth.

The problem of using derived indices has been reviewed (10.2). Because kidney weight is not directly proportional to body weight (6.2) the use of the kidney weight to body weight ratio needs careful consideration as it may lead to misinterpretation of results, particularly when comparing renal growth in subjects of diverse body weight. The problem may be overcome by applying the allometric equation $y = bx^k$, attributed to Huxley[410]. In this case, kidney weight(y) is related to the fraction (b) of body weight (x) and the exponent (k) describes the rate of change of kidney weight with respect to body weight. Comparison of renal growth (*i.e.* kidney weight) between test and control groups can be made by examining the differences in intercept (b) and slope ($x^k$). The constancy of this relationship has been established for both large and miniature swine[205,216], however, Preece[411], whilst acknowledging its usefulness in demonstrating symmetry and conformity of growth in specific circumstances, has reviewed the problems in its application since the exponent (k) does not necessarily remain constant for the whole period of growth and may vary quite unpredictably.

## 15.2 Measurement of Renal Growth in Section IV Experiments

It is apparent that renal growth assessed from radiographic measurements is subject to considerable error and was not therefore considered for the Section IV investigation. Sonographic measurements may be better, but accurate results depend on more advanced equipment than that available. Renal growth was assessed only from the direct measure of kidney weight at the end of the study period. Because VUR, unlike ureteric obstruction, is unlikely to

affect the water content of the kidney this was considered acceptable. Since a change in kidney weight with time cannot be established it was important, therefore, to aim to follow individual pigs with VUR and/or compensatory growth for the same periods of both time and equivalent somatic growth. It remains necessary, however, to account for any residue of variability apparent as disparate body weight and due to variations in growth rate between individual pigs. This was obviated in **Experiment II** by using a paired kidney model with unilateral VUR which allows the weight of the kidney subjected to VUR to be examined in relation to the contralateral kidney without VUR. In the solitary kidney model in **Experiment** I the influence of somatic growth (or body weight) was eliminated by calculating the ratio of kidney to body weight. However, the same importance of maintaining a similarity in body weight between pigs applies to derived indices of kidney weight as it does to the analysis of glomerular filtration rate (**11.1.6**).

# Section IV

# An Investigation of the Effects of Vesicoureteric Reflux on Renal Growth and Function

TWO EXPERIMENTAL STUDIES IN GOTTINGEN MINIATURE PIGS

**Experiment I**

The effects of vesicoureteric reflux on renal growth and function. Evaluation in pigs with **solitary kidneys** undergoing growth and compensatory growth following unilateral nephrectomy in comparison with non-refluxing control animals. The effects of reflux examined in the presence and absence of bladder outflow obstruction.

**Experiment II**

The effects of unilateral vesicoureteric reflux on renal growth and function. Evaluation in pigs with **paired kidneys and unilateral reflux** in comparison with non-refluxing control animals. The effect of reflux examined in the presence and absence bladder outflow obstruction.

CHAPTER 16

# INVESTIGATION DESIGN AND PROTOCOL

## 16.1 Investigation Design

The investigation was undertaken in 2 experiments using pigs with either a solitary kidney (**Experiment I**) or those retaining paired kidneys (**Experiment II**). In both experiments the influence of VUR was examined in growing pigs over a period of about 5 months.

In **Experiment I** the effects of VUR are measured by comparing glomerular filtration rate, urinary concentrating ability, $^{99m}$Tc-DMSA renal uptake and renal growth in pigs with reflux, with similarly prepared non-refluxing controls. The fact that compensatory growth and augmented $^{99m}$Tc-DMSA uptake normally occur in the remaining kidney following uninephrectomy might help to exaggerate any differences in renal function or growth consequent on VUR.

In **Experiment II** an alternative but complimentary approach is made. By retaining both kidneys, comparison is possible not only between animals with and without unilateral reflux, but also, within the refluxing group, between the refluxing and non-refluxing kidney subjected, in an individual animal, to precisely the same experimental conditions apart from the presence or absence of VUR.

In **Experiment II**, VUR is induced in all test animals on the left side. The effects of VUR are documented as the relative (left/[left

+ right] kidney growth and relative uptake of $^{99m}$Tc-DMSA. Glomerular filtration rate and urinary concentrating ability are assessed in **Experiment II** but only to establish the functional homogeneity of the experimental groups.

In **Experiment I** and **Experiment II** the effects of VUR were tested in pigs with normal bladder function and in those with bladder outflow obstruction and abnormal bladder function. In both experiments, test and control pigs are required for each of these bladder function conditions, making 4 groups of animals in each experiment (Figure 27).

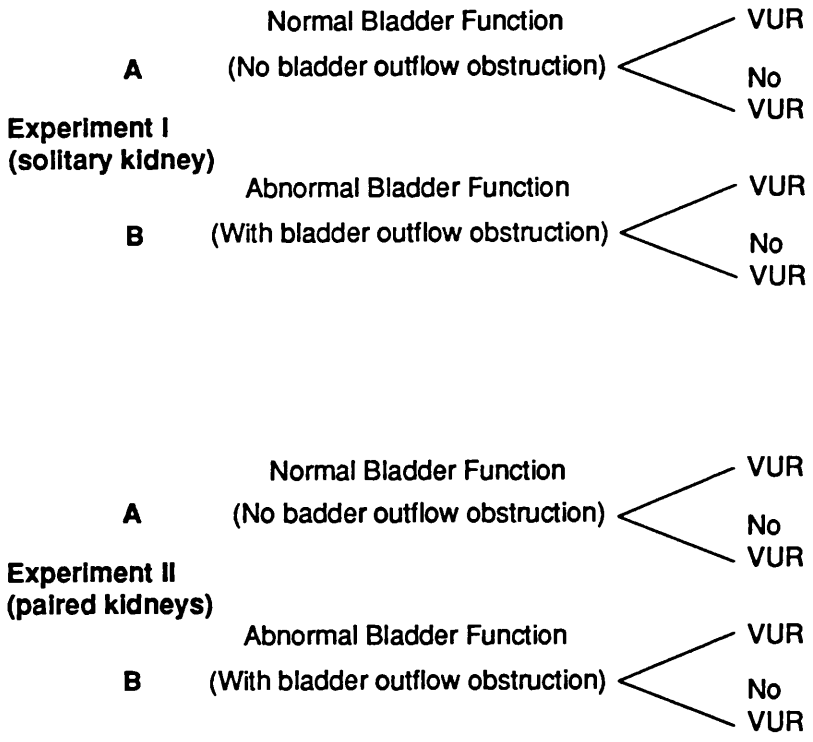



Fig.27 Investigation Design

## 16.2 Investigation Protocol: Experiments I and II

Pigs in the 8 groups of the combined experiments were studied in parallel. Litter mate animals were distributed between groups of both experiments but with the priority of placement pairwise between a test (with VUR) group and corresponding control group. As female pigs are unsuitable for the required model of bladder outflow obstruction (9.2) male pigs were assigned to groups B of each experiment. Sequential litters of pigs were introduced to the study at intervals of about 6 to 8 weeks. The protocol for pigs in **Experiments I and II** were similar (Figure 28).

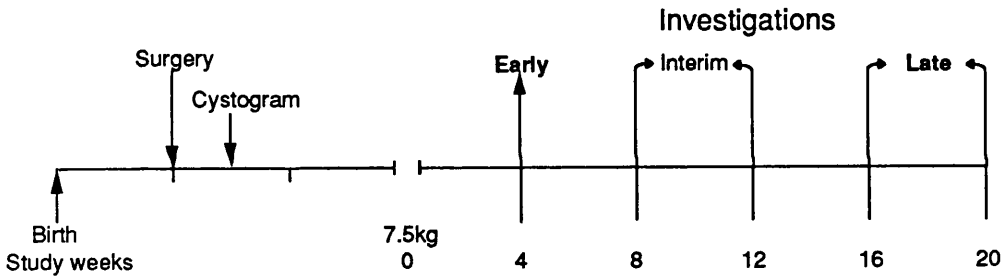



**Fig.28 Investigation Protocol (Experiments I and II).**

Surgery (in pigs 3 to 5 weeks of age): Unilateral nephrectomy - all pigs in Experiment I; Urethral ring implanted - all group B pigs (Experiment I and Experiment II); Creation of unilateral VUR - groups A1 and B1 in Experiment I and Experiment II. Cystography (10 to 14 days after surgery) - groups A1 and B1 of both experiments.

Three discrete investigation periods were designated. These were; **Early**, study week 4; **Interim**, between study weeks 8 and 12; and **Late**. The **Late** (final) time was pre-designated as study week 16 but

in practice extended between weeks 15 to 20 (16.3.4). The investigations of renal function, as well as the assessment of bladder function by cystometrography, were performed at these intervals as detailed in Table 14.

The pigs were weighed weekly and entered the study period when they had achieved a weight between 7 and 8kg (study week 0). After completion of the investigations at the Late time the pigs were killed, the urinary tracts removed, examined macroscopically and all kidneys weighed.

Table 14 Details of the investigations performed at Early, Interim and Late times.

| | Early (week 4) | Interim (weeks 8-12) | Late (weeks 15-20) |
|---|---|---|---|
| Experiment I | GFR<br>DMSA<br>CMG (group B) | GFR*<br><br>CMG (group B) | GFR<br>DMSA<br>CMG<br>Concentrating ability<br>Kidney weight |
| Experiment II | <br>DMSA<br>CMG (group B) | <br><br>CMG (group B) | GFR<br>DMSA<br>CMG<br>Concentrating ability<br>Kidney weight |

GFR - $^{51}$Cr-EDTA (C-slope) clearance; DMSA - Renal uptake of $^{99m}$Tc-DMSA; CMG - cystometrography to determine bladder function.

*GFR measured at the interim time was not an absolute requirement of these experiments. It was measured in Experiment I pigs, but with the exception of 2 group A2 pigs, in order to monitor the progress of GFR. In all other cases investigations were performed in all pigs of groups A and B, or group B alone (group B).

## 16.3 Discussion of Protocol

The essential aims of the experimental protocol were, firstly: to satisfy the requirement of the investigation in rapidly growing pigs, and secondly: to restrict variability both between individual pigs and between the groups to that imposed only by the required conditions of the experiment. Of particular importance was the requirement to retain a similitude of body weight between the pigs, throughout the study period, whilst maintaining a consistency in the period of study *i.e* the time to which the pigs were exposed to VUR and/or compensatory renal growth. It was impossible, both for theoretical and practical reasons, to fulfill these objectives absolutely and the protocol used represents the best possible compromise.

### 16.3.1 Preparation of the Model

Surgery was undertaken when the piglets were several weeks old and not immediately after birth to minimise their exposure to stress (9.5) thereby restricting any impediment to the potential for growth. Control pigs without VUR were not subjected to sham surgery as an investigation into the direct effects of surgical induction of VUR was not the objective and it was desired to keep the control pigs as physiologically normal as possible within the confines of essential model requirements. A pre-requisite to complete all surgery and cystography before the pigs achieved a weight of 7.5kg was rigorously applied.

### 16.3.2 Controlling Variability

As body weight is an index of growth (6.1) this was used rather than age to select the point at which the pigs entered the study period. This meant that any transient effect of surgery on body weight was accounted for. In addition, this practice gives the best opportunity for maintaining a similarity in body weight between pigs throughout the study period. It was anticipated that this, combined with the practice of investigating the pigs at specific intervals, would favour minimal variability both within and between the various experimental groups. This is important when comparing growth and function parameters between the test and control groups (10.2;15.2) and allows the effects of VUR to be more readily resolved. The practice of studying the 8 groups of pigs, particularly those in corresponding test and control groups, as a parallel series of litters exposes each group to equivalent environmental or other external effects, which might otherwise bias the performance of individual groups.

### 16.3.3 The Physiological Investigations

The measurement of GFR at the **early**, **interim** and **late** times in **Experiment** I and DMSA uptake **early** and **late** in both experiments (I and II) was to allow assessment of any effect of VUR with time. Glomerular filtration rate was investigated only **late** in **Experiment** II since this was not a primary outcome measure in this experiment. Urinary concentrating ability was measured only **late** in the study to minimise the periods in which animals were deprived of water. Bladder function was estimated at intervals in pigs with an urethral

ring (groups B) in order to ensure the correct degree of bladder outflow obstruction and functional abnormality prevailed, and was equivalent in test and control groups. In those pigs without an urethral ring (groups A), voiding behaviour was observed throughout the study and normal bladder function was confirmed by cystometrography only at the end of the study period.

### 16.3.4 The Study Period

The protocol was designed so that pigs having entered the study period were followed for about 16 weeks when the final (**late**) investigations were implemented. The number of investigations, most performed in duplicate, required for each pig at the end of the study period meant there had to be a final period of 2 - 3 weeks over which the pigs were investigated. This was especially important for preventing one investigation procedure from influencing the results of another. In addition, the number of pigs included in the combined experiments meant that some flexibility in this period was an inevitable requirement. The objective was, therefore, to begin the final investigations as close to study week 16 as was practicable and to kill the pigs for kidney weight determinations 2 weeks after this, immediately on completion of the final physiological investigations. It was anticipated that by this time the pigs would be aged about 26 weeks and by normal growth be approaching a body weight of 30kg (**9.1**). This reflects a rapid growth achievement since birth, and body weight gain throughout the study period, from week 0 when the pigs weighed 7.5kg, is comparable to following a child between the ages of 6 months and 10 years.

CHAPTER 17

# PIG MODELS: METHODS AND DISCUSSION

## 17.1 Objectives

In order to accomplish the objectives of the investigation the pigs of each experimental group were required to fulfill rapid growth and satisfy the following criteria:

1. That VUR was present unilaterally, either into a solitary kidney or one of a pair, in those pigs in whom it was induced, and was absent into all other kidneys where it was not.

2. That urine was kept sterile for the whole period of study.

3. That the kidneys showed no evidence of macroscopic scars.

4. That bladder function was normal in group A pigs (without an urethral ring) and was abnormal in group B pigs (with an urethral ring). Furthermore, that the degree of bladder abnormality was similar between reflux and control pigs of group B.

## 17.2 Methods: Pig Models

### 17.2.1 Husbandry

A total of 54 Gottingen piglets entered the study. Forty-six (23 of each gender) were the normal healthy progeny of 8 in-pig sows brought into the animal house 2 weeks before term. After birth, piglets were weaned at 5 to 6 weeks onto sow/mini-pig diet, approximately 25g/kg body weight/day, in 3 feeds. After the weaning period pigs were fed twice daily with the majority of feed given at the end of the day. Water was normally provided *ad libitum* and bedding comprised of cedar wood chips and straw both previously autoclaved. Animals were housed, generally in their litter groups, in clean, spacious and well ventilated conditions with an ambient temperature of 66-70°C. Male pigs were castrated during one of the surgical procedures described below. An additional 8 male piglets were purchased after weaning at 4-5 weeks.

### 17.2.2 Anaesthesia

All pigs were anaesthetised for surgery and invasive procedures, this included intra-venous injections and blood sampling but not intra-muscular injections. In addition renal imaging, for determining renal uptake of $^{99m}$Tc-DMSA, was performed under anaesthesia.

At all times gaseous anaesthesia was administered through a paediatric Magill circuit fitted with a conical mask. Anaesthesia was induced with 4% Halothane in a mixture of $N_2O$ and $O_2$ in a ratio of 2:1, using a high total flow rate (6-12 l/min). For maintenance

of anaesthesia a lower flow rate (3-6 l/min) and Halothane concentration (1-1.5%) was used with the animals breathing spontaneously.

### 17.2.3 Surgical Preparation of Models

Vesicoureteric reflux was induced (groups A1 and B1) by resection of the intra-vesical ureter as detailed by Ransley and Risdon[7].

Male pigs in groups B had a ring, 5-7mm in diameter, placed around the urethra and below the immature prostate[7]. In order to avoid any influence on the kidney of acute post-operative bladder outflow obstruction the 'ring' was inserted 7 to 14 days before the induction of any VUR.

Unilateral nephrectomy was performed through a loin incision in all **Experiment I** pigs. Except in control pigs of group A2, where it formed a single operation, in general, it formed part of the surgical procedures for VUR and/or emplacement of the ring.

### 17.2.4 Urine Microbiology

Following surgery all animals received oral Macrodantin$^{R}$, 25-200mg/day ( $\approx$ 5mg/kg body weight) for the duration of the experiment. Urine samples for microscopy and microbiological culture were obtained by suprapubic puncture at approximately 2-weekly intervals throughout the study. After surgery and all manipulations involving suprapubic puncture a single intra-venous injection of gentamicin (2mg/kg) was given. Rectal temperatures

were taken daily throughout the study.

### 17.2.5 Ultrasonography

All pigs had regular renal and bladder ultrasound examination. These were performed daily the week following surgery and subsequently at intervals coincident with urine sampling.

### 17.2.6 Cystography

Cystography was performed only in those pigs in whom VUR was induced. The procedure was carried out using aseptic techniques and with the animals anaesthetised. Ultrasonography was used to aid localization of the bladder and to monitor the degree of bladder filling. With the pig supine, an intra-cath (16 guage) was inserted into the bladder by suprapubic puncture. This was facilitated by first increasing the bladder volume with a suprapubic injection of saline (21 guage needle). The bladder was then filled with radiographic contrast media. After positioning the pig on its side, with the back legs extended, a radiograph was taken using exposures of 80kVp, 150ma at 0.08s. Where incomplete bladder filling was observed from the initial radiograph further contrast media was instilled by way of the intra-cath and X-ray exposure repeated. Where the radiograph failed to demonstrate VUR in the presence of a full bladder a further radiograph was taken during micturition. This was encouraged by reducing the level of anaesthesia.

### 17.2.7 Urodynamics

After anaesthetising the pigs an epidural catheter (16 guage) was

introduced through a hollow needle into the bladder using ultrasound guidance and in an aseptic manner similar to that described above for cystography. A second epidural catheter was placed in the peritoneal cavity and a butterfly needle (21 guage) placed in an ear vein. All catheters were fixed firmly to the skin with elastic adhesive tape, following which the pigs were placed in a restraining cage with access to food, where they were allowed to recover from anaesthesia and adopt a standing position. Intra-vesical and intra-abdominal catheters were connected independently to suitable pressure transducers (**Appendix I**) and the 2 simultaneous pressures displayed on a chart recorder.

A diuresis was induced by the intra-venous infusion of 0.45% NaCL in 4.5% dextrose ( $\approx$ 0.5ml/min/kg body weight) and at least 2 filling and voiding cycles were observed. Residual urine volumes were assessed subjectively using ultrasound. Each cystometrogram was analysed by noting the following:

1. The presence or absence of abnormal detrusor activity during the bladder filling phase.

2. The maximum rise in detrusor pressure during voiding, described as intra-vesical pressure rise minus coincident intra-abdominal pressure.

3. The duration of void from the initiation of urine flow to complete detrusor relaxation.

4. The presence or absence of a pre-micturition rise in intra-vesical pressure and the phasic nature of the detrusor contraction.

The described urodynamic parameters together with observations of urine stream and residual volumes were ranked to provide an overall index describing either normal bladder function or the degree of abnormality (Figure 29).

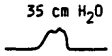
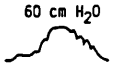
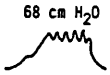
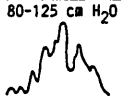
| Category | Urodynamics | | Flow/Residual |
|---|---|---|---|
| 1 | 20 cm $H_2O$  20s | Normal female voiding | |
| 2 | 35 cm $H_2O$  25s | Normal male voiding | Normal stream<br>No residual |
| 3 | 45 cm $H_2O$  50s | Prolonged void, raised end filling pressure | Slow stream<br>Small residual |
| 4 | 60 cm $H_2O$  60s | As 3, further increases in pressure and duration. Instability during filling | Slow/weak stream<br>Small residual |
| 5 | 27 cm $H_2O$  30s | Frequent low pressure voids of small volume | Weak stream<br>Small residual |
| 6 | 68 cm $H_2O$  50s | Prolonged void with phasic high pressure detrusor contractions | Barely continuous<br>weak stream<br>Small residual |
| 7 | 80-125 cm $H_2O$  >100s | As 6, with increases in duration of void, phasic detrusor contraction at high pressures | Intermittant, weak streams<br>Large residual volume |

**Fig.29 Urodynamic Parameters.** Categories 1 and 2 represent normal voiding patterns. Categories 3 to 7 indicate degrees of bladder dysfunction. The illustrated patterns and text represent typical features used in categorisation. The values recorded for pressure (cm $H_2O$) and duration of void (s) are common examples within each category. (Reprinted with permission; British Journal of Urology)

## 17.3 Discussion of the Experimental Models

The majority of the 54 pigs entered into the study satisfied the required criteria for the experimental models. Those pigs whose failure to do so was unquestionable were eliminated from further study or from the data analyses. There were some instances, however, where the decision to include or exclude the pigs was more difficult. This included some animals who were exceptions from their otherwise homogenous groups. All pigs in these categories are listed in Table 15 and are discussed.

Table 15 Pigs who failed to meet the criteria for the experimental models or who were exceptions from their otherwise homogenous groups.

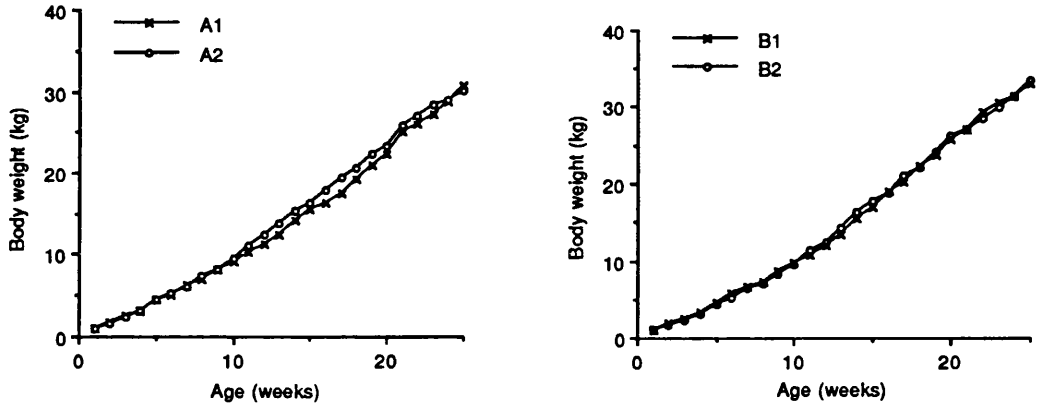
| Pig | Expt.,Group | Exception | |
|---|---|---|---|
| 284 | I, B1 | UTI early. Eliminated from study. Scars. | |
| 288 | II,B1 | " " " " " " | |
| 412* | I, B1 | Severe urodynamic abnormalities. Scars. | |
| 414 | II B1 | " " " " " | Excluded |
| 417 | II,B1 | UVJ obstruction. | |
| 402 | II,B1 | UTI, treated. " " " " | |
| 274 | II,AI | Right rather than left unilateral VUR | |
| 275 | II,A1 | " " " " " | |
| 410 | I, B2 | Urethral Ring enlarged. | |
| 416 | I, B2 | " " " | |
| 287 | I, A2 | UTI at final study week. | |
| 277 | II,A2 | Male pig in otherwise female group. | Included |
| 279 | I, A1 | Left rather than right solitary kidney. | |
| 278 | I, A2 | " " " " " " | |
| 297 | I, A1 | Some, rather than, no, upper tract dilatation. | |
| 423 | I, B1 | Upper tract dilatation more marked than others. | |

*Pig 412 excluded only from final data analysis (see 17.3.1.5)

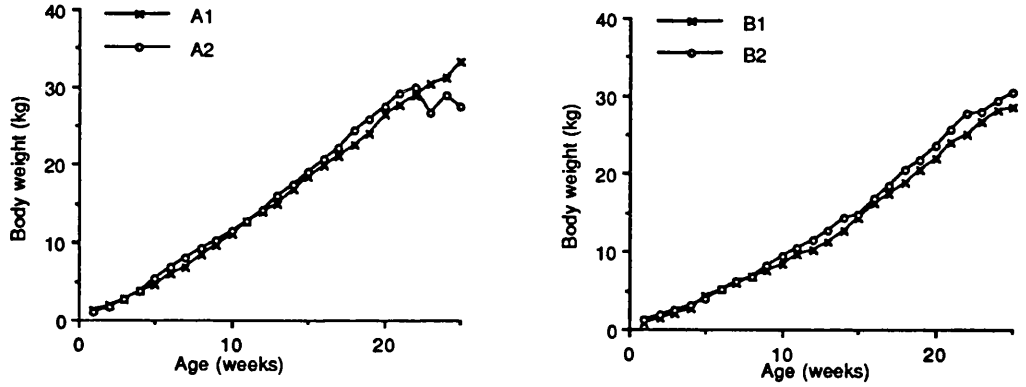
### 17.3.1 The Criteria

#### 17.3.1.1 Growth

By the end of the study period the pigs in both experiments were approximately 6 months of age and weighed about 30kg, therefore, the requirement for substantial and rapid growth was achieved. The weekly body weights of pigs entered into both experiments are shown plotted against age in Figure 30, as the mean for each group.




Experiment I




Experiment II

Fig.30 Mean Body Weight With Age for Pigs in Individual Groups.

### 17.3.1.2 Vesicoureteric Reflux

Cystography showed that VUR was present in all pigs in reflux groups but only on the operated side. As well as confirming only unilateral VUR, this corroborates previous observations that VUR does not occur naturally in Gottingen pigs. For this reason it was assumed that in control pigs VUR did not occur, and this was not confirmed cystographically. For the unequivocal exclusion of VUR a micturating cystogram is needed and because this investigation in the pig is difficult, and potentially hazardous, its practice was restricted. It was for this same reason that cystography was not repeated on the pigs in reflux groups at the end of the study period, especially as the difficulties are compounded in large pigs.

The assured and appropriate presence or absence of VUR was supported by the appearance of the ureterovesical juntion at *post mortem* examination (Figure 31).

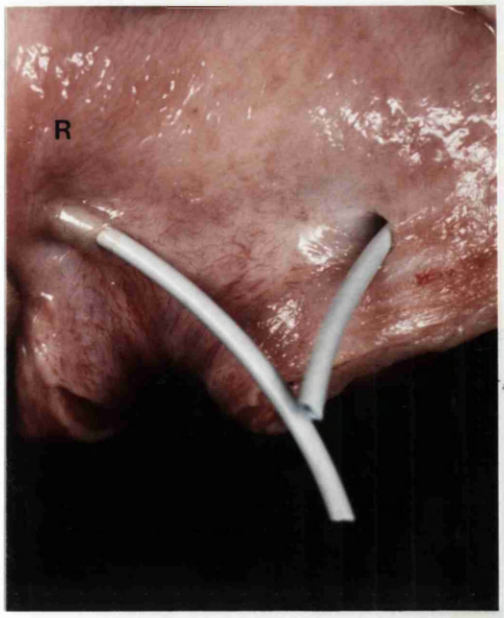



**Fig.31 *Post Mortem* Examination of the Ureterovesical Junctions.**
The intra-vesical ureter is long on the right (unoperated) side and absent on the left (operated) side.

In all pigs in reflux groups of both experiments there was complete absence of an intra-vesical tunnel on the operated side. By contrast, a long intra-vesical ureter was observed on the unoperated side in all pigs in test groups, and bilaterally in all control group pigs. In only one pig with VUR was there fibrosis at the ureteric orifice. The associated (refluxing) ureter was dilated and the kidney both appeared abnormal and was smaller than the contralateral kidney. This latter was also noticed on the initial $^{99m}$Tc-DMSA image. These appearances suggested that some ureterovesical obstruction had occurred and data from this pig were excluded from analysis.

**17.3.1.3 Urinary Infection**

Only 4 of the 54 pigs entered into the study had positive urinary cultures (E.coli and/or Sp.Klebsiella) at any time. This developed early in the study in 2 pigs which were excluded from further investigation. A third pig developed a brief episode of urinary infection later in the study period; was continued in the investigation, but excluded from analysis. The left (refluxing) kidney weight value (left/[left + right]) from this pig was 3.7 standard deviations below the expected mean and thus appeared to be an outlier. The data from a fourth pig whose urinary infection occurred in the final study week were retained in the analysis. At the time of infection all investigations had been completed with the exception of a second (duplicate) $^{99m}$Tc-DMSA uptake study. All previous samples from this pig and those from all other pigs retained

in the investigation analysis indicated that the required condition of urine sterility had been met.

#### 17.3.1.4 Bladder Function

The observations from each urodynamics study performed are tabulated in appendices IV and V. Included is a category, ranked 1 - 7, designated to each urodynamics investigation, which provided an index of either normal bladder function or the degree of dysfunction (see 17.2.7).

In both experiments, pigs without an urethral ring (groups A) had normal bladder function confirmed by cystometrography at the end of the study periods. Detrusor activity was only ever associated with micturition when there was a simple and brief bladder contraction at a peak detrusor pressure of up to 30cm $H_2O$ (females category 1, males category 2). *Post mortem* examination showed that all these pigs had macroscopically normal bladders and urethras.

All pigs with an urethral ring (groups B) had abnormal bladder function. By the end of the study periods all voided at higher detrusor pressures than the group A pigs. Voiding was slow, incomplete and sometimes associated with a pre-micturition rise in pressure (category 3). In some animals there was detrusor instability, and with voiding there were often pronounced pre- and post-void contractions (category 4). At *post mortem* examination urethral stenosis, induced by the implanted ring, was confirmed in

all cases. In every pig the bladder was thick walled indicating detrusor hypertrophy; this was most marked in 2 of the 4 pigs with the most severe degrees of bladder dysfunction (see below) and was least marked in 3 of the Experiment II pigs (421,429,424).

The objective in pigs assigned to groups B was to produce a stable degree of bladder outflow obstruction with prolonged voiding, raised detrusor pressures and abnormal storage dynamics. The majority of pigs satisfied this objective for the whole period of study but there was an unavoidable tendency for the 'ring' to progressively restrict the urethra as the pigs grew during the study period. This meant, inevitably, that some pigs early in the study failed to reach the required degree of urodynamic abnormality, whilst this was exceeded by others at the end of the study period. These latter, severe and unacceptable degrees of urodynamic abnormality (category 6 and 7) occurred in 4 pigs (412,414,410,416). Two of these 4 pigs were retained in the data analyses since more moderate functional abnormalities were restored after enlarging the urethral ring. In 2 others, gross urodynamic abnormalities persisted and the data from these pigs were excluded, in whole or in part, from the data analyses.

Despite some variability in the degree of bladder abnormality in individual group B pigs retained in the study, the distribution of the various urodynamic categories between the reflux and control groups of separate experiments was similar (Figure 32).

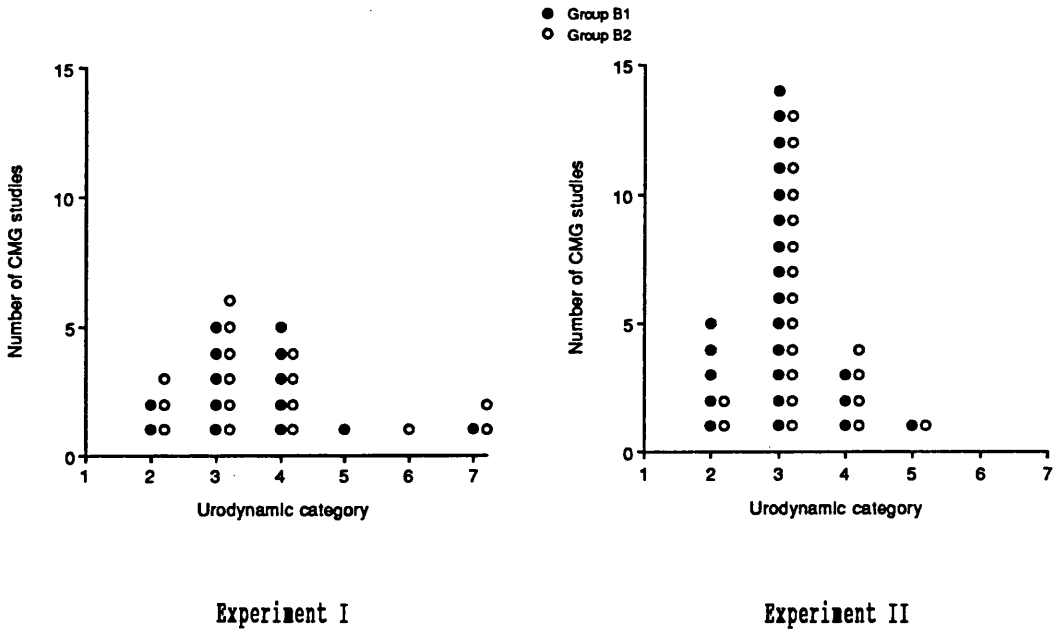



Fig.32 Urodynamic Categories for Each of the Urodynamic Studies in Pigs With Bladder Outflow Obstruction (see text and 17.2.7).

### 17.3.1.5 Renal Scarring

Four pigs had macroscopic renal scarring at *post mortem*. In 2 of these, scarring was associated with urinary infection, which excluded the pigs from further study (**17.3.1.3**). In the other 2 pigs scarring was associated with grossly abnormal bladder function (**17.3.1.4**). One of these (Pig 414; Experiment II) was excluded from all (**early** and **late**) data analysis. The other (Pig 412; Experiment I) was excluded from the **late** data analysis but was retained in the appraisal of **early** data. This was accepted because at the **early** time urodynamic abnormalities were less severe (category 5) and $^{99m}$Tc-DMSA imaging determined that renal scarring was absent.

Abnormal kidneys in the absence of macroscopic scarring were observed only in 2 pigs excluded either because of urinary infection (17.3.1.3) or because of obstruction (17.3.1.2). The kidneys from all other pigs were macroscopically normal, although in some there was residual fetal lobulation, confirmed by microscopic examination. Of the pigs included in the data analyses at both the **early** and **late** times the requirement for no evidence of macroscopic scars was upheld.

### 17.3.2 Other Components of the Models

As well as having the required attributes, the pig models had other components which could influence the interpretation of results. Since it was important to restrict variability in order to optimise sensitivity in detecting any effects of VUR these components needed to be restricted where possible and where not their influence acknowledged.

#### 17.3.2.1 The Side of the Solitary Kidney or of Reflux

Two of the pigs with paired kidneys and unilateral VUR (**Experiment II**) were excluded from analysis because they had right sided VUR whilst in all others this was left sided. Retaining only pigs with left VUR allowed any natural bias (in growth or function) towards either the right or left kidney to be identified and accounted for during comparison with control animals.

In **Experiment I**, there were 2 pigs who had a right solitary kidney

whilst in all others this was left sided. These 2 pigs were retained in the data analysis since in this experiment individual kidneys are compared simply to those of other pigs, therefore, variation between individual pigs is likely to exceed any small natural difference in performance between left and right kidneys.

#### 17.3.2.2 Upper Tract Appearances

Ultrasound examination showed that upper tract dilatation was not a feature of the experimental models, even during the post-operative periods. In later stages of the study splitting of the calyceal echoes was seen on occasions. On the whole, this correlated with the *post mortem* observations of marginally dilated ureters.

*Post mortem* examination showed that of the pigs with normal bladder function only one had mild ureteric dilatation. In all others the ureters were of normal calibre, irrespective of the presence of VUR. In pigs with abnormal bladder function, mild ureteric dilatation was observed in association with VUR. Apart from pigs excluded with severe urodynamic abnormalities this was most marked in one other.

Despite the exceptions, (one group A pig, one group B pig), the within group variation in ureteric dilatation was minimal and no pigs were excluded because of this feature alone. All kidneys retained in the study appeared normal on sequential ultrasonography throughout the study period and at *post mortem*, irrespective of the presence or absence of VUR.

### 17.3.2.3 Sex

Both experiments required pigs with normal (groups A) and abnormal (groups B) bladder function as a result of bladder outflow obstruction. Both experiments used male and female pigs, but because females are unsuitable for the model of bladder outflow obstruction (9.2.3) all pigs with **abnormal** bladder function were male. In **Experiment I** all pigs with **normal** bladder function were female, but in **Experiment II**, however, one male was included.

The influence of sex as an independent variable was not a consideration of these experiments. The use, and on the whole confinement, of separate sexes in either group A or group B provides 2 diverse conditions of urodynamic variation: *i.e.* very low pressures in females (groups A); elevated and abnormal pressures in males with bladder outflow obstruction (groups B). In this situation, therefore, bladder function is intrinsically related to, and therefore confounded with, sex. However, it was considered that the effect of sex itself on any outcome of VUR was likely to be small compared with that of bladder function. Thus, any effects on outcome of the confounded variables (sex + bladder function) are most likely to be attributed to bladder function alone. The possibility that sex may influence the values of renal growth and function between groups A (with normal bladder function) and B (with abnormal bladder function), can be accounted for statistically.

## 17.4 Model Conclusions

The experiments using the pig models described allow evaluation of the effects of sterile VUR persisting in pigs during a period of prodigious growth, in the absence of macroscopic renal scarring. The model for VUR is one where gross ureteric and pelvi-calyceal dilatation are absent. The use of pig models with normal and abnormal bladder function allow the effects of VUR to be tested at 2 levels of hydrodynamic insult. That severe urodynamic abnormalities, associated with renal scarring, occurred in some pigs in whom bladder outflow obstruction was induced, indicates that those models with bladder outflow obstruction exemplify the most extreme conditions under which the functional consequences of VUR alone can be assessed experimentally in the pig during a time of rapid growth.

CHAPTER 18

# MEASUREMENT OF THE EFFECTS OF VUR ON RENAL GROWTH AND FUNCTION

## 18.1 Final Experimental Design

After excluding those pigs discussed in the previous chapter, 47 remained; 23 in **Experiment I** and 24 in **Experiment II**. Their distribution throughout the experimental groups is shown in Figure 33.

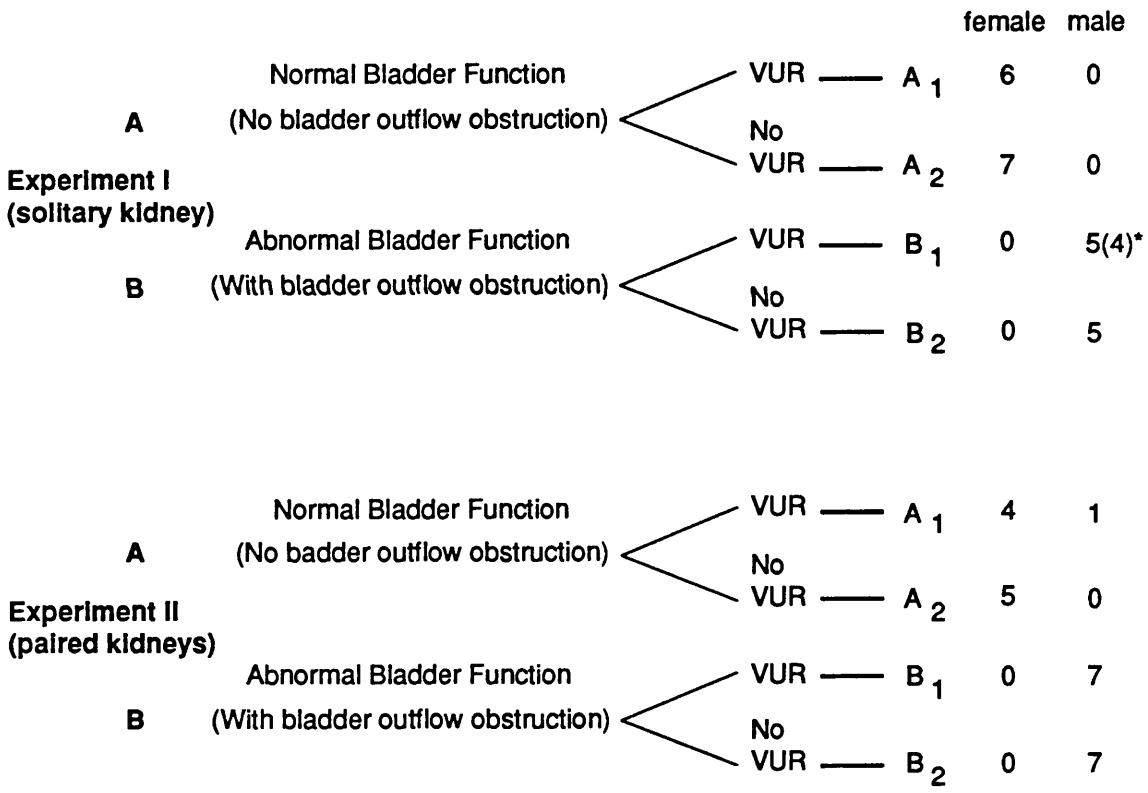



**Fig.33 Final Investigation Design**
*One pig omnited from Late analysis (17.3)

## 18.2 Methods: Measurement of Renal Growth and Function

The methods of measurement have been detailed and discussed in previous chapters. In this chapter the application of these methods to the investigation of VUR are described.

### 18.2.1 Glomerular Filtration Rate

Glomerular filtration rate (GFR) was measured at the intervals stated (16.2) using the $^{51}$Cr-EDTA C-slope method (**Chapters 10,11**). Accurately timed blood samples (5ml) were collected 100min and 200min after an initial injection of $^{51}$Cr-EDTA (0.2Mbq; ≈ 5$\mu$Ci; in 0.5ml 0.9% NaCl/kg body weight) given intra-venously through an ear vein. Venous blood samples were collected either from the ear opposite to the one used for injection or from superficial veins of the legs. For each separate injection and sampling procedure the animals were given transient light anaesthesia. The time from induction of anaesthesia to complete arousal was noted. Where this exceeded 10min on any one occasion the whole procedure was abandoned and repeated on another day.

The GFR determinations were always performed between the same hours of the day (12.00h to 16.00h) and at a time when the pigs had not been subject to other physiological investigations for at least 3 days. In general, 2 independent GFR determinations were performed at each time point, usually on 2 consecutive days. In the solitary kidney pigs (**Experiment I**) at the **late** (final) time a third GFR estimation was performed if either the second estimate varied from

the first by more than 15%, or when a delay of more than 5 days occurred between the first or duplicate GFR estimates and killing the animal. In all cases the final measurements were made within a 10 day period prior to termination of the experiment.

**In Experiment I** and **Experiment II** each individual estimate of GFR was expressed as the absolute (ml/min) and as the body weight ratio (ml/min/kg). Additionally, in **Experiment I**, GFR ml/min/g kidney weight was derived from the final GFR estimates. The values recorded for each pig at each time point were the mean of the duplicate or triplicate estimates but excluding the occasional measurement where there was low activity (<5 times background) in the second blood sample.

### 18.2.2 Urinary Concentrating Ability

Urinary concentrating ability was measured at the end of the study period (**late**), using the water deprivation test (**14.4**). In general this was performed on 2 occasions separated by one week. In group A pigs in **Experiment II** a second test was not performed if the maximal concentration achieved on the first occasion was equal to or more than 1000 mosmol/kg water.

Drinking water was withdrawn in the evening (from 17.00h) and on the following morning urine samples were collected by suprapubic aspiration after 16h and 18h of dehydration, emptying the bladder on each sampling occasion. These procedures were assisted by

ultrasound imaging. Macrodantin$^{R}$ was not given for 30 hr prior to collection of these urine samples. The osmolality of each urine sample was measured immediately and in duplicate by freezing point determination. If the osmolality of the second (18h) urine sample was greater than the first (16h) by more than 10% a further sample was collected between 19h and 20h of dehydration.

The urinary concentrating ability for each pig was assessed as the mean of the highest osmolality (maximal urinary concentration) achieved on each test occasion.

### 18.2.3 Renal Uptake of DMSA

Renal uptake of $^{99m}$Tc-DMSA was estimated at **early** and **late** time points by applying the methods and precautions detailed (**Chapter 13**). The dose preparation and procedures for injection and imaging were the same as described (**13.4**). Renal counts were acquired (**13.4.1**) at accurately timed intervals approximately 6h and 24h after injection and true renal counts calculated as before (**13.4.1.1**).

Absolute uptake by individual kidneys (**Experiment** I and **II**) and left relative uptake (**Experiment II**) was calculated as described (**13.4.1**) but the method used to determine the dose activity was different to that adopted previously. This was because in the present experiments larger doses (prepared per kg body weight) were required which meant it was necessary to take account of count rate efficiency (**13.1**). In order to avoid either attenuating the dose counts at

source or making a correction for loss of count rate efficiency the dose activity was calculated by relating the weight of the dose to the activity of a known weight of standard. This latter comprised of about 0.3ml $^{99m}$Tc-DMSA drawn from the same preparation as the dose. An account was made for the residue of dose remaining in the syringe and butterfly extension after injection. Both dose weight and standard weight were determined from the difference in weights between the full and empty (pre-weighed) syringe (1ml). The activity of the standard and residue were recorded on full field of view for 100s using the same marked area of the gamma camera face as that used for recording renal activity.

The activity of the effective dose injected was calculated:

$$D = D_1 - D_2$$

where:

$$D_1 = \frac{\text{counts of standard} \times \exp(-0.1149 \times t_1) \times \text{weight of dose}}{\text{weight of standard (g)}}$$

$$D_2 = \text{residue count} / \exp(-0.1149 \times t_2)$$

and

$t_1$ = time (hours decimilised) between recording standard counts and injection of dose.
$t_2$ = time (hours decimilised) between injection of dose and recording residue counts.

For each investigation the recorded $^{99m}$Tc-DMSA renal uptake was the mean of the 6h and 24h estimations. At the end of the study period (late) the recorded result for each pig was the mean of 2 duplicate investigations separated by one week. In general, the pigs were killed following acquisition of the 24h renal activity in the final uptake study. The activity of the isolated kidneys taken from 18 pigs in **Experiment I** and 23 pigs in **Experiment II** was recorded and

the uptake calculated from these *ex situ* kidneys was compared with that obtained from the 24h acquisition *in situ*.

### 18.2.4 Renal Growth

Renal growth was assessed by the weight (g) of the kidneys at the end of the study (**Chapter 15**). Kidneys were weighed after removal of the capsule, ureter and any adhering fat. Surface moisture was removed with absorbant paper.

In **Experiment I**, kidney weight per kg body weight was derived and in **Experiment II**, left relative weight was calculated as the percentage ratio of left kidney weight to total renal weight ($100 \times L/[L+R]$).

## 18.3 Statistics

The data from **Experiments** I and II were analysed separately using the same methods of interactive modelling. For each parameter of renal growth or function, outcome measures from the 4 groups ( A1, A2, B1, B2,) were examined together, simultaneously testing the effects of VUR in the 2 conditions of bladder function. Regression analysis was applied to absolute values (kidney weight, GFR and maximal urinary concentration) and to log transformed ratio and percentage values (GFR/kg body weight, GFR/g kidney weight, absolute DMSA uptake, left relative DMSA uptake, kidney weight/kg body weight, left relative kidney weight).

The normal distribution of each outcome measure was assessed from the distribution of residuals for the regression model fitted. Each residual value (X) was standardised (X-mean of X / SD) and plotted against its expected value (Figure 34).

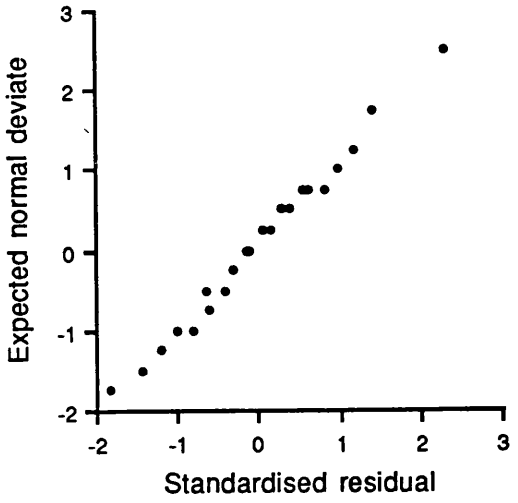



**Fig.34 Normal Probability Plot.** Sorted, standardised residuals (X-axis) plotted against their expected normal deviates (Y-axis).

The regression models are demonstrated in Figures 35a,b. Both figures assume an effect of VUR (X-axis; A2-A1,B2-B1) on a specific outcome measure (Y-axis). In addition, both assume a difference in the outcome measure 'Y' between the 2 conditions of bladder function but in the absence of VUR; *i.e.* A2-B2 $\neq$ 0. Figure 35a assumes the magnitude of VUR effects is the same in both conditions of bladder function, *i.e.* A2 - A1 = B2 - B1, and denotes no interaction between VUR and bladder function. Figure 35b assumes the magnitude of VUR effects is different for each condition of bladder function, *i.e.* A2 - A1 $\neq$ B2 - B1, and denotes interaction.

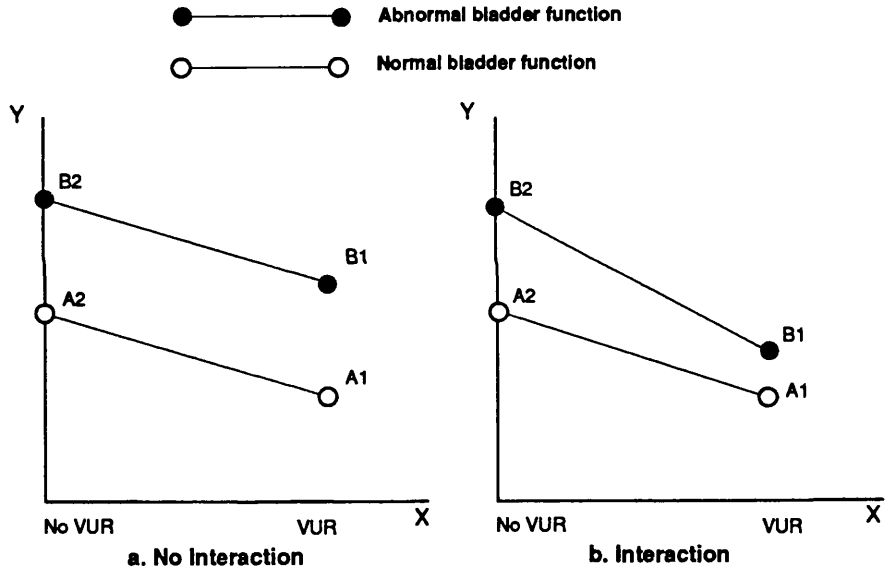



**Fig.35 The Regression Models.** Graphic representation of the mean values for a specific outcome measure (Y) in the absence (A2,B2) and presence (A1,B1) of VUR at 2 levels of bladder function (A - normal, B - abnormal).

For each outcome measure the interaction between VUR and bladder function was tested. If there was no significant interaction

($p>0.1$), the non-interactive model (Fig. 35a) was fitted allowing estimation of the effects of abnormal bladder function alone (or group B effects) and of VUR, in each case taking the other variable into account. For each index of renal growth or function the 95% confidence limit (mean +/- SE × ≈ $2;t_{0.025,df}$) was calculated for the difference in that index effected by VUR.

For the outcome measures obtained at the end of the study (**late**) the interactive effect of VUR and bladder function was assessed by comparing the VUR effects in group A with those in group B, *i.e.* (A2 - A1) - (B2 - B1). The 95% confidence limits (mean +/- SE × ≈ $2;t_{0.025,df}$) for these differences between the A and B groups was calculated. Although the 95% confidence limits may be large, since the standard errors will reflect variability throughout the 4 groups including that attributed to VUR, they provide an estimate for the influence of bladder function on any effects of VUR. Since group B values are subtracted from group A values a negative bias in these estimates will denote a greater severity of VUR effects in the presence of abnormal bladder function.

The same regression methods were applied separately to age and body weight at the **early** and **late** time points to determine the difference in these variables between the test pigs with VUR and the control pigs without.

Regression analysis was further applied to renal $^{99m}$Tc-DMSA uptake results from **Experiment** I to test for any interaction between VUR

effects and either the pigs age or body weight at the time of unilateral nephrectomy.

In both experiments the method of Bland and Altman[412] was used to assess the agreement between DMSA uptake recorded with the kidney in place (*in situ*) and that recorded from the same kidney after its isolation (*ex situ*).

CHAPTER 19

# RESULTS: EXPERIMENT I

## 19.1 Experimental Design

All pigs were followed from the time they entered the study at week 0 for a minimum of 16 weeks. The maximum period was 20 weeks. The mean number of study weeks for pigs in each group are shown in Table 16. Also shown for each group are the mean values for age and body weight at the times of surgery, study week 0, and the **early** and **late** investigations. The data for individual pigs are shown in **Appendix IV.**

At the times of both the **early** and **late** investigations each group of pigs were of similar age and had body weights sufficiently similar for the purpose of comparing parameters of renal growth and function. At the end of the study period there was no significant interaction between bladder function and VUR on either age ($t_{17}$=0.54) or body weight ($t_{17}$=0.43). The estimated differences in both age and weight of pigs with VUR in comparison with control pigs without VUR are shown in Table 17. The differences were not significant (age:$t_{18}$=1.18; weight $t_{18}$=1.53).

Table 16 Mean values for study period, ages and body weights for each group.

| | Normal Bladder Function | | Abnormal Bladder Function | |
|---|---|---|---|---|
| Group | A1 Reflux | A2 Control | B1 Reflux | B2 Control |
| Number of Pigs | 6 | 7 | 5(4)[1] | 5 |
| Mean Weeks of Study | | | | |
| Study week 0 to death | 18.4 | 19.4 | 17.6 | 17.6 |
| Mean Age (weeks) at: | | | | |
| Surgery (nephrectomy) | 5.0 | 4.4 | 3.8 | 4.2 |
| (reflux) | 4.7 | | 3.8 | |
| Study week 0 | 8.8 | 6.7 | 8.2 | 7.8 |
| Investigations | | | | |
| Early (study week 4) | 13.0 | 11.8 | 12.4 | 12.4 |
| Late (start) | 25.0 | 24.3 | 23.4[1] | 24.0 |
| (end) | 27.2 | 26.1 | 25.8[1] | 25.4 |
| Mean Body Weight (kg) at: | | | | |
| Surgery (nephrectomy) | 4.4 | 4.4 | 3.7 | 3.6 |
| (reflux) | 4.7 | | 3.7 | |
| Study weeek 0 | 7.4 | 7.5 | 7.3 | 7.1 |
| Investigations | | | | |
| Early (study week 4) | 12.7 | 12.2 | 12.3 | 12.7 |
| Late (start) | 29.8 | 29.4 | 31.2[1] | 31.0 |
| (end) | 34.3 | 32.2 | 35.0[1] | 33.8 |

[1]One pig excluded from late data analysis (see 17.3)

Table 17 Differences in age and body weight attributed to VUR.

| | 95% Confidence Limits |
|---|---|
| Age at end | -0.55 to +2.05 weeks |
| Body weight at end | -0.60 to +4.19 kg |

## 19.2 Renal Growth

The kidney weight and kidney weight per kg body weight values for individual pigs are included in Appendix IV and the mean values for each group shown below (Table 18).

Table 18 Solitary kidney weight mean values for each group.

| | Normal Bladder function | | Abnormal Bladder Function | |
|---|---|---|---|---|
| Group | A1 Reflux | A2 Control | B1 Reflux | B2 Control |
| Number of Pigs | 6 | 7 | 4 | 5 |
| Mean kidney weight (g) | 104.5 | 110.0 | 127.0 | 135.8 |
| Mean kidney weight (g/kg b.wt.) | 3.06 | 3.40 | 3.62 | 3.94 |

**Kidney weight.** In group A pigs with normal bladder function the mean kidney weight value was similar for reflux group A1 and for non-reflux group A2. In group B animals with abnormal bladder function the mean value was slightly lower for reflux group B1 than for non-reflux group B2. There was no significant interaction between VUR and bladder function ($t_{17}$=0.21). There was a significant ($P<0.01$) difference between group A (female) and group B (male) pigs ($t_{18}$=3.16). After accounting for this difference there was no significant effect of VUR on kidney weight ($t_{18}$=0.90).

**Kidney weight per kg body weight.** In both groups A and B mean kidney weight per kg body weight values were slightly lower for reflux groups A1 and B1 compared with those for corresponding

non-reflux groups A2,B2. There was no significant interaction between VUR and bladder function ($t_{17}$=0.25); no significant difference between the group A and group B values ($t_{18}$=1.12) and no significant effect of VUR on kidney weight per kg of body weight ($t_{18}$=0.98).

The estimated change in both kidney weight and kidney weight per kg body weight attributed to VUR is shown in Table 19.

Table 19 Change in kidney weight indices attributed to VUR.

| | 95% Confidence Limits |
|---|---|
| Kidney weight | -22.4 to +8.66 g |
| Kidney weight/b.wt | -0.674 to +0.067 g/kg |

## 19.3 Renal Function

### 19.3.1 Glomerular Filtration Rate

The results of individual and mean GFR determinations for each pig at the **early**, **interim**, and **late** time points are tabulated in **Appendix IV**. These include for each pig the mean values for GFR per kg body weight at each time point, and GFR per g kidney weight (**late**).

The mean GFR values for individual animals at each time point are shown in Figure 36 for pigs with normal bladder function (group A) and in Figure 37 for those with abnormal bladder function (group B).

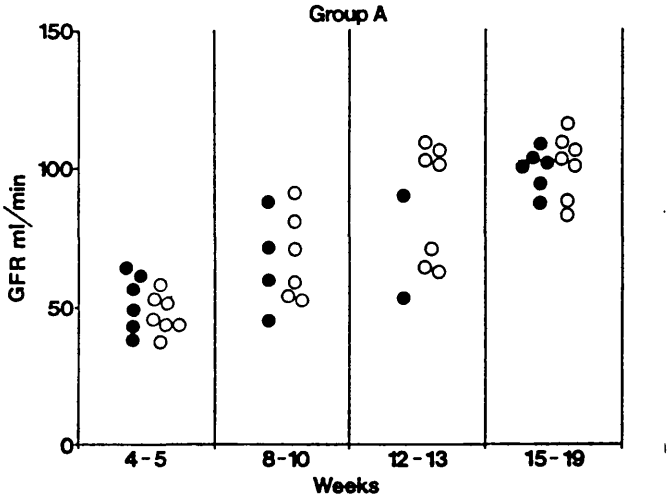



Fig.36 Glomerular Filtration Rates at the Various Time Points for Pigs in Groups A; With (●) and Without (○) VUR. (Reproduced with permission; British Journal of Urology)

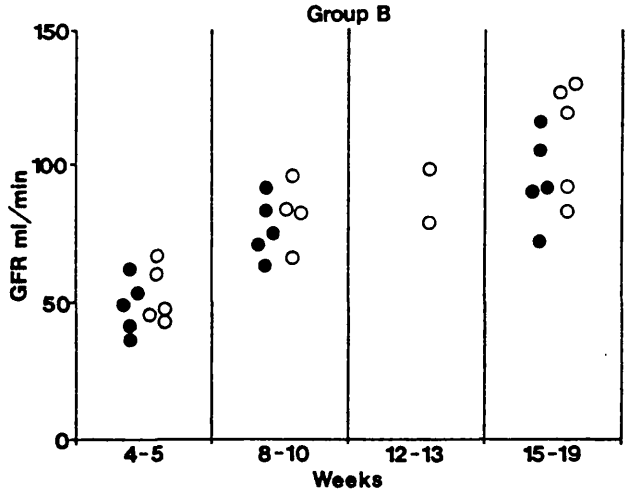



Fig.37 Glomerular Filtration Rates at the Various Time Points for Pigs in Groups B; With (●) and Without (○) VUR. (*pig 412 - see 17.3). (Reproduced with permission; British Journal of Urology)

The mean GFR values for individual pigs are shown plotted sequentially against pig weight with separate plots for groups A1,A2 (Figure 38) and B1,B2 (Figure 39).

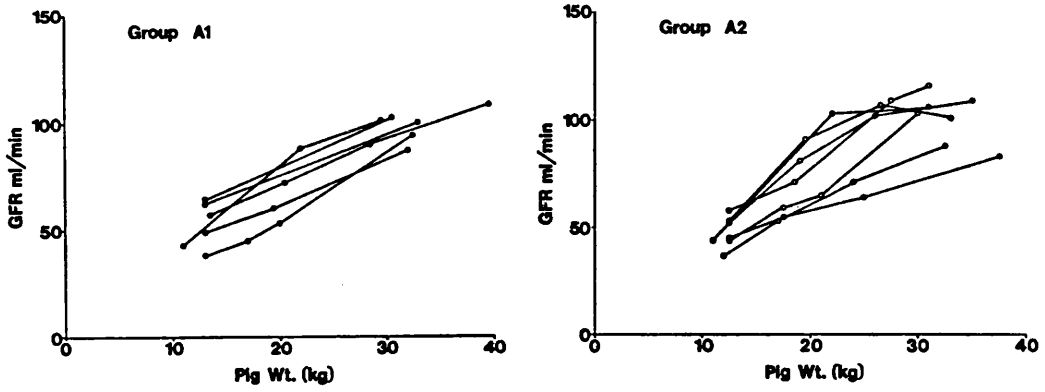



Fig.38 Glomerular Filtration Rate Against Body Weight for Pigs With (A1) and Without (A2) VUR. Lines represent individual animals. (Reproduced with permission: British Journal of Urology).

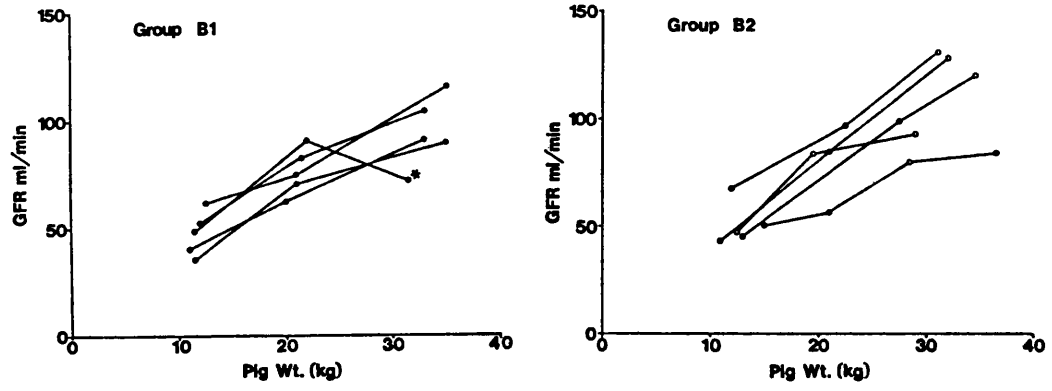



Fig.39 Glomerular Filtration Rate Against Body Weight for Pigs With (B1) and Without (B2) VUR. Lines represent individual animals. (*412 - see 17.3: Reproduced with permission; British Journal of Urology).

The mean values for GFR ml/min and GFR ml/min/kg body weight for each group are shown below for the **early** (study weeks 4-5) and **late** (final) determinations. The table also includes GFR ml/min/g kidney weight calculated from the **late** data.

Table 20 Mean values for indices of GFR.

| | Normal Bladder Function | | Abnormal Bladder Function | |
|---|---|---|---|---|
| Group | A1 Reflux | A2 Control | B1 Reflux | B2 Control |
| Number of Pigs | 6 | 7 | 5 (4)[1] | 5 |
| Early (weeks 4-5) | | | | |
| ml/min | 52.0 | 47.6 | 48.1[1] | 50.5 |
| ml/min/kg body weight | 4.04 | 3.85 | 4.03[1] | 3.95 |
| Late (final) | | | | |
| ml/min | 98.7 | 100.9 | 100.5 | 110.2 |
| ml/min/kg body weight | 2.99 | 3.15 | 2.98 | 3.28 |
| ml/min/g kidney weight | 0.995 | 0.915 | 0.789 | 0.818 |

[1]. One pig (412) excluded from analysis of late data (see section 17.3)

**GFR ml/min.** At both the **early** and **late** times mean GFR ml/min values for reflux groups A1,B1 were similar to those for corresponding control groups A2,B2. There was no significant interaction between bladder function and VUR on these values either at the **early** ($t_{18}$=0.88) or at the **late** ($t_{17}$=0.75) time and no significant effect of VUR either **early** ($t_{19}$=0.372) or **late** ($t_{18}$=0.897).

**GFR ml/min/kg body weight.** At the **early** time mean GFR ml/min/kg body weight values for reflux groups A1,B1 were similar to or

slightly higher than those in control groups A2,B2. At the **late** time mean values for reflux groups were slightly lower than those for control groups. There was no significant interaction between bladder function and VUR either **early** ($t_{18}$=0.17) or **late** ($t_{17}$=0.37) and no significant effect of VUR either **early** ($t_{19}$=0.50) or **late** ($t_{18}$=1.17).

**GFR ml/min/g kidney weight**. In male pigs the mean value for GFR ml/min/g kidney weight was slightly lower for reflux group B1 than for control group B2. In female pigs the mean value was slightly higher for the reflux group than for the control group. Interaction between bladder function and VUR was again absent ($t_{17}$=0.57) but there was a significant difference ($t_{18}$=2.43; P<0.05) between groups A (females) and groups B (males). After accounting for this there was no significant effect of VUR ($t_{18}$=0.080).

The estimated changes in the various indices of GFR attributed to VUR are shown in Table 21.

Table 21 Change in GFR attributed to VUR.

| | | 95% Confidence Limits |
|---|---|---|
| GFR | Early | -6.62 to +9.52 ml/min |
| | Late | -17.3 to +6.80 ml/min |
| GFR/b.wt. | Early | -0.43 to +0.80 ml/min/kg |
| | Late | -0.56 to +0.16 ml/min/kg |
| GFR/kidney weight | | -0.10 to +0.12 ml/min/g |

### 19.3.2 Urinary Concentrating Ability

The individual and mean maximal urinary osmolalities for each pig at the end of the study periods are included in **Appendix 1V**. The mean values for each group are shown in Table 22.

**Table 22** Mean values for maximal urinary osmolality.

| Group | Normal Bladder Function A1 Reflux | Normal Bladder Function A2 Control | Abnormal Bladder Function B1 Reflux | Abnormal Bladder Function B2 Control |
|---|---|---|---|---|
| Number of Pigs | 6 | 7 | 4 | 5 |
| Late (Final) mosmol/kg water | 891.1 | 1072.0 | 929.7 | 1092.2 |

Maximal urinary osmolality mean values were lower for reflux groups A1,B1 than for non-reflux control groups A2,B2. There was no significant interaction between bladder function and VUR ($t_{17}$=2.20). The effect of VUR was significant ($t_{19}$=3.79;P<0.01) and is estimated below in Table 23.

**Table 23** Change in maximal urinary concentration attributed to VUR.

| | 95% Confidence Limits |
|---|---|
| Urinary Concentration | -266 to -80.1 mosmol/kg water |

### 19.3.3 Renal Uptake of $^{99m}$Tc-DMSA

The mean absolute uptake values for each group are shown in Table 24. These include estimates made from the kidneys in place (*in situ*) at the **early** and **late** times as well as those calculated from the isolated (*ex situ*) kidneys from 18 animals. The results for each individual pig are shown in **Appendix IV.**

Table 24 Mean values for $^{99m}$Tc-DMSA absolute renal uptake.

| | | Normal Bladder Function | | Abnormal Bladder Function | |
|---|---|---|---|---|---|
| | Group | A1 Reflux | A2 Control | B1 Reflux | B2 Control |
| *In situ* Kidneys | | | | | |
| Number of Pigs | | 6 | 7 | 5(4)[1] | 5 |
| Early (weeks 4-5) | % uptake | 40.9 | 44.7 | 38.6 | 42.3 |
| Late (final) | % uptake | 38.4 | 43.5 | 38.0 | 40.0 |
| *Ex situ* Kidneys | | | | | |
| Number of Pigs | | 5 | 5 | 4(3)[1] | 4 |
| % uptake | | 38.1 | 43.0 | 39.4 | 41.4 |

[1]One pig (412) excluded from late data analysis (see 17.3)

**Comparison of *In situ* and *Ex situ* Estimates.** Absolute uptake calculated from the final 24h imaging procedure with the kidneys in place (*in situ*) was compared with that obtained from the same kidneys after their isolation (*ex situ*). The mean of the differences between 18 paired estimates was -0.3%. The 95% confidence limit (mean +/- SE 2.11) was -0.311 to +0.710 % uptake showing good agreement between the 2 estimates.

***In situ* Absolute Uptake**. The mean values at both the **early** and **late** times were lower for reflux groups A1,B1 than for control groups A2,B2 without reflux. These differences were not effected by an interaction between VUR effects and either the pigs age (**early** $t_{18}$=0.61; **late** $t_{17}$=1.10) or weight (**early** $t_{18}$=0.44; **late** $t_{17}$=0.23) at the time of unilateral nephrectomy.

There was no significant interaction between bladder function and VUR at either the **early** ($t_{18}$=0) or **late** ($t_{17}$=0.90) times. The effect of VUR was just significant (0.05>P>0.02) both **early** ($t_{19}$=2.20) and **late** ($t_{18}$=2.41).

***Ex situ* Estimates Absolute Uptake**. Mean values for absolute uptake calculated from the *ex situ* kidneys of 18 animals were slightly lower for reflux groups A1,B1 than for non-reflux control groups A2,B2. After establishing that there was no significant interaction between bladder function and VUR, and after accounting for bladder (group B) effects alone, any effects attributed to VUR just failed to reach statistical significance ($t_{14}$=2.05). (The difference between the reflux and control groups was just significant ($t_{15}$=2.15;P=0.05) when the 2 groups (A and B) were compared simply.)

The changes attributed to VUR in the absolute renal uptake of $^{99m}$Tc-DMSA measured both with the kidneys *in situ* at the **early** and **late** times and with the kidneys *ex situ* are shown in Table 25.

Table 25 Change in $^{99m}$Tc-DMSA renal uptake attributed to VUR.

| | 95% Confidence Limits |
|---|---|
| *In situ* | |
| Uptake Early | -6.98 to -0.31 % of dose |
| Late | -6.91 to -0.56 % of dose |
| *Ex situ* | |
| Uptake | -8.07 to +0.05 % dose |

## 19.4 Interactive Effects Between VUR and Bladder Function

The influence of abnormal bladder function in changing the outcome of VUR on measures of renal growth and function at the late time, is shown in Table 26 as the mean of the difference between VUR effects in group A and group B; *i.e* [A2-A1] - [B2-B1].

Table 26 Effects of abnormal bladder function on the outcome of VUR. The mean and estimates for the difference in the outcome of VUR attributed to abnormal bladder function in comparison with normal bladder function i.e. [A2-A1] - [B2-B1].

| Outcome Measure | Mean | 95% Confidence Limit | |
|---|---|---|---|
| | | Measured Units | % Difference |
| Kidney wt./body wt. | +0.10g/kg | -0.67 to +1.03g/kg | 80 to 133% |
| GFR/body wt. | -0.15 ml/min | -0.82 to +0.74 ml/min/kg | 74 to 123% |
| GFR/kidney wt. | -0.06 ml/min/g | -0.26 to +0.18ml/min/g | 73 to 120% |
| Max. Osmolality | +18.6 mosmol/kg water | -177 to +214 mosmol/kg water | 83 to 120% |
| DMSA uptake | +3.21% dose | -7.14 to +11.7 % dose | 91 to 127% |

The 95% confidence limits (mean +/- SE × $\approx 2; t_{0.025,df}$) for the differences [A2-A1] - [B2-B1] are included both as real values and as a percentage, *i.e.* the percentage change to any VUR effect in group A that may be expected in the presence of abnormal bladder function.

In no case was there a pronounced negative bias denoting a greater effect of VUR in group B (with abnormal bladder function). The negative bias apparent for GFR both as the body weight and the kidney weight ratio was very small. A positive bias observed for maximal concentrating ability was also very small and insufficient to infer a greater effect in group A (with normal bladder function).

The results presented here both supplement and corroborate those showing no significant interaction between VUR and bladder function on outcome measures of kidney weight and renal function at the end of the study period.

CHAPTER 20

# RESULTS: EXPERIMENT II

## 20.1 Experimental Design

All pigs were followed from the time they entered the study at week 0 for a minimum of 15 weeks. The maximum period of study was 21 weeks. The mean number of study weeks for pigs in each group are shown in Table 27. Also included for each group are the mean values for age and body weight at the times of surgery, study week 0, and the **early** and **late** investigations. The data for individual pigs are shown in **Appendix V**.

At the times of both the **early** and **late** investigations, ages and body weights of the pigs were sufficiently similar for the purpose of comparing parameters of renal growth and function. At the end of the study period there was no significant interaction between bladder function and VUR on either age ($t_{19}$=0.10) or body weight ($t_{19}$=1.36). The estimated differences in both age and weight attributed to pigs with unilateral VUR in comparison with those without VUR are shown in Table 27. The differences were not significant (age:$t_{20}$=1.32; weight $t_{20}$=1.03).

Table 27 Mean values for study period, ages and body weights for each group.

| | Normal Bladder Function | | Abnormal Bladder Function | |
|---|---|---|---|---|
| Group | A1 Reflux | A2 Control | B1 Reflux | B2 Control |
| Number of Pigs | 5 | 5 | 7 | 7 |
| Mean Weeks of Study | | | | |
| Study week 0 to death | 17.6 | 18.2 | 18.3 | 17.3 |
| Mean Age (weeks) at: | | | | |
| Surgery (VUR) | 4.6 | | 5.0 | |
| Study week 0 | 8.0 | 6.6 | 9.4 | 8.1 |
| Investigations | | | | |
| Early (study week 4) | 11.4 | 10.6 | 13.4 | 12.4 |
| Late (start) | 23.6 | 23.2 | 26.6 | 24.3 |
| (end) | 25.8 | 24.8 | 27.7 | 26.0 |
| Mean Body Weight (kg) at: | | | | |
| Surgery (VUR) | 4.6 | | 5.0 | |
| Study week 0 | 7.5 | 7.3 | 7.5 | 7.4 |
| Investigations | | | | |
| Early (study week 4) | 12.3 | 12.1 | 13.4 | 12.4 |
| Late (start) | 31.8 | 31.9 | 28.7 | 32.1 |
| (end) | 34.5 | 33.6 | 31.4 | 34.9 |

Table 28 Differences in final age and body weight attributed to pigs with VUR compared with those without VUR.

| | 95% Confidence Limits |
|---|---|
| Age at end | -0.77 to +3.61 weeks |
| Body weight at end | -5.42 to +1.71 kg |

## 20.2 Renal Growth

The left and right kidney weights and relative left kidney weights; i.e. $100 \times L/[L+R]$; for individual pigs are included in **Appendix V** and the mean values for each group shown in Table 29.

Table 29 Individual and left relative kidney weight mean values for each group.

| | Normal Bladder Function | | Abnormal Bladder Function | |
|---|---|---|---|---|
| Group | A1 Reflux | A2 Control | B1 Reflux | B2 Control |
| Number of Pigs | 5 | 5 | 7 | 7 |
| Kidney weight | | | | |
| Left (g) | 66.6 | 67.8 | 63.3 | 75.6 |
| Right (g) | 64.6 | 64.4 | 62.9 | 73.1 |
| Relative kidney weight | | | | |
| Left (%) | 50.8 | 51.4 | 50.1 | 50.7 |

**Left relative kidney weight.** For 15 of the 24 pigs left relative kidney weight values were slightly greater than 50% indicating a tendency for the left kidney to be larger than the right. This is reflected in the mean values for each group although for group B1 is barely apparent. There was no significant difference between the A and B groups ($t_{20}=1.60$), no significant interaction between bladder function and VUR ($t_{19}=0.037$) and no significant effect of left unilateral VUR on left relative kidney weight ($t_{20}=1.23$).

The estimated change to left relative kidney weight attributed to left unilateral VUR is shown in Table 30.

Table 30 Change in left relative kidney weight attributed to VUR.

| | 95% Confidence Limits |
|---|---|
| Relative kidney weight | |
| Left | -1.42 to +0.34% |

## 20.3 Renal Function

### 20.3.1 Glomerular Filtration Rate and Concentrating Ability

Glomerular filtration rate (GFR) and maximal urine osmolality values for each pig at the late time are included in Appendix V and the mean values for each group shown in Table 31.

Table 31 Mean values for GFR and maximal urine osmolality (Late).

| | Normal Bladder Function | | Abnormal Bladder Function | |
|---|---|---|---|---|
| Group | A1 Reflux | A2 Control | B1 Reflux | B2 Control |
| Number of Pigs | 5 | 5 | 7 | 7 |
| GFR ml/min | 121.0 | 120.8 | 103.7 | 122.7 |
| GFR ml/min/kg body weight | 3.63 | 3.49 | 3.39 | 3.79 |
| Osmolality (mosmol/kg water) | 1137 | 1139 | 1125 | 1094 |

The GFR ml/min mean values were lower for group B1 than for other groups. Mean values for GFR ml/min/kg body weight were similar in all 4 groups, as were mean values for maximum urinary osmolality. Statistical tests on all these parameters showed no significant difference between the A and B groups, no significant interaction between VUR and bladder function and no significant effect of VUR (Table 32). The changes in indices of GFR and in maximal urinary concentration attributed to the presence of unilateral VUR are shown in Table 33.

Table 32 Statistics results: Glomerular filtration rate, urinary concentrating ability.

| | Interaction (VUR + Bladder Function) | Difference Attributed to: Bladder Function (A-B group) | VUR |
|---|---|---|---|
| | | Effects | Effects |
| GFR | | | |
| ml/min | $t_{19}=1.37$ | $t_{20}=1.10$ | $t_{20}=1.59$ |
| ml/min/kg body weight | $t_{19}=0.13$ | $t_{20}=0.12$ | $t_{20}=0.82$ |
| Osmolality (mosmol/kg water) | $t_{19}=0.39$ | $t_{20}=0.65$ | $t_{20}=0.40$ |

Table 33 Effects of unilateral VUR on GFR and maximal urinary concentrating ability.

| | 95% Confidence Limits |
|---|---|
| GFR | -25.7 to +3.72 ml/min |
| GFR/body weight | -0.58 to +0.29 ml/min/kg |
| Urine concentration | -70.13 to +87.30 mosmol/kg water |

### 20.3.2 Renal Uptake of $^{99m}$Tc-DMSA

The mean values for absolute uptake and left relative uptake for each group at the **early** and **late** times are shown in Table 34. Individual results for each pig are included in **Appendix V**.

Table 34 Mean values for absolute uptake of $^{99m}$Tc-DMSA by left and right kidneys and for left relative uptake.

| | Normal Bladder Function | | Abnormal Bladder Function | |
|---|---|---|---|---|
| Group | A1 Reflux | A2 Control | B1 Reflux | B2 Control |
| Number of Pigs | 5(4)[1] | 5 | 7 | 7 |
| Early (week 4) | | | | |
| Absolute uptake % Left | 22.0[1] | 20.7 | 22.6 | 21.9 |
| Right | 20.9[1] | 20.5 | 23.2 | 22.1 |
| Left relative uptake % | 51.3[1] | 50.2 | 49.4 | 49.9 |
| Late (final) | | | | |
| Absolute uptake % Left | 19.8 | 20.8 | 23.1 | 21.3 |
| Right | 20.6 | 21.6 | 23.6 | 22.4 |
| Left relative uptake % | 49.0 | 49.1 | 49.4 | 48.8 |

[1]Data from one pig not recorded due to technical problems.

**Comparison of Final *In Situ* and *Ex* Situ Estimates.** Absolute uptake calculated in 23 pigs from the final 24h imaging procedure with the kidneys in place (*in situ*) was compared with that calculated from the same kidneys (n=46) after their isolation at *post mortem* (*ex situ*). Values from individual pigs are included in **Appendix V**. The results of the differences between the 2 estimates are shown in Table 35.

Table 35 Comparison between paired (*in situ* and *ex situ*) estimates of left relative and absolute $^{99m}$Tc-DMSA renal uptake.

| | Final *In situ*-*Ex situ* difference | | 95% Confidence Limits (mean +/- SE × 2.07) |
|---|---|---|---|
| | Mean | SD | |
| Absolute uptake % | | | |
| Left | -0.64 | 1.46 | -1.24 to +0.04 |
| Right | +0.73 | 1.63 | -0.02 to +1.44 |
| Relative uptake % | | | |
| Left | -1.30 | 1.26 | -1.85 to +0.75 |

There was good agreement between the *in situ* and *ex situ* estimates for both absolute uptake by left and right kidneys and for left relative uptake.

***In situ* Absolute Uptake.** Left and right kidney absolute uptake mean values for all groups at the **early** and **late** times were similar (Table 34).

**In *situ* Left Relative Uptake.** The mean relative uptake values for reflux groups A1,B1 were similar to those for control groups A2,B2 at both the **early** and **late** times (table 34). There was no significant interaction between VUR and bladder function either **early** ($t_{18}$=0.88) or **late** ($t_{19}$=0.60) and no significant effect of VUR either **early** ($t_{19}$=0.14) or **late** ($t_{20}$=0.51).

The estimated changes in left relative uptake of $^{99m}$Tc-DMSA attributed to VUR are shown in Table 36.

Table 36 Change in left relative uptake of $^{99m}$Tc-DMSA attributed to VUR.

| | 95% Confidence Limits |
|---|---|
| Left relative uptake | |
| Early | -1.72 to +1.93% |
| Late | -0.92 to +1.03% |

## 20.4 Interactive Effects Between VUR and Bladder Function

The influence of abnormal bladder function in changing the outcome of VUR on left relative kidney weight and left relative $^{99m}$Tc-DMSA uptake at the late time, is shown in Table 37 as the mean of the difference between VUR effects in group A (normal bladder function) and group B (abnormal bladder function); *i.e.* [A2-A1] - [B2-B1]. The 95% confidence limits (mean +/- SE × ≈ 2; $t_{0.025,df}$) for these differences are included both as real values and as a percentage difference, *i.e.* the percentage change to any VUR effects in group A that may be expected in the presence of abnormal bladder function.

Table 37 Effects of abnormal bladder function on the outcome of VUR. The mean and estimates for the difference in the outcome of VUR attributed to abnormal bladder function in comparison with normal bladder function *i.e.* [A2 - A1] - [B2 - B1].

| Outcome Measure | Mean | 95% Confidence Limits | |
|---|---|---|---|
| | | Measured Units | % Difference |
| Left relative: | | | |
| Kidney Weight | -0.025% of L+R | -1.84 to +1.79% of L+R | 96 to 103% |
| DMSA uptake | +0.74% of L+R | -1.82 to +3.43% of L+R | 96 to 107% |

For both relative kidney weight and for relative DMSA uptake the mean of the difference between groups A and B in any effects of VUR was close to zero. A negative bias in the estimates (95% confidence limits), denoting a greater effect of VUR in group B, was either absent or very small. These results both supplement and corroborate those showing no significant interaction between left unilateral VUR and bladder function on left relative kidney weight or left relative DMSA uptake.

CHAPTER 21

## DICUSSION OF RESULTS: EXPERIMENT I AND II

This investigation performed as 2 separate but parallel experiments using either single kidney or 2-kidney models with unilateral VUR has allowed, by statistical comparison with control animals, quantitation of the effects of VUR on parameters of renal growth and function. These effects relate only to the primary hydrodynamic component of VUR since urinary infection and renal scarring were excluded by design.

The influence of VUR was tested on kidney weight, glomerular filtration rate (GFR), urinary concentrating ability and renal uptake of $^{99m}$Tc-DMSA. The results showed that after an approximately 5-month period of rapid growth urinary concentrating ability in the single kidney models was significantly reduced by VUR, otherwise there were little or no significant effects of VUR on kidney growth or any other parameter of renal function.

By including in both experiments animals with normal and abnormal bladder function the effects of VUR were examined in 2 contrasting urodynamic conditions. Those prevailing in pigs with abnormal bladder function represented the limits to which the pig kidney may be subjected to hydrodynamic insult without developing macroscopic scarring. Under these conditions, in neither experiment, did abnormal bladder function produce any significant enhancement of the

effects of VUR on any parameter of renal growth or function.

## 21.1 Statistical Methods

The regression methods applied to determine the effects of VUR used to advantage the total number of pigs in each separate experiment and permitted examination of any interaction between VUR and bladder function, *i.e.* the severity of the hydrodynamic insult imposed by the 2 bladder conditions: group A and group B. The absence of any significant interaction (P always more than 0.1) in all parameters tested, and the 95% confidence limits for the effects of interaction, demonstrated that the effects of VUR were virtually the same in pigs with bladder outflow obstruction and abnormal bladder function (groups B) as in those with normal bladder function (groups A). This in turn meant that for each parameter an estimate of the effects of VUR could be calculated after taking into account any inherent differences between the A and B groups in the absence of reflux (**18.3**).

Such inherent differences as did exist between the A and B groups were a summation of the effects of sex and bladder function (since groups A were predominantly female and groups B male; **17.3.2.3**). The effects of sex, however, enabled VUR to be tested in 2 urodynamic conditions of extreme diversity. It was only where parameters concerned kidney weight (absolute kidney weight, GFR per g kidney weight) that significant differences were observed between the A and B groups. In these instances the differences are ascribed to sex as

it is recognised that this influences kidney weight. These sex effects did not interfere with the assessment of interaction which considers the relative difference in parameter values between the control groups without VUR and the test groups with VUR (A2 - A1 compared with B2 - B1; **18.3**)). Furthermore, as the inherent differences between the A and B groups were always accounted for mathematically they did not distort the calculated estimates for the effects of VUR, derived from the combined results for groups A and B.

### 21.2 Body Weight and Age

By the end of the study period (**late**) the pigs with VUR in **Experiment I** were slightly older and weighed a little more than those without VUR. In **Experiment II** those with VUR were slightly older than control pigs but weighed less. There was, however, no significant difference in either age or body weight between any of the 4 groups of each experiment, at either the **early** or **late** times. The influence of VUR on somatic growth was not a primary consideration of this investigation but maintaining a uniformity in age and especially body weight was essential for comparing both absolute and derived measures of kidney weight and renal function. The importance of this even in relation to ratio values has been discussed (**10.2**).

### 21.3 Renal Growth

In both experiments renal growth was assessed from the kidney weights at the end of the study period. The effects of VUR on renal

growth were tested in 2 different models of kidney growth: *i.e.* normal growth in pigs with both kidneys in place (**Experiment II**) and augmented growth in pigs with a solitary kidney following unilateral nephrectomy (**Experiment I**).

That normal renal growth had been achieved in the 2-kidney models was supported by the observation that combined kidney weights (about 120-130g) were as expected when compared with other documented miniature pig data (**6.2.3**) and were of a similar order to that of the human (**6.2.1**) of comparable body weight (about 30kg).

That exceptional renal growth was achieved by the solitary kidney is evidenced by a comparison of kidney weight values from control pigs in the 2 experiments. This comparison is permissible since both experiments were performed in parallel and all pigs were of similar ages and body weights. By the end of the study, mean solitary kidney weight in females was 85% that for total renal mass of females with normally paired kidneys, and in males this was 90%.

The effects of VUR on the compensatory response by a solitary kidney following unilateral nephrectomy was not itself an issue of this investigation. The solitary kidney undergoing compensatory growth synchronous with normal growth was used merely to maximise the sensitivity of any effects of VUR on renal growth. Despite the nearly twice normal renal growth by the solitary kidney models the calculated estimates for the effects of VUR showed only a tendency for a small reduction in that growth. This was apparent both from

the absolute values of kidney weight (95% confidence limits; -22.4 to +8.7g) and from the kidney weight per kg body weight ratios. These small effects attributed to VUR were not statistically significant and therefore may have occurred by chance.

Although renal growth was less in the 2-kidney pigs it was nevertheless substantial and these animals were no less valuable for examining renal growth. By restricting VUR to the left side alone and by considering left relative kidney weight in comparison with control pigs without VUR the 2-kidney models allow detection of any unnatural assymetry in kidney weight. That a natural tendency for the left kidney to be bigger than the right was detected by this practice testifies to the sensitivity of the 2-kidney models used in this way. The results from the 2-kidney pigs showed that VUR induced no detectable effect at all on left relative kidney weight, therefore, the absence of any significant effects of VUR on renal growth are further substantiated.

There have been no other satisfactory experimental studies where young growing animals have been used to accurately assess the effects of VUR on renal growth (3.2). The results for kidney weight from studies using older canine models do not conflict with the observations from this investigation that VUR does not significantly affect renal growth[152-154,156]. This is further corroborated by those clinical investigations which consider the effects of VUR in the absence of scarring and urinary infection[115-117].

## 21.4 Renal Function

**Experiment I** used the solitary kidney model especially to allow testing of the effects of VUR on glomerular filtration rate (GFR), urinary concentrating ability and absolute uptake of $^{99m}$Tc-DMSA (DMSA) renal uptake. **Experiment II**, was acknowledged suitable only for considering relative renal function, measured by the uptake of DMSA. In the 2-kidney pigs, in this experiment, GFR and urinary concentrating ability were measured only to confirm functional homogeneity between the groups. That this was so and that GFR and urinary concentrating ability were not affected by unilateral VUR was not unexpected because any effect of left unilateral VUR on left renal function may be masked or even compensated for by the right contralateral kidney. In this situation, therefore, the model is powerful only for considering the effects of VUR on left relative renal uptake of DMSA.

### 21.4.1 Glomerular Filtration Rate

The results from **Experiment I** showed that during the course of the study period the expected increase in GFR with age and body weight was observed both for pigs with and without VUR and individual GFR values were comparable to those obtained in preliminary studies (**11.1.4; 11.2**). Other studies documenting GFR through a sustained period of pig growth are limited but individual values from this study were similar to those documented for another miniature pig variety[162] but slightly higher than those reported for large pigs[266,268]. Values recorded at the end of the study period were within the ranges documented for children between ages 12 to 14 years (7.2).

There was a decrease in GFR per kg body weight between the **early** measurements at study weeks 4/5 and those at the end of the study period (**late**). This observation in pigs has not been acknowledged previously but was not unexpected as preliminary studies in the pig (**11.1**), together with documented values for GFR in the pig (**7.3**) and the human (**7.2**), indicate that GFR has a curvilinear relationship with body weight. The decline in GFR per kg body weight did appear, however, to be greater for pigs with VUR than for those without. These differences were not investigated statistically because they may be artefactual. When a derived index changes with time and one of its components is itself time related (body weight) these differences may be artefactually influenced by small timing variations between the first (**early**) and second (**late**) measurements. For this same reason no interpretation of these effects can be made. Similarly, the change in absolute GFR (ml/min) between the **early** and **late** times was not considered because differences in body weight at these times, although small, interfere with the precision of such an assessment. The effects of VUR on GFR were examined only by comparing test and control animals at either **early** or **late** times separately when body and kidney weights were sufficiently similar to allow comparison of GFR as the absolute (ml/min) and as body and kidney weight ratio values.

The estimated changes in GFR indices attributed to VUR showed that whilst GFR both as absolute and as the body weight ratio was unnaffected by VUR at the **early** time, there was at the **late** time a tendency for diminished GFR in association with VUR. These effects

after approximately 5 months of rapid growth were small, not statistically significant and, therefore, may have arisen by chance. The absence of any direct effect of VUR on GFR is further supported by the observation of no detectable effect of VUR on GFR per g kidney weight at the end of the study period.

The only other experimental investigation of the effects of VUR on GFR in the growing pig was that of Helin[107] who demonstrated a reduction in GFR in kidneys exposed to unilateral VUR when compared with contralateral kidneys without VUR. These quite different results may be ascribed to the methods of measuring GFR and/or to the pig model used. In the Helin[107] study GFR was estimated in anaesthetised pigs by an invasive technique involving catheterisation of both renal arteries and veins. Of perhaps more significance was the choice and use of the pig model. The urinary tract of the Landrace pig chosen by Helin may be different to that of other pig varieties since the upper tracts have been reported[173] to appear wide and the ureters tortuous. Furthermore, it is only in the Landrace pig that naturally occurring VUR has been observed (**5.3.2**). In the Gottingen pig used in this present investigation the gross anatomy of the urinary tract is similar to that of the human (**9.5**) and wide or grossly dilated ureters were not a feature of the models for VUR (**17.3.2.2**). In the use of the Helin[107] pig model there is no discussion concerning the size or morphology of the ureters and kidneys so ureteric dilatation, obstruction and renal scarring cannot be excluded which makes the results difficult to interpret.

The results from **Experiment I** showing no significant effect of VUR on GFR are consistent with those of the few clinical investigations which consider this issue in the absence of renal scarring and urinary infection[93,118].

### 21.4.2 Urinary Concentrating Ability

That maximal urinary concentration was achieved in all pigs on each test occasion was established by observing similar urinary osmolalities in at least 2 urine samples collected at 2h intervals after 16h dehydration. The results showed that concentrating ablity in control pigs without VUR, from both experiments, was the same or greater than that reported previously for large pigs and similar to that in the human (5.2). Full concentrating capacity was retained by pigs with unilateral VUR and by pigs with a solitary kidney but without VUR. However, in solitary kidney pigs with VUR, concentrating ability was significantly impaired. It was more apparent in pigs with normal bladder function than in those with abnormal bladder function but this may be explained by the smaller number of animals in this latter group. The overall mean reduction attributed to VUR was small in value being between 266 and 81 mosmol/kg water (95% confidence limits) and it is noteworthy that the majority of individual values (**Appendix IV**) exceeded 800 mosmol/kg water taken in clinical practice as the lower limit of normal. Nevertheless, an effect of VUR on urinary concentrating ability was conclusively demonstrated.

A decrease in urinary concentrating capacity of a similar order to

that demonstrated in this investigation has been shown in children with VUR in the absence of renal scarring[89]. However, unlike the results from pigs with paired kidneys and unilateral VUR (**Experiment II**), a decreased urinary concentration was found in children with unilateral VUR. In the clinical study the influence of a preceeding urinary infection, known to reduce concentrating ability for up to 12 weeks after its eradication[105], cannot be disregarded as a period of only 6 weeks was allowed between any urinary infection and the concentrating test.

The mechanism(s), by which VUR diminishes concentrating ability cannot be deduced from this investigation (**14.1**). Its occurrence, secondary to an effect on GFR is unlikely, however, since GFR was not significantly affected by VUR. Also, the effect is unlikely to be mediated through a systemic endocrine function because concentrating ability was not impaired in the 2-kidney pig models with unilateral VUR. Effects mediated through small changes in tubular function, or renal blood flow cannot be eliminated, especially as an effect of VUR on effective renal plasma flow has been reported[114]. It should be noted, however, that affects of VUR on urinary concentrating ability may result directly from the influence on the medullary concentration gradient of refluxed urine in the renal pelvis. These effects may be enhanced in the event of intra-renal reflux into susceptical papillae as it is not difficult to envisage a disruption of countercurrent mechanisms in loops of Henle by the retrograde flow of urine through tubules. Such a mechanism, if operative, would

undoubtedly revert to normal with the cessation of VUR by whatever means.

### 21.4.3 Renal Uptake of DMSA

In **Experiment I** and **Experiment II** the uptake of DMSA was determined as the percentage of the administered dose sequestered in individual kidneys (*i.e.* absolute uptake). In **Experiment II** uptake by the left kidney relative to the total (left + right) was derived from absolute uptake values. Although relative uptake can be derived simply from the true renal counts and does not depend upon calculating absolute uptake values these latter were determined in the 2-kidney pigs to ascertain that the expected efficieny of DMSA renal accumulation of was met.

There are a number of factors associated with errors in the measurement of DMSA uptake (12.2) and the methods used in this investigation aimed to minimise their influence. When deriving relative uptake, errors associated with estimating the effective dose administered are obviated since they apply to left and right kidneys equally.

In both paired and solitary kidneys absolute uptake values were similar to those described for pigs previously (13.4)[374], irrespective of the presence of VUR or bladder outflow obstruction. Uptake by solitary kidneys was about twice that achieved by individual kidneys of pairs. These observations are not unlike those described for the human but data for normal and solitary

kidneys is scarce[356,361,373].

Both experiments had the common objective of testing the effects of VUR on DMSA uptake when this was calculated from the accumulated radio-activity measured with the kidneys in place (*in situ*). This permitted uptake measurements during growth both **early** and **late** in the study and allowed duplicate uptake studies to be performed at the **late** time. Measurement of uptake at 6h and 24h after the dose injection ensured that counts were acquired only after uptake had reached a maximum and stable level (plateau). The uptake values calculated from single measurements of radio-activity in kidneys after their isolation (*ex situ* values) were obtained only to gain a perspective on the errors associated with *in situ* estimates, of which the depth of the kidney below the skin surface is of most significance. This is of crucial importance because for $^{99m}$Tc radio-tracer an error of 1cm in depth is equivalent to 13% error in renal activity[376,377]. Even when considering relative uptake it cannot be assumed that separate kidneys of pairs are equidistant from the skin surface[376] and indeed differences between left and right kidneys were noted in individual pigs of this investigation. In order to evaluate these errors the absolute uptake values calculated from the kidneys *in situ* at 24h were compared with those of the same kidney immediately after its isolation. Although the *in situ* and *ex situ* values were not identical, the mean of the difference (*in situ* - *ex situ*) between them was less than 0.5% absolute uptake for the solitary kidneys (**Experiment I**) and less than 1% (absolute uptake) for both the right and left kidneys of pairs (**Experiment II**). In

both experiments the variation in these differences was small indicating a good agreement between the 2 values. The *in situ* and *ex situ* differences in the 2-kidney pigs showed that uptake by the left kidney was slightly underestimated whilst that of the right kidney was slightly overestimated, producing a small negative bias in left relative uptake. This did not interfere with the analysis of the influence of VUR because left relative uptake in pigs with left unilateral VUR was compared with left relative uptake in control pigs without VUR.

In the 2-kidney pigs where unilateral VUR was always left sided there was no detectable effect of VUR on left relative DMSA uptake. These results are supported by one of few studies on the influence of VUR on DMSA uptake. Goldraich and Goldraich *et al*[361] found that absolute uptake was normal in children wth low grade VUR but was reduced in those with high grade VUR. However, diminished uptake was never found in the absence of renal scarring, and many scarred kidneys had normal uptake.

In the solitary kidney pigs of **Experiment I**, however, there was associated with VUR a detectable but small reduction in uptake (95% confidence limits; -7 to -0.3% of dose) which was the same at both the **early** and **late** times. On both occasions this reduction was statistically significant but only at the 5% level. On neither occasion could the reduction be ascribed to any small differences in the ages or weights of the pigs at the time of unilateral nephrectomy.

A reduced uptake by solitary kidneys with VUR, was also apparent from the results of the kidneys after their isolation (*ex situ* kidneys) but this just failed to reach statistical significance. Most likely, this was because not all pigs were included in this aspect of the study as it was not always possible to kill the animals immediately following the final uptake study. These *ex situ* results are further limited to only a single uptake estimate and therefore may be subject to more inherent variability. Nevertheless, estimates for the differences in uptake between kidneys with and without VUR (95% confidence limits -8 to +0.05 % dose) calculated from this data does indicate that the observations made from the kidneys *in situ* are unlikely to be artefactually associated with kidney depth. This is further supported by the good agreement in 24h uptake values found between the measurements made from the same kidneys in and out of the body.

In this investigation all factors likely to contribute to errors or variability in measures of absolute uptake of DMSA have been constrained or considered and since the reduction in uptake associated with VUR was significant at the 5% level some certainty has to be ascribed to the observation. However, the changes in absolute uptake attributed to VUR were very small and the possibility remains that they may have arisen by chance. For greater certainty a difference significant at the 1% confidence level is required and this was not met.

The difference in results of the 2 experiments in showing a dectable effect of VUR on renal DMSA uptake may be ascribed to the enhanced uptake in solitary kidneys compared with that in individual kidneys of pairs and/or to the intrinsic difference between the solitary and 2-kidney models. In the solitary kidney model, increased demands for growth and function are imposed by compensatory adaption superimposed on normal development (**Chapter 8**), and this model was used in the knowledge that it may provide a sensitive indicator for the influence of VUR. In the solitary kidney pigs both GFR, uncorrected for kidney weight, and kidney weight itself tended to be marginally diminished in the presence of VUR. If a premise that these reductions were attributed to VUR is accepted, despite their failure to reach statistical significance, then the observation of a small but significant reduction in DMSA uptake by VUR is both consistant and explicable. This is because DMSA renal uptake most likely represents an amalgamation of inter-related parameters (**12.1**) and the diminution in uptake found in association with VUR in solitary kidneys may possibly be related to a summation of small influences of VUR on renal performance or some determinant of this such as renal blood flow (**Chapter 2**).

Alternatively, the decreased uptake in solitary kidneys with VUR may be associated directly with the decrease in urinary concentrating ability also seen in solitary kidneys with VUR, but this is unlikely. The observed decrease in urinary concentrating ability (in solitary kidney pigs) was of such clear profoundity that it is reasonable to assume that VUR influences urinary concentrating ability the same in

the one kidney of a pair with unilateral VUR as it does in a solitary kidney with VUR, and, that the inability to detect this in the 2-kidney pigs was because of the presence of a normal contralateral kidney. Given these assumptions, a reduced left relative DMSA uptake in the 2-kidney pigs with left unilateral VUR might be expected but was not observed.

Whilst it may be acceptable, and indeed correct, to dismiss the observation of an association between VUR and impaired renal uptake of DMSA as a statistical artefact the possibility remains that VUR has, in addition to its effect on urinary concentrating ability, a physiological effect upon the kidney, which can be detected as diminished uptake of DMSA but only in the solitary kidney undergoing prodigous growth and development and with normally enhanced DMSA renal uptake. These changes were of such small magnitude that they cannot be credited with any biological or pathological significance, furthermore, they are unlikely to be detected in the clinical setting where variables cannot be rigorously controlled.

## 21.5 Conclusions

This investigation using the pig model has shown that vesicoureteric reflux *per se* can have a significant effect in diminishing urinary concentrating ability. The effect in impairing concentrating ability is small and unlikely to be of pathological significance. In normally paired kidneys there is no effect of VUR on renal uptake of DMSA. In solitary kidneys undergoing growth and compensatory

growth VUR may induce a small diminution in enhanced DMSA uptake, but this is unlikely to be of biological or pathological significance. There is no significant effect of VUR on renal growth or glomerular filtration rate.

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The daily management of the experiments, anaesthesia, urine sampling and measurements of glomerular filtration rate, renal uptake of $^{99m}$Tc-dimercaptosuccinic acid, urinary concentrating ability and kidney weights were performed entirely by me. I performed all the data analysis with advice from a medical statistician (Department of Epidemiology, Institute of Child Health). The daily animal husbandry was carried out by staff of the Animal House but with my involvement. The microbiological examination of urine samples was performed by others in routine pathology laboratories (Hospital for Sick Children).

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morphology (Chapter 9). Otherwise, my contribution to the Section III experiments, presented to support the use of the pig model and the methods of measurement, was the same or similar to that described above.

As well as all data analysis, the interpretations of all the experimental results presented in this thesis are entirely my own, although for the most part they are in accordance with those of my supervisors. The historical survey, interpretation and discussion of the literature are my own work, as is all discussion of the various experimental studies. The preparation of the manuscripts, now published (see enclosures), and describing much of the work concerned with this thesis was a collaborative venture and my participation at least equalled that of my co-authors.

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Margaret L. Godley

April 1991

# APPENDICES

I. Details: Animals, Equipment, Materials.

II. Tabulated Results: Chapter 9; **Papillary Morphology.**

III. Tabulated Results: Chapter 11; **GFR: C-slope Evaluation.**

IV. Tabulated Results: Chapter 19; **Experiment I.**

V. Tabulated Results: Chapter 20; **Experiment II.**

## Animals

| | |
|---|---|
| Large white pigs (for GFR evaluation) | Chelmwood Herd, Dunmow, Essex, U.K. |
| Large white pigs (for papillary morphology)<br>Gottingen pigs | Royal Veterinary College, Boltons Park Farm, South Mimms, Herts., U.K. |
| Froxfield pigs | Froxfield Rabbits, Broadway Farm, Froxfield, Hants., U.K. |

## Equipment

| | |
|---|---|
| Pig restraining cage (made to specification) | Agricultural College, Reading, Berks., U.K. |
| Harvard 975 infusion pump | Harvard Apparatus Co. Inc., 150 Dover Road, Millis, Massachussets 02054, U.S.A. |
| Burkard Koolspin centrifuge (with angle rotar) | Burkard Scientific Ltd., Uxbridge, Middx., U.K. |
| LKB 1282 Compugamma well counter | LKB Medical Ltd., South Croydon, London, U.K. |
| Philips SonoDiagnost R (with linear array transducer) | Philips Medical Ltd., Kelvin House, Glenthorne Road, Hammersmith, London, U.K. |
| Dean mobile x-ray machine 300Ma | Todd Research, Robjohns Road, Chelmsford, Essex, U.K. |

| | |
|---|---|
| Scinticamera V gamma camera<br>Collimator NE 8922<br>(Parallel multihole, low energy, 10.5" field of view) | Nuclear Enterprises,<br>Sighthill,<br>Edinburgh, Scotland, U.K. |
| 2-Channel chart recorder EM 720<br>Preamplifiers EM 722<br>Pressure transducers EM 750 | Elcomatic Ltd.,<br>Kirktonfield Road,<br>Neilston,<br>Glasgow, Scotland, U.K. |
| Advanced Micro-osmometer 3MO | Vital Scientific,<br>Partridge Green,<br>Sussex, U.K. |

**Materials**

| | |
|---|---|
| SMI Micropipettes | Alpha Laboratories,<br>169 Oldfield Lane,<br>Greenford, Middx., U.K. |
| Bard Intracaths 1617<br>Portex Epidural Catheters 100/382/116 | Arterial Medical Services Ltd.,<br>Arterial House,<br>303 Chase Road,<br>Southgate, London, U.K. |
| Halothane (Fluothane) | ICI Pharmaceuticals,<br>Alderley Park,<br>Macclesfield,<br>Cheshire, U.K. |
| Radiographic contrast medium<br>Isopaque cysto 100mgI/ml | Nycomed (UK) Ltd.,<br>2111 Coventry Road<br>Sheldon,<br>Birmingham, U.K. |
| Radiopharmaceuticals:<br>Technetium generator<br>Dimercaptosuccinic acid (CIS)<br>$^{51}$Cr-EDTA | Amersham International plc.,<br>Little Chalfont,<br>Berks, U.K. |

| | |
|---|---|
| Gentamicin (Cidomycin) | Roussel Laboratories Ltd.,<br>Broadwater Park,<br>Whitehall Lane,<br>Egham, Surrey, U.K. |
| Nitrofurantoin (Macrodantin) | Norwich Eaton Ltd.,<br>Rusham Park,<br>Whitehall Lane,<br>Egham, Surrey, U.K. |

* * * * * * * *

## For Computing:

Calculations for glomerular filtration rate and for $^{99m}$Tc-DMSA renal uptake were done at first manually and then using a programmable calculator (TI 59 with printer PC-100C; Texas Instuments).

Regression analysis was done using a personal computer running GLIM 3.77 (the Generalised Linear Interactive Modelling system; The Royal Statistical Society). Other statistics were performed with the aid of a calculator (as above) or personal computer running 'Statgraphics', STSC; Inc..

The main text of the thesis was prepared on an Apricot Xi personal computer running SuperWriter (ACT (UK) Ltd.) and printed (QuickJet with Epson FX80 emulation; Hewlett Packard) in Elite 12cpi. The appendices were produced using an Apple Macintosh personal computer.

**Table II.i.** **Large White Pigs** (n = 20). The numbers of papillary types found in upper pole, mid-zone and lower pole of 34 normal kidneys.

| Pig | | Simple & Compound Papillae Type I | II | III | Simple & Compound Papillae Type I | II | III | Simple & Compound Papillae Type I | II | III |
|---|---|---|---|---|---|---|---|---|---|---|
| | | UPPER | | | MIDDLE | | | LOWER | | |
| 1 | Left | 2 | 0 | 2 | 2 | 1 | 0 | 0 | 0 | 1 |
| | Right | 1 | 0 | 1 | 4 | 0 | 2 | 0 | 0 | 1 |
| 2 | Left | 1 | 1 | 3 | 2 | 0 | 2 | 0 | 0 | 1 |
| 3 | Left | 6 | 0 | 1 | 3 | 2 | 0 | 0 | 0 | 1 |
| 4 | Left | 0 | 4 | 0 | 5 | 1 | 0 | 1 | 0 | 1 |
| 5 | Left | 1 | 2 | 2 | 5 | 0 | 0 | 1 | 0 | 1 |
| 6 | Left | 0 | 2 | 0 | 5 | 1 | 0 | 0 | 2 | 0 |
| | Right | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| 7 | Left | 2 | 0 | 1 | 3 | 1 | 0 | 0 | 0 | 1 |
| | Right | 0 | 0 | 1 | 4 | 1 | 1 | 0 | 1 | 1 |
| 8 | Left | 0 | 2 | 1 | 1 | 0 | 2 | 1 | 0 | 2 |
| | Right | 0 | 0 | 2 | 4 | 0 | 1 | 5 | 0 | 2 |
| 9 | Left | 2 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 2 |
| 10 | Left | 0 | 0 | 1 | 3 | 0 | 1 | 0 | 0 | 2 |
| 11 | Left | 1 | 1 | 2 | 2 | 3 | 1 | 0 | 1 | 1 |
| | Right | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 2 |
| 12 | Left | 0 | 1 | 1 | 1 | 3 | 0 | 0 | 2 | 1 |
| | Right | 0 | 0 | 2 | 1 | 0 | 1 | 1 | 1 | 1 |
| 13 | Left | 1 | 0 | 1 | 2 | 1 | 0 | 2 | 0 | 1 |
| | Right | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 |
| 14 | Left | 1 | 0 | 2 | 3 | 4 | 0 | 0 | 0 | 2 |
| | Right | 0 | 0 | 1 | 3 | 1 | 0 | 1 | 0 | 4 |
| 15 | Left | 0 | 0 | 2 | 0 | 2 | 2 | 1 | 0 | 2 |
| | Right | 0 | 1 | 1 | 0 | 0 | 4 | 2 | 0 | 1 |
| 16 | Left | 2 | 0 | 1 | 0 | 2 | 1 | 0 | 1 | 1 |
| | Right | 0 | 3 | 1 | 0 | 3 | 1 | 0 | 0 | 1 |
| 17 | Left | 0 | 2 | 1 | 0 | 1 | 2 | 1 | 0 | 2 |
| | Right | 0 | 0 | 2 | 3 | 0 | 1 | 0 | 2 | 2 |
| 18 | Left | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 |
| | Right | 1 | 0 | 2 | 0 | 1 | 1 | 0 | 1 | 1 |
| 19 | Left | 0 | 0 | 2 | 1 | 0 | 3 | 1 | 0 | 2 |
| | Right | 0 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 1 |
| 20 | Left | 0 | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 1 |
| | Right | 0 | 0 | 1 | 0 | 2 | 0 | 1 | 0 | 2 |

**Table II.ii. Froxfield Miniature Pigs** (n = 8). The numbers of papillary types found in upper pole, mid-zone and lower pole of 16 normal kidneys.

| Pig | | Simple & Compound Papillae Type I (UPPER) | II (UPPER) | III (UPPER) | Simple & Compound Papillae Type I (MIDDLE) | II (MIDDLE) | III (MIDDLE) | Simple & Compound Papillae Type I (LOWER) | II (LOWER) | III (LOWER) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Left | 0 | 0 | 2 | 1 | 0 | 1 | 0 | 1 | 2 |
| | Right | 0 | 1 | 1 | 5 | 0 | 0 | 1 | 1 | 1 |
| 2 | Left | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| | Right | 0 | 0 | 1 | 1 | 2 | 1 | 0 | 2 | 0 |
| 3 | Left | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| | Right | 0 | 0 | 1 | 2 | 0 | 1 | 0 | 1 | 0 |
| 4 | Left | 2 | 0 | 1 | 4 | 0 | 0 | 0 | 2 | 0 |
| | Right | 0 | 2 | 0 | 4 | 0 | 0 | 2 | 0 | 0 |
| 5 | Left | 0 | 1 | 2 | 5 | 1 | 0 | 0 | 1 | 1 |
| | Right | 2 | 0 | 1 | 4 | 0 | 0 | 0 | 1 | 1 |
| 6 | Left | 0 | 0 | 2 | 6 | 0 | 0 | 2 | 0 | 1 |
| | Right | 0 | 0 | 2 | 3 | 3 | 0 | 3 | 0 | 1 |
| 7 | Left | 0 | 0 | 1 | 0 | 3 | 1 | 1 | 0 | 1 |
| | Right | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 0 | 1 |
| 8 | Left | 0 | 0 | 1 | 4 | 0 | 1 | 0 | 3 | 0 |
| | Right | 0 | 0 | 1 | 8 | 0 | 0 | 2 | 0 | 1 |

**Table II.iii. Gottingen Miniature Pigs** (n = 30). The numbers of papillary types found in upper, mid-zone and lower pole of 59 normal kidneys.

| Pig | | Simple & Compound Papillae Type I | II | III | Simple & Compound Papillae Type I | II | III | Simple & Compound Papillae Type I | II | III |
|---|---|---|---|---|---|---|---|---|---|---|
| | | UPPER | | | MIDDLE | | | LOWER | | |
| 1 | Left | 4 | 0 | 0 | 3 | 0 | 0 | 5 | 0 | 0 |
| | Right | 2 | 1 | 0 | 5 | 0 | 0 | 1 | 1 | 0 |
| 2 | Left | 0 | 1 | 1 | 4 | 1 | 0 | 0 | 2 | 1 |
| | Right | 0 | 1 | 1 | 3 | 0 | 0 | 3 | 2 | 0 |
| 3 | Left | 4 | 0 | 1 | 7 | 0 | 0 | 7 | 0 | 1 |
| 4 | Left | 2 | 1 | 1 | 5 | 0 | 0 | 6 | 0 | 1 |
| | Right | 5 | 0 | 1 | 8 | 0 | 0 | 7 | 0 | 0 |
| 5 | Left | 4 | 0 | 0 | 2 | 0 | 0 | 2 | 1 | 1 |
| | Right | 3 | 0 | 1 | 3 | 0 | 1 | 4 | 0 | 1 |
| 6 | Left | 3 | 0 | 1 | 4 | 0 | 0 | 3 | 0 | 1 |
| | Right | 2 | 0 | 1 | 2 | 0 | 1 | 4 | 0 | 0 |
| 7 | Left | 2 | 0 | 1 | 5 | 0 | 0 | 3 | 0 | 0 |
| | Right | 4 | 0 | 1 | 5 | 0 | 0 | 1 | 1 | 0 |
| 8 | Left | 2 | 1 | 0 | 8 | 0 | 0 | 3 | 0 | 0 |
| | Right | 3 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 |
| 9 | Left | 1 | 0 | 1 | 1 | 1 | 0 | 5 | 1 | 0 |
| | Right | 3 | 1 | 0 | 4 | 0 | 0 | 2 | 0 | 1 |
| 10 | Left | 0 | 3 | 0 | 2 | 0 | 0 | 4 | 1 | 0 |
| | Right | 2 | 1 | 0 | 3 | 0 | 0 | 2 | 1 | 0 |
| 11 | Left | 1 | 1 | 1 | 4 | 0 | 0 | 4 | 0 | 0 |
| | Right | 1 | 1 | 0 | 4 | 0 | 0 | 4 | 0 | 0 |
| 12 | Left | 1 | 1 | 1 | 3 | 0 | 0 | 2 | 0 | 1 |
| | Right | 1 | 0 | 1 | 4 | 0 | 0 | 1 | 1 | 0 |
| 13 | Left | 3 | 2 | 0 | 4 | 0 | 0 | 1 | 2 | 0 |
| | Right | 0 | 1 | 1 | 5 | 0 | 0 | 2 | 0 | 1 |
| 14 | Left | 2 | 0 | 1 | 3 | 0 | 0 | 2 | 0 | 0 |
| | Right | 3 | 0 | 0 | 3 | 0 | 0 | 2 | 0 | 1 |
| 15 | Left | 2 | 0 | 1 | 5 | 0 | 0 | 1 | 1 | 0 |
| | Right | 5 | 0 | 1 | 2 | 0 | 0 | 2 | 0 | 0 |

Continued .../.

Table II.iii. (Continued)

| Pig | | Simple & Compound Papillae Type I | II | III | Simple & Compound Papillae Type I | II | III | Simple & Compound Papillae Type I | II | III |
|---|---|---|---|---|---|---|---|---|---|---|
| | | UPPER | | | MIDDLE | | | LOWER | | |
| 16 | Left | 6 | 0 | 0 | 3 | 0 | 0 | 7 | 0 | 0 |
| | Right | 6 | 1 | 0 | 5 | 1 | 0 | 5 | 0 | 0 |
| 17 | Left | 6 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 |
| | Right | 3 | 1 | 0 | 7 | 0 | 0 | 3 | 0 | 0 |
| 18 | Left | 5 | 0 | 0 | 4 | 0 | 0 | 2 | 0 | 0 |
| | Right | 3 | 1 | 0 | 3 | 0 | 0 | 3 | 0 | 0 |
| 19 | Left | 7 | 0 | 0 | 3 | 0 | 0 | 5 | 0 | 0 |
| | Right | 4 | 0 | 0 | 2 | 0 | 0 | 7 | 0 | 0 |
| 20 | Left | 0 | 0 | 2 | 6 | 0 | 0 | 4 | 0 | 0 |
| | Right | 3 | 0 | 1 | 4 | 0 | 0 | 1 | 2 | 0 |
| 21 | Left | 5 | 0 | 0 | 5 | 0 | 0 | 4 | 0 | 0 |
| | Right | 2 | 1 | 0 | 3 | 1 | 0 | 2 | 1 | 0 |
| 22 | Left | 9 | 0 | 0 | 3 | 0 | 0 | 6 | 0 | 0 |
| | Right | 6 | 0 | 0 | 5 | 0 | 0 | 8 | 0 | 0 |
| 23 | Left | 1 | 1 | 0 | 4 | 0 | 0 | 7 | 0 | 0 |
| | Right | 1 | 2 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |
| 24 | Left | 3 | 1 | 0 | 6 | 0 | 0 | 4 | 0 | 0 |
| | Right | 4 | 1 | 0 | 4 | 0 | 0 | 5 | 0 | 0 |
| 25 | Left | 3 | 0 | 0 | 5 | 1 | 0 | 3 | 1 | 0 |
| | Right | 4 | 0 | 0 | 5 | 0 | 0 | 4 | 0 | 0 |
| 26 | Left | 2 | 1 | 0 | 6 | 0 | 0 | 4 | 0 | 0 |
| | Right | 3 | 0 | 0 | 6 | 0 | 0 | 5 | 0 | 0 |
| 27 | Left | 0 | 1 | 0 | 6 | 0 | 0 | 2 | 1 | 0 |
| | Right | 0 | 0 | 2 | 5 | 0 | 0 | 2 | 0 | 1 |
| 28 | Left | 3 | 0 | 0 | 7 | 0 | 0 | 2 | 1 | 0 |
| | Right | 6 | 1 | 0 | 4 | 0 | 0 | 6 | 0 | 0 |
| 29 | Left | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 1 | 0 |
| | Right | 4 | 0 | 0 | 4 | 0 | 0 | 7 | 0 | 0 |
| 30 | Left | 1 | 0 | 1 | 5 | 0 | 0 | 2 | 0 | 2 |
| | Right | 2 | 0 | 2 | 4 | 0 | 0 | 1 | 0 | 1 |

**Table III.i.** **Pigs with solitary kidneys followed early.** UV/P and C-slope $^{51}$Cr-EDTA clearance values.

| Pig | Body Weight (kg) | Clearance (ml/min) UV/P | C- slope |
|---|---|---|---|
| 1 | 6.0 | 19 | 22 |
| | 8.7 | 30 | 29 |
| | 11.7 | 49 | 40 |
| | 17.5 | 55 | 58 |
| | 33.7 | 85 | 96 |
| | 42.0 | 116 | 122 |
| 2 | 3.8 | 12 | 12 |
| | 6.2 | 17 | 22 |
| | 8.9 | 28 | 32 |
| | 11.4 | 46 | 46 |
| | 14.9 | 62 | 51 |
| | 17.5 | 58 | 61 |
| | 23.8 | 63 | 78 |
| | 30.5 | 77 | 99 |
| | 43.0 | 104 | 140 |
| 3 | 2.5 | 4 | 6 |
| | 4.4 | 9 | 10 |
| | 6.2 | 15 | 15 |
| | 8.2 | 24 | 27 |
| | 14.9 | 36 | 40 |
| | 17.4 | 47 | 52 |
| | 23.7 | 42 | 55 |
| | 31.5 | 44 | 66 |
| | 34.0 | 65 | 80 |

**Table III.ii.** **Pigs with paired kidneys followed early.** UV/P and C-slope $^{51}$Cr-EDTA clearance values.

| Pig | Body Weight (kg) | Clearance (ml/min) UV/P | C- slope |
|---|---|---|---|
| 4 | 2.9 | 8 | 9 |
| | 4.8 | 14 | 20 |
| | 7.1 | 29 | 16 |
| | 10.0 | 49 | 41 |
| | 11.5 | 51 | 48 |
| | 14.2 | 51 | 56 |
| | 21.1 | 74 | 84 |
| | 28.1 | 79 | 91 |
| | 38.0 | 103 | 125 |
| 5 | 3.0 | 11 | 9 |
| | 4.7 | 16 | 19 |
| | 6.8 | 29 | 37 |
| | 8.4 | 39 | 44 |
| | 14.7 | 50 | 56 |
| | 18.8 | 62 | 66 |
| | 26.2 | 67 | 82 |
| | 32.5 | 78 | 72 |
| | 38.8 | 85 | 100 |
| 6 | 3.3 | 10 | 8 |
| | 5.2 | 15 | 15 |
| | 7.5 | 25 | 32 |
| | 9.6 | 36 | 46 |
| | 17.1 | 49 | 56 |
| | 22.0 | 65 | 77 |
| | 28.0 | 70 | 83 |
| | 36.5 | 81 | 85 |
| | 41.0 | 89 | 100 |

**Table III.iii. Pigs with solitary kidneys followed late. UV/P and C-slope $^{51}$Cr-EDTA clearance values.**

| Pig | Body Weight (kg) | Clearance (ml/min) UV/P | C- slope |
|---|---|---|---|
| 7 | 14.7 | 40 | 41 |
| | 21.6 | 53 | 71 |
| | 28.2 | 47 | 53 |
| | 35.0 | 60 | 70 |
| | 44.0 | 77 | 87 |
| | 50.5 | 76 | 100 |
| | 57.0 | 93 | 112 |
| 8 | 16.1 | 48 | 48 |
| | 22.4 | 57 | 68 |
| | 28.4 | 54 | 57 |
| | 34.0 | 76 | 77 |
| | 44.6 | 77 | 99 |
| | 48.5 | 89 | 116 |
| | 53.3 | 111 | 130 |
| 9 | 21.9 | 55 | 66 |
| | 32.0 | 79 | 104 |
| | 39.0 | 85 | 98 |
| | 47.0 | 102 | 104 |
| | 52.0 | 102 | 120 |
| | 60.0 | 104 | 141 |
| | 68.0 | 123 | 160 |
| 10 | 25.7 | 47 | 70 |
| | 36.0 | 76 | 102 |
| | 43.6 | 88 | 116 |
| | 51.3 | 111 | 120 |
| | 56.4 | 125 | 158 |
| | 67.0 | 123 | 160 |
| | 76.0 | 146 | 160 |

**Table III.iv. Pigs with paired kidneys followed late.** UV/P and C-slope $^{51}$Cr-EDTA clearance values.

| Pig | Body Weight (kg) | Clearance (ml/min) UV/P | C- slope |
|---|---|---|---|
| 11 | 15.5 | 52 | 61 |
| | 23.1 | 80 | 87 |
| | 27.5 | 69 | 77 |
| | 37.0 | 91 | 97 |
| | 47.4 | 95 | 102 |
| | 52.5 | 106 | 142 |
| | 57.0 | 121 | 134 |
| 12 | 13.8 | 42 | 46 |
| | 21.2 | 58 | 80 |
| | 33.5 | 71 | 92 |
| | 42.0 | 71 | 104 |
| | 51.5 | 91 | 122 |
| | 58.0 | 94 | 123 |
| 13 | 24.9 | 72 | 95 |
| | 36.0 | 100 | 144 |
| | 41.9 | 84 | 128 |
| | 50.4 | 105 | 115 |
| | 55.0 | 127 | 127 |
| | 64.0 | 143 | 171 |
| | 73.0 | 150 | 176 |
| 14 | 22.2 | 79 | 92 |
| | 32.0 | 88 | 104 |
| | 40.0 | 88 | 119 |
| | 47.8 | 116 | 112 |
| | 52.0 | 129 | 130 |
| | 60.0 | 139 | 160 |
| | 67.0 | 176 | 208 |

**Table IV.i.** Ages (weeks) and body weights (kg) of individual pigs at surgery, study week 0, the **Early** (weeks 4/5) investigation time, and at the beginning and end of the final (**Late**) investigation period (weeks 16 - 18 approx).

| Group | Pig | Surgery** | | Study Week 0 | | Early | | Late Period | |
|---|---|---|---|---|---|---|---|---|---|
| | | Age | Weight | Age | Weight | Age | Weight | Age | Weight |
| **Normal Bladder Function** | | | | | | | | | |
| A1 - Reflux | 279 | *5 | *6.0 | 7 | 7.0 | 11 | 13.0 | 22 - 26 | 27.0 - 35.5 |
| | 291 | 3 | 3.2 | 9 | 6.5 | 14 | 13.0 | 24 - 26 | 28.0 - 30.5 |
| | 296 | *8 | *6.0 | 9 | 8.0 | 13 | 12.5 | 26 -28 | 29.5 - 35.5 |
| | 297 | 5 | 4.9 | 11 | 7.5 | 15 | 13.0 | 27 - 29 | 30.5 - 34.0 |
| | 408 | 3 | 2.6 | 8 | 7.5 | 12 | 11.0 | 25 - 26 | 28.5 - 31.0 |
| | 419 | 6 | 5.5 | 9 | 8.0 | 13 | 13.5 | 26 - 28 | 35.0 - 40.0 |
| A2 - No Reflux | 278 | 5 | 5.0 | 7 | 7.0 | 11 | 12.7 | 25 - 26 | 30.0 - 33.0 |
| | 281 | 5 | 5.2 | 8 | 9.0 | 12 | 12.5 | 24 - 27 | 27.0 - 30.5 |
| | 290 | 3 | 3.1 | 7 | 7.0 | 11 | 12.5 | 23 - 25 | 33.5 - 33.0 |
| | 295 | 4 | 3.3 | 8 | 6.5 | 13 | 12.5 | 25 - 26 | 26.0 - 30.0 |
| | 299 | 5 | 6.5 | 7 | 7.5 | 11 | 12.0 | 24 - 26 | 30.0 - 32.5 |
| | 407 | 3 | 2.6 | 6 | 7.5 | 12 | 11.0 | 23 - 25 | 28.5 - 31.5 |
| | 418 | 6 | 5.8 | 9 | 8.0 | 13 | 12.5 | 26 - 28 | 31.5 - 35.0 |
| **Abnormal Bladder Function** | | | | | | | | | |
| B1 - Reflux | 286 | 2 | 2.4 | 7 | 7.5 | 11 | 11.5 | 22 - 25 | 33.5 - 36.6 |
| | 401 | 5 | 5.0 | 7 | 7.0 | 11 | 11.5 | 22 - 25 | 28.5 - 34.0 |
| | ***412 | 5 | 3.8 | 10 | 7.5 | 14 | 13.0 | 25 - 29 | 29.5 - 34.5 |
| | 413 | 2 | 2.1 | 8 | 7.5 | 13 | 13.0 | 22 - 24 | 31.0 - 33.0 |
| | 423 | 6 | 5.0 | 9 | 7.0 | 13 | 12.5 | 26 - 29 | 32.5 - 37.0 |
| B2 - No Reflux | 285 | 7 | 6.5 | 7 | 6.5 | 12 | 15.0 | 23 - 25 | 34.0 - 38.0 |
| | 287 | 2 | 1.8 | 9 | 8.0 | 13 | 13.0 | 25 - 27 | 33.5 - 35.0 |
| | 298 | 5 | 4.3 | 6 | 7.0 | 11 | 11.0 | 21 - 23 | 30.5 - 32.0 |
| | 410 | 5 | 3.1 | 9 | 6.5 | 14 | 12.5 | 24 - 26 | 26.5 - 29.5 |
| | 416 | 2 | 2.1 | 8 | 7.5 | 12 | 12.0 | 24 - 26 | 30.5 - 34.5 |

* At induction of VUR only - unilateral nephrectomy performed separately (Pig 279 age 7 weeks, weight 7 kg: Pig 296 age 4 weeks, weight 3.3 kg).

** Surgery - induction of VUR and unilateral nephrectomy except where indicated.

*** Pig excluded from analysis of late data.

**Table IV.ii.** Urodynamic characteristics recorded at the **Late** time for female pigs with normal bladder function (no bladder outflow obstruction).

| Group | Pig | UDCs | Increased EFP | Void Duration (s) | PVP (cm $H_2O$) | Residual | Grade |
|---|---|---|---|---|---|---|---|
| A1 - Reflux | 279 | No | No | 20 | 30 | No | 1 |
| | 291 | No | No | 28 | 18 | No | 1 |
| | 296 | No | No | 32 | 15 | No | 1 |
| | 297 | No | No | 20 | 20 | No | 1 |
| | 408 | No | No | 28 | 24 | No | 1 |
| | *419 | No | No | * | * | No | * |
| A2 - No Reflux | 278 | No | No | 24 | 25 | No | 1 |
| | 281 | No | No | 12 | 20 | No | 1 |
| | 290 | No | No | 40 | 30 | No | 1 |
| | 295 | No | No | 16 | 10 | No | 1 |
| | 299 | No | No | 56 | 15 | No | 1 |
| | 407 | No | No | 24 | 30 | No | 1 |
| | 418 | No | No | 28 | 0 | No | 1 |

* Lost data.
UDCs - unstable detrusor contractions; Increased EFP - rise in end fill pressure;
PVP - peak voiding pressure.

**Table IV.iii.** Urodynamics characteristics recorded at **Early, Interim** and **Late** times for male pigs with bladder outflow obstruction and abnormal bladder function.

| Group | Pig | Study Week | UDCs | Increased EFP | Void Duration (s) | PVP (cm $H_2O$) | Residual | Grade |
|---|---|---|---|---|---|---|---|---|
| B1 - Reflux | 286 | 4 | Yes | Yes | 70 | 50 | Yes | 4 |
| | | 12 | Yes | Yes | 90 | 70 | Yes | 4 |
| | | 17 | Yes | Yes | 70 | 70 | Yes | 4 |
| | 401 | 5 | No | No | 50 | 20 | No | 2 |
| | | 12 | No | No | 60 | 60 | Yes | 3 |
| | | 16 | No | Yes | 70 | 45 | Yes | 3 |
| | *412 | 4 | Yes | No | 25 | 30 | Yes | 5 |
| | | 12 | Yes | Yes | 50 | 65 | Yes | 6 |
| | | 17 | Yes | Yes | >150 | 120 | Yes | 7 |
| | 413 | 4 | No | No | 25 | 30 | No | 2 |
| | | 10 | No | No | 40 | 55 | Yes | 3 |
| | | 15 | No | Yes | 50 | 70 | Yes | 3 |
| | 423 | 4 | No | Yes | 30 | 45 | Yes | 3 |
| | | 11 | Yes | Yes | 30 | 55 | Yes | 4 |
| | | 18 | Yes | No | 90 | 55 | Yes | 4 |
| B2 - No Reflux | 285 | 5 | No | No | 35 | 35 | No | 2 |
| | | 10 | Yes | Yes | 55 | 40 | Yes | 3 |
| | | 17 | Yes | Yes | 40 | 55 | Yes | 4 |
| | 287 | 4 | No | No | 50 | 45 | No | 3 |
| | | 9 | No | No | 45 | 40 | No | 3 |
| | | 15 | No | Yes | 60 | 40 | No | 3 |
| | 298 | 4 | No | No | 10 | 30 | No | 2 |
| | | 10 | No | No | 15 | 20 | No | 2 |
| | | 15 | Yes | Yes | 50 | 45 | Yes | 4 |
| | 410 | 4 | No | Yes | 25 | 45 | No | 3 |
| | | 10 | Yes | Yes | 25 | 35 | Yes | 4 |
| | | **14 | Yes | Yes | 52 | 105 | Yes | 7 |
| | | 15 | No | No | 30 | 45 | No | 2 |
| | 416 | 4 | No | Yes | 50 | 30 | Yes | 3 |
| | | **11 | No | Yes | 200 | 110 | Yes | 7 |
| | | 16 | No | Yes | 50 | 55 | Yes | 4 |

* Pig excluded from analysis of late data.
** Ring enlarged after this study.
UDCs - unstable detrusor contractions; Increased EFP - rise in end fill pressure;
PVP - peak voiding pressure.

Table IV.iv. $^{51}$Cr-EDTA glomerular filtration rate (ml/min and ml/min/kg body weight) at Early time.

| Group | Pig | Glomerular Filtration Rate ml/min 1 | 2 | Mean | ml/min/kg Mean |
|---|---|---|---|---|---|
| **Normal Bladder Function** | | | | | |
| A1 - Reflux | 279 | 38.2 | ** | 38.2 | 2.94 |
| | 291 | 63.2 | 63.8 | 63.6 | 4.89 |
| | 296 | 63.7 | 59.5 | 61.6 | 4.92 |
| | 297 | 48.8 | ** | 48.8 | 3.75 |
| | 408 | 44.8 | 41.3 | 43.1 | 3.92 |
| | 419 | 56.8 | ** | 56.8 | 4.21 |
| A2 - No Reflux | 278 | 58.2 | - | 58.2 | 4.58 |
| | 281 | 45.7 | 43.3 | 44.5 | 3.56 |
| | 290 | 52.7 | *90.0 | 52.7 | 4.22 |
| | 295 | *66.3 | 44.3 | 44.3 | 3.54 |
| | 299 | 39.3 | 34.2 | 36.8 | 3.07 |
| | 407 | 45.4 | 42.8 | 44.1 | 4.01 |
| | 418 | 52.3 | ** | 52.3 | 4.18 |
| **Abnormal Bladder Function** | | | | | |
| B1 - Reflux | 286 | 34.7 | 36.3 | 35.5 | 3.09 |
| | 401 | 47.7 | 34.2 | 41.0 | 3.73 |
| | 412 | 45.8 | 52.0 | 48.9 | 4.25 |
| | 413 | 52.7 | ** | 52.7 | 4.39 |
| | 423 | 62.2 | ** | 62.2 | 4.98 |
| B2 - No Reflux | 285 | 48.1 | 52.3 | 50.2 | 3.35 |
| | 287 | 35.4 | 54.0 | 44.7 | 3.44 |
| | 298 | 46.6 | 39.3 | 43.0 | 3.91 |
| | 410 | 47.4 | ** | 47.4 | 3.79 |
| | 416 | 67.2 | ** | 67.2 | 5.60 |

* Results excluded (low counts).
** Duplicate studies either not performed or abandoned for technical reasons.

Table IV.v. $^{51}$Cr-EDTA glomerular filtration rate at Interim times (weeks 8-10 and/or weeks 12-13).

| Group | Pig | Glomerular Filtration Rate (ml/min) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Weeks 8 - 10 | | | Weeks 12 - 13 | | |
| | | 1 | 2 | Mean | 1 | 2 | Mean |
| **Normal Bladder Function** | | | | | | | |
| A1 - Reflux | 279 | 47.8 | 42.8 | 45.3 | 60.9 | 45.2 | 53.1 |
| | 291 | - | - | - | - | - | - |
| | 296 | - | - | - | - | - | - |
| | 297 | 57.6 | 62.5 | 60.1 | - | - | - |
| | 408 | 96.8 | 78.7 | 87.8 | - | - | - |
| | 419 | 73.1 | 70.5 | 71.8 | 87.8 | 93.9 | 90.1 |
| A2 - No Reflux | 278 | * | 70.8 | 70.8 | 104 | 112 | 108 |
| | 281 | 52.7 | 58.1 | 55.4 | 64.5 | 63.2 | 63.9 |
| | 290 | 89.2 | 93.6 | 91.4 | 108 | 106.0 | 107 |
| | 295 | 59.1 | * | 59.1 | 65.2 | * | 65.2 |
| | 299 | 50.1 | 55.7 | 52.9 | 71.4 | 70.3 | 70.9 |
| | 407 | - | - | - | 101 | 111.0 | 106 |
| | 418 | 81.0 | 81.7 | 81.4 | 110 | 94.4 | 102 |
| **Abnormal Bladder Function** | | | | | | | |
| B1 - Reflux | 286 | 81.5 | 59.5 | 70.5 | - | - | - |
| | 401 | 63.8 | 62.4 | 63.1 | - | - | - |
| | 412 | 93.5 | 87.7 | 90.6 | - | - | - |
| | 413 | 83.1 | * | 83.1 | - | - | - |
| | 423 | 71.1 | 79.0 | 75.1 | - | - | - |
| B2 - No Reflux | 285 | 56.5 | 55.6 | 56.1 | 78.8 | 78.1 | 78.5 |
| | 287 | - | - | - | 99.8 | 95.1 | 97.5 |
| | 298 | 83.5 | 84.0 | 83.8 | - | - | - |
| | 410 | 77.7 | 87.4 | 83.8 | - | - | - |
| | 416 | 85.7 | 96.3 | 91.0 | - | - | - |

* Duplicate studies not performed or abandoned for technical reasons.

Table IV.vi. $^{51}$Cr-EDTA glomerular filtration rate (ml/min and ml/min/kg body weight at Late time.

| Group | Pig | Glomerular Filtration Rate ml/min | | | | ml/min/kg |
|---|---|---|---|---|---|---|
| | | 1 | 2 | *3 | Mean | Mean |
| **Normal Bladder Function** | | | | | | |
| A1 - Reflux | 279 | 113.6 | 84.3 | 86.5 | 94 | 2.82 |
| | 291 | 110.1 | 93.0 | - | 102 | 3.33 |
| | 296 | 101.2 | 98.8 | - | 100 | 2.99 |
| | 297 | 91.3 | 82.8 | 87.6 | 87 | 2.71 |
| | 408 | 98.4 | 102.9 | - | 101 | 3.41 |
| | 419 | 107.4 | 108.1 | - | 108 | 2.75 |
| A2 - No Reflux | 278 | 120.0 | 112.4 | - | 116 | 3.75 |
| | 281 | 80.0 | 85.6 | - | 83 | 2.71 |
| | 290 | 100.3 | 99.4 | 101.8 | 101 | 3.03 |
| | 295 | 96.2 | 110.2 | - | 103 | 3.44 |
| | 299 | 92.4 | 84.4 | - | 88 | 2.72 |
| | 407 | 101.2 | 110.5 | - | 106 | 3.41 |
| | 418 | 108.3 | 116.9 | 102.1 | 109 | 3.12 |
| **Abnormal Bladder Function** | | | | | | |
| B1 - Reflux | 286 | 86.0 | 90.0 | 92.2 | 90 | 2.56 |
| | 401 | 84.6 | 82.0 | 104.7 | 91 | 2.89 |
| | **412 | 72.1 | 72.3 | - | 72 | 2.29 |
| | 413 | 102.5 | 107.3 | - | 105 | 3.18 |
| | 423 | 121.9 | 101.8 | - | 116 | 3.34 |
| B2 - No Reflux | 285 | 76.8 | 82.9 | 89.2 | 83 | 2.34 |
| | 287 | 121.5 | 116.3 | - | 119 | 3.45 |
| | 298 | 123.0 | 130.5 | - | 127 | 3.96 |
| | 410 | 89.8 | 93.5 | - | 92 | 3.16 |
| | 416 | 124.3 | 135.7 | - | 130 | 3.76 |

* Third estimate made on occasions (see text 18.2.1.).
** Data excluded from analysis (Pig with renal scarring).

**Table IV.vii.** Maximal urinary osmolality at the Late time.

| Group | Pig | Osmolality (mosmol/kg $H_2O$) | | |
|---|---|---|---|---|
| | | 1 | 2 | Mean |
| **Normal Bladder Function** | | | | |
| A1 - Reflux | 279 | 945 | 946 | 946 |
| | 291 | 874 | 946 | 910 |
| | 296 | 807 | 876 | 842 |
| | 297 | 795 | 923 | 859 |
| | 408 | 933 | 933 | 933 |
| | 419 | 840 | 873 | 856 |
| A2 - No Reflux | 278 | 1069 | 1068 | 1069 |
| | 281 | 1065 | 1065 | 1065 |
| | 290 | 1211 | 1199 | 1205 |
| | 295 | 1218 | 1212 | 1215 |
| | 299 | 1068 | 1078 | 1073 |
| | 407 | 999 | 855 | 927 |
| | 418 | 981 | 919 | 950 |
| **Abnormal Bladder Function** | | | | |
| B1 - Reflux | 286 | 974 | 950 | 962 |
| | 401 | 888 | 885 | 887 |
| | *412 | 612 | 646 | 629 |
| | 413 | 959 | 1157 | 1058 |
| | 423 | 903 | 720 | 812 |
| B2 - No Reflux | 285 | 1043 | 1014 | 1029 |
| | 287 | 1320 | 1322 | 1321 |
| | 298 | 1141 | 1155 | 1148 |
| | 410 | 952 | 915 | 934 |
| | 416 | 1043 | 1014 | 1029 |

* Data excluded from analysis (renal scarring).

**Table IV.viii.** Renal uptake of $^{99m}$Tc - DMSA at **Early** and **Late** times.

| Group | Pig | Absolute Uptake DMSA (%) | | | |
|---|---|---|---|---|---|
| | | **Early** | **Late** | | |
| | | | 1 | 2 | Mean |
| **Normal Bladder Function** | | | | | |
| A1 - Reflux | 279 | 37.7 | 36.0 | 34.7 | 35.4 |
| | 291 | 38.1 | 38.3 | 39.1 | 38.8 |
| | 296 | 38.2 | 39.2 | * | 39.2 |
| | 297 | 37.7 | 40.8 | 39.6 | 40.2 |
| | 408 | 50.4 | 38.1 | 36.1 | 37.1 |
| | 419 | 44.6 | 40.0 | 40.3 | 40.2 |
| A2 - No Reflux | 278 | 45.6 | 43.3 | 41.8 | 42.6 |
| | 281 | 44.8 | 46.1 | 43.8 | 45.0 |
| | 290 | 43.7 | 42.3 | 42.1 | 42.2 |
| | 295 | 43.6 | 40.1 | 38.8 | 39.5 |
| | 299 | 40.1 | 46.0 | 47.3 | 46.7 |
| | 407 | 50.3 | 48.3 | 50.5 | 48.9 |
| | 418 | 45.1 | 41.5 | 38.9 | 40.2 |
| **Abnormal Bladder Function** | | | | | |
| B1 - Reflux | 286 | 33.5 | 33.5 | 36.8 | 35.2 |
| | 401 | 42.2 | 33.1 | 34.1 | 33.6 |
| | **412 | 39.5 | ** | ** | ** |
| | 413 | 36.3 | 43.0 | 45.0 | 44.0 |
| | 423 | 42.5 | 41.3 | 38.9 | 40.1 |
| B2 - No Reflux | 285 | 40.1 | 34.1 | 31.2 | 32.7 |
| | 287 | 40.0 | 46.1 | 41.9 | 44.0 |
| | 298 | 49.4 | 41.0 | 39.9 | 40.5 |
| | 410 | 40.4 | 44.1 | 40.3 | 42.2 |
| | 416 | 41.7 | * | 41.8 | 41.8 |

* No results due to technical problems.

** Pig with severe bladder outflow obstruction and renal scarring (**Late**).

**Table IV.ix.** Absolute renal uptake of $^{99m}$Tc - DMSA, 24 hours after the dose injection. Uptake calculated from the kidneys in place (*In Situ*)[1] and from the same kidneys after their isolation at post mortem (*Ex Situ*)[2].

| Group | Pig* | Absolute Uptake DMSA (%) | |
|---|---|---|---|
| | | *In Situ* | *Ex Situ* |
| **Normal Bladder Function** | | | |
| A1 - Reflux | 291 | 39.1 | 36.5 |
| | 296 | 42.4 | 39.7 |
| | 297 | 39.3 | 40.4 |
| | 408 | 34.3 | 34.0 |
| | 419 | 41.3 | 40.2 |
| A2 - No Reflux | 290 | 42.5 | 43.5 |
| | 295 | 39.3 | 35.4 |
| | 299 | 48.6 | 50.5 |
| | 407 | 47.8 | 46.8 |
| | 418 | 39.3 | 40.6 |
| **Abnormal Bladder Function** | | | |
| B1 - Reflux | 401 | 34.7 | 38.6 |
| | 412 | 35.7 | 35.7 |
| | 413 | 45.0 | 42.7 |
| | 423 | 39.3 | 37.1 |
| B2 - No Reflux | 287 | 43.7 | 43.3 |
| | 298 | 39.9 | 39.2 |
| | 410 | 40.8 | 40.8 |
| | 416 | 40.0 | 42.6 |

[1] *In Situ* absolute uptake calculated from renal counts corrected for background, decay and attenuation by tissue (see 13.4.1).

[2] *Ex Situ* absolute uptake calculated from renal counts corrected for background and decay.

* Not all pigs were included in this aspect of the study (see 18.2.3)

**Table IV.x.** Kidney weights (in g and g/kg body weight) at the end of the experiment (**Late**).

| Group | Pig | Kidney Weight | |
|---|---|---|---|
| | | g | g/kg |
| **Normal Bladder Function** | | | |
| A1 - Reflux | 279 | 101 | 2.85 |
| | 291 | 108 | 3.54 |
| | 296 | 110 | 3.14 |
| | 297 | 90 | 2.65 |
| | 408 | 101 | 3.26 |
| | 419 | 117 | 2.89 |
| A2 - No Reflux | 278 | 114 | 3.46 |
| | 281 | 105 | 3.44 |
| | 290 | 109 | 3.30 |
| | 295 | 97 | 3.23 |
| | 299 | 101 | 3.11 |
| | 407 | 129 | 4.10 |
| | 418 | 115 | 3.29 |
| **Abnormal Bladder Function** | | | |
| B1 - Reflux | 286 | 144 | 4.00 |
| | 401 | 123 | 3.62 |
| | *412 | 140 | 4.05 |
| | 413 | 120 | 3.64 |
| | 423 | 121 | 3.27 |
| B2 - No Reflux | 285 | 123 | 3.24 |
| | 287 | 164 | 4.69 |
| | 298 | 172 | 5.36 |
| | 410 | 93 | 3.15 |
| | 416 | 127 | 3.68 |

* Data excluded from analysis (renal scarring).

**Table V.i.** Ages (weeks) and body weights (kg) of individual pigs at surgery, study week 0, the **Early** (week 4) investigation time and the beginning and end of the final (**Late**) investigation period (weeks 16-18 approx).

| Group | Pig | Surgery * | | Study Week 0 | | Early | | Late Period | |
|---|---|---|---|---|---|---|---|---|---|
| | | Age | Weight | Age | Weight | Age | Weight | Age | Weight |
| **Normal Bladder Function** | | | | | | | | | |
| A1 - Unilateral Reflux | | | | | | | | | |
| | ** 274 | 5 | 7.5 | 6 | 8.0 | 10 | 16.0 | 20 - 22 | 31.0 - 36.0 |
| | ** 275 | 5 | 6.0 | 7 | 7.5 | 11 | 12.5 | 22 - 24 | 24.5 - 26.0 |
| | 280 | 5 | 5.3 | 7 | 7.0 | 11 | 12.5 | 25 - 26 | 37.0 - 41.5 |
| | 289 | 2 | 1.7 | 8 | 7.7 | 10 | 13.0 | 23 - 26 | 33.5 - 37.0 |
| | 293 | 7 | 6.5 | 8 | 7.5 | 12 | 12.0 | 24 - 27 | 30.0 - 31.0 |
| | 403 | 6 | 7.0 | 7 | 7.5 | 11 | 11.5 | 21 - 23 | 30.5 - 32.0 |
| | 411 | 3 | 2.4 | 10 | 7.5 | 13 | 12.5 | 25 - 27 | 28.0 - 31.0 |
| A2 - No Reflux | | | | | | | | | |
| | 277 | | | 6 | 7.5 | 10 | 13.0 | 22 - 23 | 34.5 - 34.0 |
| | 282 | | | 6 | 8.0 | 10 | 13.5 | 21 - 24 | 31.5 - 35.0 |
| | 294 | | | 7 | 7.0 | 11 | 12.5 | 24 - 25 | 30.0 - 32.0 |
| | 405 | | | 5 | 6.5 | 9 | 11.5 | 20 - 22 | 33.5 - 35.0 |
| | 409 | | | 9 | 7.5 | 13 | 10.0 | 29 - 30 | 30.0 - 32.0 |
| **Abnormal Bladder Function** | | | | | | | | | |
| B1 - Unilateral Reflux | | | | | | | | | |
| | 283 | 5 | 6.8 | 7 | 7.5 | 11 | 12.5 | 23 - 25 | 34.0 - 39.0 |
| | ** 402 | 5 | 4.0 | 8 | 7.0 | 13 | 11.5 | 24 - 26 | 33.5 - 35.5 |
| | ** 414 | 2 | 1.9 | 8 | 7.5 | 12 | 11.5 | 24 - 25 | 30.0 - 32.0 |
| | ** 417 | 2 | 1.9 | 6 | 7.0 | 10 | 11.5 | 21 - 23 | 31.0 - 31.5 |
| | 421 | 5 | 3.2 | 9 | 7.5 | 13 | 10.5 | 27 - 29 | 33.5 - 37.5 |
| | 425 | 5 | 4.5 | 10 | 7.0 | 14 | 10.5 | 27 - 28 | 27.5 - 30.0 |
| | 426 | 5 | 4.5 | 10 | 6.5 | 14 | 10.0 | 27 - 28 | 27.0 - 30.0 |
| | 427 | 5 | 4.5 | 11 | 8.0 | 15 | 11.5 | 28 - 29 | 28.0 - 29.0 |
| | 428 | 5 | 5.5 | 11 | 8.5 | 15 | 11.0 | 28 - 29 | 26.0 - 27.5 |
| | 429 | 5 | 5.5 | 8 | 7.5 | 12 | 9.5 | 24 - 26 | 25.0 - 27.0 |
| B2 - No Reflux | | | | | | | | | |
| | 292 | 2 | 3.4 | 5 | 6.5 | 10 | 14.5 | 20 - 22 | 31.5 - 32.0 |
| | 400 | 3 | 2.8 | 5 | 7.5 | 9 | 12.5 | 20 - 21 | 31.0 - 31.5 |
| | 415 | 2 | 1.5 | 9 | 8.0 | 13 | 16.0 | 24 - 26 | 31.5 - 35.5 |
| | 420 | 5 | 4.1 | 8 | 7.5 | 13 | 14.5 | 26 - 28 | 34.0 - 39.0 |
| | 422 | 5 | 2.7 | 8 | 7.5 | 12 | 11.5 | 26 - 28 | 35.0 - 39.0 |
| | 424 | 5 | 6.0 | 8 | 7.5 | 12 | 12.0 | 25 - 26 | 34.0 - 35.0 |
| | 430 | 6 | 5.5 | 14 | 7.0 | 18 | 13.0 | 29 - 31 | 29.0 - 32.0 |

* Surgery: Group B1 at time of creation of VUR (implantation of urethral ring normally 10-14 days earlier); Group B2 implantation of urethral ring.

** Pigs excluded from data analysis (see 17.3.).

**Table V.ii.** Urodynamic characteristics recorded at the **Late** time for pigs with normal bladder function (no bladder outflow obstruction).

| Group | Pig | UDCs | Increased EFP | Void Duration(s) | PVP ($cmH_2O$) | Residual | Grade |
|---|---|---|---|---|---|---|---|
| **Normal Bladder Function** | | | | | | | |
| A1 - Unilateral Reflux | * 274 | No | No | 8 | 25 | No | 1 |
| | * 275 | No | No | 60 | 25 | No | 2 |
| | 280 | No | No | 15 | 30 | No | 1 |
| | 289 | No | No | 20 | 15 | No | 1 |
| | 293 | No | No | 25 | 30 | No | 1 |
| | 403 | No | No | 30 | 10 | No | 1 |
| | 411 | No | No | 30 | 25 | No | 1 |
| A2 - No Reflux | 277 | No | No | 70 | 25 | No | 2 |
| | 282 | No | No | 5 | 30 | No | 1 |
| | 294 | No | No | 15 | 20 | No | 1 |
| | 405 | No | No | 5 | 10 | No | 1 |
| | 409 | No | No | 10 | 5 | No | 1 |

* Pigs excluded from study (see 17.3.)
UDCs - unstable detrusor contractions; Increased EFP - rise in end fill pressure;
PVP - peak voiding pressure

**Table V.iii.** Urodynamic characteristics recorded at **Early, Interim** and **Late** times. Pigs in Group B1 (unilateral reflux) with bladder outflow obstruction and abnormal bladder function.

| Group | Pig | Study Week | UDCs | Increased EFP | Void Duration(s) | PVP ($cmH_2O$) | Residual | Grade |
|---|---|---|---|---|---|---|---|---|
| **Abnormal bladder function** | | | | | | | | |
| B1-Unilateral Reflux | 283 | 4 | Yes | Yes | 50 | 55 | No | 4 |
| | | 12 | Yes | Yes | 115 | 55 | Yes | 4 |
| | | 16 | Yes | No | 50 | 70 | Yes | 3 |
| | *402 | 4 | Yes | Yes | 55 | 40 | No | 3 |
| | | 10 | Yes | Yes | >100 | 65 | Yes | 5/6 |
| | | 17 | Yes | Yes | >100 | 65 | Yes | 5/6 |
| | *414 | 4 | No | Yes | 35 | 55 | No | 4 |
| | | **10 | Yes | Yes | >100 | >100 | Yes | 6 |
| | | 12 | No | No | 35 | 55 | No | 2 |
| | | 16 | Yes | Yes | 90 | 100 | Yes | 4 |
| | *417 | 5 | No | No | 30 | 50 | Yes | 2 |
| | | 10 | No | No | 35 | 55 | Yes | 2 |
| | | 15 | No | Yes | 35 | 75 | Yes | 3 |
| | 421 | 4 | No | Yes | 40 | 35 | Yes | 3 |
| | | 11 | Yes | Yes | 50 | 55 | Yes | 3 |
| | | 18 | No | Yes | 70 | 75 | Yes | 4 |
| | 425 | 4 | No | No | 20 | 40 | Yes | 2 |
| | | 10 | No | Yes | 25 | 40 | Yes | 3 |
| | | 18 | No | Yes | 30 | 40 | Yes | 3 |
| | 426 | 4 | Yes | No | < 10 | 25 | No | 5 |
| | | 10 | No | No | 25 | 40 | Yes | 2 |
| | | 17 | No | Yes | 40 | 45 | Yes | 3 |
| | 427 | 4 | No | No | 20 | 40 | No | 2 |
| | | 10 | No | Yes | 40 | 40 | Yes | 3 |
| | | 18 | No | Yes | 20 | 50 | Yes | 3 |
| | 428 | 4 | No | No | 15 | 30 | No | 2 |
| | | 10 | No | No | 25 | 40 | Yes | 3 |
| | | 17 | No | Yes | 85 | 40 | Yes | 3 |
| | 429 | 4 | No | Yes | 45 | 40 | Yes | 3 |
| | | 10 | No | Yes | 60 | 40 | Yes | 3 |
| | | 16 | No | Yes | 50 | 40 | Yes | 3 |

* Pigs excluded from data analysis (See 17.3).

** Ring enlarged after this study.

UDCs - unstable detrusor contractions; Increased EFP - rise in end fill pressure;
PVP - peak voiding pressure.

**Table V.iv.** Urodynamic characteristics recorded at **Early, Interim** and **Late** times. Pigs in Group B2 (no reflux) with bladder outflow obstruction and abnormal bladder function.

| Group | Pig | Study Week | UDCs | Increased EFP | Void Duration(s) | PVP ($cmH_2O$) | Residual | Grade |
|---|---|---|---|---|---|---|---|---|
| **Abnormal Bladder Function** | | | | | | | | |
| B2 - No Reflux | 292 | 7 | No | No | 25 | 55 | No | 2 |
| | | 11 | No | No | >100 | 60 | Yes | 3 |
| | | 16 | No | No | 40 | 75 | Yes | 3 |
| | 400 | 4 | Yes | Yes | 20 | 50 | No | 4 |
| | | 10 | Yes | Yes | 50 | 60 | Yes | 4 |
| | | 16 | No | No | 20 | 65 | Yes | 3 |
| | 415 | 4 | No | No | 25 | 40 | No | 3 |
| | | 10 | Yes | Yes | 50 | 55 | Yes | 3 |
| | | 16 | Yes | No | 60 | 70 | Yes | 4 |
| | * 420 | 4 | Yes | Yes | 45 | 35 | No | 3 |
| | | 17 | Yes | Yes | 90 | 55 | No | 4 |
| | 422 | 5 | No | No | 60 | 37 | No | 3 |
| | | 11 | No | Yes | 95 | 55 | Yes | 3 |
| | | 18 | No | Yes | 100 | 55 | Yes | 3 |
| | 424 | 4 | No | No | 20 | 30 | No | 2 |
| | | 10 | No | No | 45 | 55 | Yes | 3 |
| | | 18 | No | Yes | 45 | 50 | Yes | 3 |
| | 430 | 4 | Yes | No | < 10 | 15 | No | 5 |
| | | 10 | No | Yes | 80 | 40 | Yes | 3 |
| | | 17 | No | Yes | 40 | 55 | Yes | 3 |

* Interim urodynamics not analysed - data lost.
UDCs - unstable detrusor contractons; Increased EFP - rise in end fill pressure;
PVP - peak voiding pressure.

**Table V.v.** $^{51}$Cr-EDTA glomerular filtraton rate (ml/min and ml/min/kg body weight) at Late time.

| Group | Pig | Glomerular Filtration Rate ml/min | | | ml/min/kg | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | Mean | 1 | 2 | Mean |
| **Normal Bladder Function** | | | | | | | |
| A1 - Unilateral Reflux | ** 274 | 112 | 113 | 113 | 3.50 | 3.43 | 3.47 |
| | ** 275 | 89 | 76 | 83 | 3.55 | 3.05 | 3.30 |
| | 280 | 124 | 132 | 128 | 3.35 | 3.56 | 3.45 |
| | 289 | 123 | 128 | 126 | 3.10 | 3.36 | 3.22 |
| | 293 | 117 | 107 | 112 | 4.02 | 3.67 | 3.84 |
| | 403 | 105 | 111 | 108 | 3.28 | 3.46 | 3.37 |
| | 411 | 136 | 126 | 131 | 4.52 | 4.21 | 4.37 |
| A2 - No Reflux | | | | | | | |
| | 277 | 105 | 122 | 112 | 3.04 | 3.52 | 3.28 |
| | 282 | 114 | 108 | 111 | 3.61 | 3.32 | 3.46 |
| | 294 | 107 | 110 | 109 | 3.33 | 3.43 | 3.38 |
| | 405 | 136 | 121 | 129 | 4.01 | 3.55 | 3.78 |
| | 409 | 144 | 141 | 143 | 4.79 | 4.39 | 4.59 |
| **Abnormal Bladder Function** | | | | | | | |
| B1 - Unilateral Reflux | 283 | 95 | 114 | 105 | 2.79 | 3.08 | 2.93 |
| | ** 402 | 109 | 106 | 108 | 3.25 | 3.17 | 3.21 |
| | ** 414 | 102 | 109 | 106 | 3.39 | 3.40 | 3.40 |
| | ** 417 | 137 | *** | 137 | 4.34 | *** | 4.34 |
| | 421 | 109 | 115 | 112 | 3.07 | 3.06 | 3.07 |
| | 425 | 97 | 118 | 108 | 3.52 | 3.93 | 3.73 |
| | 426 | 133 | *** | 133 | 4.43 | *** | 4.43 |
| | 427 | 97 | 80 | 89 | 3.46 | 2.75 | 3.11 |
| | 428 | 110* | 89 | 89 | *** | 3.23 | 3.23 |
| | 429 | 88 | 91 | 90 | 3.52 | 3.42 | 3.47 |
| B2 - No Reflux | | | | | | | |
| | 292 | 140 | 135 | 138 | 4.43 | 4.28 | 4.36 |
| | 400 | 127* | 110 | 110 | *** | 3.49 | 3.49 |
| | 415 | 134 | 128 | 131 | 4.26 | 3.59 | 3.93 |
| | 420 | 112 | 116 | 114 | 3.03 | 2.98 | 3.00 |
| | 422 | 140 | 160 | 150 | 4.02 | 4.36 | 4.19 |
| | 424 | 146 | 118 | 132 | 4.29 | 3.37 | 4.83 |
| | 430 | 85 | 83 | 84 | 2.93 | 2.76 | 2.85 |

* Results excluded (low counts)

** Pigs excluded from data analysis (see 17.3).

*** Data unavailable due to technical problems

**Table V.vi.** Maximal urinary osmolality at the **Late** time.

| Group | Pig | Osmolality (mosmol/kg/$H_2O$) 1 | 2 | Mean |
|---|---|---|---|---|
| **Normal Bladder Function** | | | | |
| A1 - Unilateral Reflux | * 274 | ** 952 | | |
| | * 275 | 1012 | | |
| | 280 | 1094 | | |
| | 289 | 1185 | | |
| | 293 | 1258 | | |
| | 403 | 1141 | | |
| | 411 | 1006 | | |
| A2 - No Reflux | 277 | 1000 | | |
| | 282 | 1165 | | |
| | 294 | 1267 | | |
| | 405 | 1120 | | |
| | 409 | 1144 | | |
| **Abnormal Bladder Function** | | | | |
| B2 - Unilateral Reflux | 283 | 1011 | 1009 | 1010 |
| | * 402 | 1115 | 1000 | 1058 |
| | * 414 | 985 | 940 | 963 |
| | * 417 | 960 | 773 | 867 |
| | 421 | 941 | 927 | 934 |
| | 425 | 1173 | 1037 | 1105 |
| | 426 | 1134 | 1171 | 1152 |
| | 427 | 1230 | 1240 | 1235 |
| | 428 | 1249 | *** | 1249 |
| | 429 | 1231 | 1155 | 1193 |
| B2 - No Reflux | 292 | 1146 | *** | 1146 |
| | 400 | 1196 | *** | 1196 |
| | 415 | 949 | 926 | 938 |
| | 420 | 1067 | 995 | 1031 |
| | 421 | 1171 | 1110 | 1141 |
| | 424 | 1011 | 992 | 1002 |
| | 430 | 1185 | 1226 | 1206 |

* Pigs excluded from analysis (see 17.3.).

** Mean of 2 estimations (where maximum osmolality exceeded 1000 mosmol/kg/$H_2O$ a second estimate was not performed in groups A1 and A2).

*** Data unavailable: dehydration not maintained.

**Table V.vii.** Renal uptake of $^{99m}$Tc-DMSA for pigs in group A (normal bladder function). Absolute uptake by left (L) and right (R) kidneys and left relative uptake (100.L[L + R]) at **Early** and **Late** times.

| Group | Pig | Kidney | Absolute Uptake (%) Early | Late 1 | Late 2 | Late Mean | Relative Uptake (%) Early | Relative Uptake (%) Late |
|---|---|---|---|---|---|---|---|---|
| **Normal Bladder Function** | | | | | | | | |
| A1 - Unilateral Reflux | | | | | | | | |
| | *274 | L | 19.7 | 18.9 | 22.8 | 20.9 | 52.3 | 49.1 |
| | | R | 18.0 | 18.6 | 24.5 | 21.6 | | |
| | *275 | L | 23.2 | 20.4 | 21.8 | 21.1 | 52.7 | 49.6 |
| | | R | 20.8 | 20.9 | 21.8 | 21.4 | | |
| | 280 | L | ** | 20.4 | 17.2 | 18.8 | ** | 47.5 |
| | | R | ** | 21.8 | 19.7 | 20.8 | | |
| | 289 | L | 21.3 | 17.1 | 18.5 | 17.8 | 48.3 | 47.6 |
| | | R | 22.8 | 19.0 | 20.1 | 19.6 | | |
| | 293 | L | 17.8 | 20.2 | 20.0 | 20.1 | 50.9 | 50.3 |
| | | R | 17.2 | 18.8 | 21.0 | 19.9 | | |
| | 403 | L | 25.0 | 22.1 | 20.3 | 21.2 | 50.9 | 51.2 |
| | | R | 24.1 | 20.8 | 19.6 | 20.2 | | |
| | 411 | L | 23.9 | 20.3 | 22.1 | 21.2 | 55.2 | 48.3 |
| | | R | 19.4 | 22.0 | 23.4 | 22.7 | | |
| A2 - No Reflux | | | | | | | | |
| | 277 | L | 20.3 | 20.8 | 22.0 | 21.4 | 52.9 | 48.5 |
| | | R | 18.1 | 21.7 | 23.6 | 22.7 | | |
| | 282 | L | 19.9 | 18.4 | 21.9 | 20.2 | 50.0 | 49.2 |
| | | R | 19.9 | 19.5 | 22.1 | 20.8 | | |
| | 294 | L | 21.3 | 19.7 | 19.8 | 19.8 | 49.1 | 47.4 |
| | | R | 22.1 | 22.3 | 22.1 | 22.0 | | |
| | 405 | L | 23.3 | 25.4 | 20.6 | 23.0 | 53.2 | 50.2 |
| | | R | 20.5 | 23.9 | 21.7 | 22.8 | | |
| | 409 | L | 18.7 | 20.3 | 18.6 | 19.5 | 45.9 | 50.0 |
| | | R | 22.0 | 19.6 | 19.3 | 19.5 | | |

* Pigs excluded from data analysis (17.3.).

** Data unavailable due to technical problems.

**Table V.viii.** Renal uptake of $^{99m}$Tc-DMSA for pigs in group B (abnormal bladder function). Absolute uptake by left (L) and right (R) kidneys and left relative uptake (100.L/[L + R]) at **Early** and **Late** times.

| Group | Pig | Kidney | Absolute Uptake (%) Early | Late 1 | Late 2 | Late Mean | Relative Uptake (%) Early | Late |
|---|---|---|---|---|---|---|---|---|
| **Abnormal Bladder Function** | | | | | | | | |
| B1 - Unilateral Reflux | | | | | | | | |
| | 283 | L | 20.9 | 22.4 | 20.6 | 21.4 | 49.5 | 48.6 |
| | | R | 21.2 | 23.0 | 22.2 | 22.6 | | |
| | *402 | L | 18.6 | 17.6 | 18.1 | 17.9 | 47.2 | 46.7 |
| | | R | 20.8 | 20.3 | 20.4 | 20.4 | | |
| | *414 | L | 23.6 | 23.5 | 21.6 | 22.6 | 47.9 | 46.2 |
| | | R | 25.7 | 27.6 | 24.9 | 26.3 | | |
| | *417 | L | 19.6 | 19.5 | 18.3 | 18.9 | 41.1 | 38.3 |
| | | R | 28.1 | 30.8 | 29.9 | 30.4 | | |
| | 421 | L | 24.0 | 18.8 | 19.0 | 18.9 | 50.0 | 50.8 |
| | | R | 24.0 | 18.5 | 18.1 | 18.3 | | |
| | 425 | L | 21.5 | 25.0 | 27.1 | 26.1 | 49.4 | 50.2 |
| | | R | 22.0 | 24.9 | 26.9 | 25.9 | | |
| | 426 | L | 20.5 | 23.5 | ** | 23.5 | 50.4 | 50.8 |
| | | R | 20.2 | 22.8 | ** | 22.8 | | |
| | 427 | L | 23.3 | 19.5 | 20.6 | 20.1 | 46.5 | 46.2 |
| | | R | 26.8 | 23.3 | 23.5 | 23.4 | | |
| | 428 | L | 25.5 | 25.8 | 28.8 | 27.3 | 52.4 | 49.8 |
| | | R | 23.2 | 23.7 | 31.2 | 27.5 | | |
| | 429 | L | 22.5 | 22.1 | 26.6 | 24.4 | 47.5 | 49.5 |
| | | R | 24.9 | 24.2 | 25.6 | 24.9 | | |

Continued.../.

Table V.viii. Continued

| Group | Pig | Kidney | Absolute Uptake (%) Early | Late 1 | Late 2 | Late Mean | Relative Uptake (%) Early | Late |
|---|---|---|---|---|---|---|---|---|
| B2 - No Reflux | | | | | | | | |
| | 292 | L | 17.2 | 19.7 | 17.7 | 18.7 | 49.6 | 48.3 |
| | | R | 17.5 | 20.9 | 19.0 | 20.0 | | |
| | 400 | L | 24.0 | 21.9 | 22.6 | 22.3 | 50.7 | 50.5 |
| | | R | 23.3 | 21.0 | 22.8 | 21.9 | | |
| | 415 | L | 24.6 | 19.8 | 21.2 | 20.5 | 49.9 | 48.2 |
| | | R | 24.7 | 21.4 | 22.5 | 22.0 | | |
| | 420 | L | 19.0 | 17.8 | 17.5 | 17.7 | 49.7 | 47.8 |
| | | R | 19.2 | 19.2 | 19.3 | 19.3 | | |
| | 422 | L | 23.4 | 23.0 | 20.4 | 21.7 | 48.4 | 49.4 |
| | | R | 24.9 | 22.9 | 21.5 | 22.0 | | |
| | 424 | L | 20.8 | ** | 22.7 | 22.7 | 50.4 | 50.3 |
| | | R | 20.5 | ** | 22.4 | 22.4 | | |
| | 430 | L | 24.4 | 25.5 | 25.4 | 25.5 | 50.1 | 46.9 |
| | | R | 24.3 | 28.2 | 29.5 | 28.9 | | |

* Pigs excluded from data analysis (see 17.3.).
** Results excluded for technical reasons

**Table V.ix.** Absolute and left relative renal uptake of $^{99m}$Tc-DMSA, 24 hours after the dose injection. Uptake calculated from the kidneys in place (*In situ*)[1] and from the same kidneys after their isolation at *post mortem* (*Ex situ*)[2]. The estimated depth of the kidney below the skin surface is included. This was measured by ultrasound (US) and at *post mortem* (PM).

| Pig* | Kidney | Depth Estimate (mm) | | Absolute Uptake(%) | | Relative Uptake (%) | |
|---|---|---|---|---|---|---|---|
| | | US | PM | *In situ* | *Ex situ* | *In situ* | *Ex situ* |
| 294 | L | 72 | 69 | 20.5 | 20.9 | 49 | 51 |
| | R | 72 | 67 | 21.8 | 20.0 | | |
| 405 | L | 73 | 73 | 19.9 | 21.9 | 48 | 52 |
| | R | 73 | 72 | 21.3 | 20.1 | | |
| 409 | L | 70 | 70 | 18.2 | 21.0 | 48 | 50 |
| | R | 70 | 70 | 19.6 | 21.4 | | |
| 289 | L | 73 | 66 | 19.7 | 21.3 | 49 | 50 |
| | R | 73 | 75 | 20.6 | 22.3 | | |
| 293 | L | 72 | 72 | 16.9 | 17.4 | 47 | 50 |
| | R | 72 | 71 | 19.1 | 17.2 | | |
| 403 | L | 65 | 67 | 19.6 | 24.0 | 53 | 53 |
| | R | 65 | 66 | 17.7 | 21.6 | | |
| 411 | L | 66 | 77 | 21.0 | 22.7 | 48 | 50 |
| | R | 66 | 74 | 22.8 | 22.4 | | |
| 292 | L | 75 | 69 | 17.3 | 19.5 | 49 | 51 |
| | R | 75 | 71 | 18.9 | 19.8 | | |
| 400 | L | 65 | 68 | 22.6 | 23.3 | 49 | 51 |
| | R | 65 | 66 | 23.3 | 22.5 | | |
| 415 | L | 72 | 70 | 21.3 | 22.3 | 49 | 51 |
| | R | 72 | 68 | 22.1 | 21.5 | | |
| 420 | L | 75 | 70 | 17.0 | 16.3 | 48 | 50 |
| | R | 75 | 72 | 18.4 | 16.5 | | |

Continued.../.

Table V.ix. Continued.

| Pig* | Kidney | Depth Estimate (mm) US | PM | Absolute Uptake(%) *In situ* | *Ex situ* | Relative Uptake (%) *In situ* | *Ex situ* |
|---|---|---|---|---|---|---|---|
| 422 | L | 77 | 70 | 20.4 | 20.9 | 50 | 50 |
| | R | 77 | 70 | 20.9 | 20.8 | | |
| 424 | L | 62 | 70 | 23.8 | 22.7 | 51 | 50 |
| | R | 62 | 70 | 22.9 | 22.6 | | |
| 430 | L | 65 | 68 | 25.7 | 26.6 | 46 | 49 |
| | R | 65 | 68 | 30.3 | 27.6 | | |
| 402 | L | 75 | 74 | 18.1 | 19.0 | 47 | 47 |
| | R | 75 | 76 | 20.4 | 21.5 | | |
| 414 | L | 68 | 70 | 21.6 | 22.2 | 46 | 47 |
| | R | 68 | 70 | 25.7 | 24.6 | | |
| 417 | L | 70 | ** | 17.9 | 19.0 | 38 | 40 |
| | R | 70 | ** | 29.5 | 28.4 | | |
| 421 | L | 71 | 72 | 19.4 | 19.4 | 51 | 51 |
| | R | 71 | 70 | 18.4 | 18.3 | | |
| 425 | L | 66 | 71 | 28.0 | 15.9 | 50 | 50 |
| | R | 66 | 71 | 27.6 | 25.4 | | |
| 426 | L | 68 | 70 | 16.8 | 15.6 | 49 | 50 |
| | R | 68 | 70 | 17.5 | 15.4 | | |
| 427 | L | 64 | 70 | 21.3 | 21.7 | 46 | 48 |
| | R | 64 | 70 | 24.8 | 23.3 | | |
| 428 | L | 63 | 69 | 29.4 | 29.1 | 47 | 50 |
| | R | 63 | 70 | 32.6 | 28.8 | | |
| 429 | L | 62 | 63 | 27.8 | 26.3 | 52 | 52 |
| | R | 62 | 62 | 25.4 | 24.1 | | |

* Not all pigs were included in this aspect of the study (see 18.2.3.).

** Data not recorded.

1 *In situ* uptake calculated from renal counts corrected for background, decay and attenuation by tissue (13.4.1.).

2 *Ex situ* uptake calculated from renal counts corrected for background and decay.

**Table V.x.** Kidney weights at the end of the experiment (**Late**). Left (L) and right (R) kidney weights and left relative (100.L/[L + R]) kidney weight.

| Group | Pig | Kidney Weight (g) Left | Right | Left Relative (%) |
|---|---|---|---|---|
| **Normal Bladder Function** | | | | |
| A1 - Unilateral Reflux | * 274 | 77 | 71 | 52.0 |
| | * 275 | 72 | 67 | 51.8 |
| | 280 | 82 | 82 | 50.0 |
| | 289 | 57 | 55 | 50.8 |
| | 293 | 75 | 73 | 50.7 |
| | 403 | 58 | 52 | 52.7 |
| | 411 | 61 | 61 | 50.0 |
| A2 - No Reflux | 277 | 86 | 83 | 50.8 |
| | 282 | 56 | 52 | 51.9 |
| | 294 | 66 | 61 | 52.0 |
| | 405 | 63 | 57 | 52.5 |
| | 409 | 68 | 69 | 49.6 |
| **Abnormal Bladder Function** | | | | |
| B2 - Unilateral Reflux | 283 | 72 | 71 | 50.6 |
| | * 402 | 71 | 82 | 46.4 |
| | * 414 | 61 | 63 | 49.2 |
| | * 417 | 56 | 85 | 39.7 |
| | 421 | 63 | 59 | 51.6 |
| | 425 | 69 | 68 | 50.4 |
| | 426 | 72 | 71 | 50.3 |
| | 427 | 54 | 56 | 49.1 |
| | 428 | 52 | 51 | 50.5 |
| | 429 | 61 | 64 | 48.8 |
| B2 - No Reflux | 292 | 86 | 86 | 50.0 |
| | 400 | 78 | 74 | 51.3 |
| | 415 | 69 | 65 | 51.5 |
| | 420 | 73 | 74 | 49.6 |
| | 422 | 66 | 66 | 50.0 |
| | 424 | 100 | 91 | 52.4 |
| | 430 | 57 | 57 | 50.0 |

* Pigs excluded from analysis (see 17.3.).

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# Section VIII: Sterile Reflux Nephropathy in the Pig

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## 25. High Pressure Sterile Vesicoureteral Reflux and Renal Scarring: An Experimental Study in the Pig and Minipig

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### *Introduction*

Whilst the association between vesicoureteral reflux (VUR) and segmental renal scarring (chronic atrophic pyelonephritis or reflux nephropathy, RN) during childhood is now generally accepted [2, 3, 10], the precise mechanisms involved remain unclear. In particular, controversy remains over the question whether scarring occurs only in the presence of urinary tract infection (UTI), or whether VUR of sterile urine can in some circumstances result in segmental kidney damage.

Our previous experimental work on RN using the pig model [8, 9] indicated that UTI is of prime importance in the scarring process. We showed that when VUR is present the renal pelvis is subjected to raised pressure during detrusor contraction at micturition. This results in reversal of the normal pressure gradient between the renal parenchyma and the pelvis, allowing retrograde flow (intrarenal reflux, IRR) back into the renal tubules. Any pathogenic organisms present in the bladder urine may thus gain access to the parenchyma and initiate renal scarring. The segmental nature of the scars is related to the morphology of individual renal papillae whose shape and distribution of papillary duct orifices determine whether or not IRR occurs in a particular renal lobe [7]. Papillary shape and propensity to allow IRR can change when subjected to abnormally raised bladder pressure in a refluxing system, and in this situation the extent of IRR, and any scarring which may ensue in the presence of UTI is increased. We have corroborated that when the urine is infected, parenchymal damage and subsequent scarring may occur rapidly in the course of only 1–3 weeks and

that this process is particularly extensive and devastating when urinary obstruction as well as VUR is present. Conversely when there is VUR alone, eradication of UTI after 1 week considerably limits the amount of scarring.

Previously [8, 9] we produced renal scarring only when UTI was present, and never in its absence. *Hodson* et al. [3], however, produced scars in the pig model in the absence of UTI when a ring was placed around the urethra to produce high pressure VUR.

In this study we report our own experience in a similar model. Particular attention has been made to monitor bladder pressures by serial conscious voiding cystometrograms, to observe changes in voiding patterns and to follow changes in the upper urinary tract and kidneys by ultrasonography and $^{99m}$Tc-dimercaptosuccinic acid (DMSA; CIS®) scanning of the kidneys in order to correlate these parameters with the pathologic changes developing in the kidney.

## *Material and Methods*

The pig was chosen as an experimental animal for reasons discussed before [8, 9].

*Experimental Model.* VUR was produced either unilaterally or bilaterally in recently weaned piglets aged between 2 and 5 weeks as described previously [8]. At the same operation a silver wire 'ring' was placed around the urethra. This 'ring' was of the form devised by *Hodson* et al. [3] and consisted of an incomplete circle with hooked ends which was closed with a silk ligature at operation (fig. 1). The ring was preformed to a known internal diameter (3–7 mm).

Following this surgical procedure all animals were maintained on prophylactic antimicrobial drugs (either Septrin® 10–30 ml pediatric suspension/day or Macrodantin® 150–300 mg/day). A cystogram was performed 2 weeks postoperatively to confirm the presence of VUR.

Routine investigations were performed as follows. Weekly urine samples for bacteriologic culture were obtained by suprapubic puncture under ultrasound control, and at the same time ultra-sonograms of both kidneys were recorded.

Voiding cystourethrograms (VCUs) were performed at approximately monthly intervals. After anesthetizing the animals with a Halothane®/$N_2O_2/O_2$ mixture, a Portex® epidural catheter was introduced percutaneously through a hollow needle into the bladder, and a second epidural catheter was placed similarly in the peritoneal cavity. The animals were then allowed to regain full conciousness in a restraining cage. A diuresis was induced by the intravenous infusion of 0.45% NaCl with 4.5% dextrose, and a number of voids were observed. Intravesical and intra-abdominal pressures were recorded independently using Elcomatic® 750 transducers with simultaneous recording of the two traces on a two-channel chart recorder (Elcomatic® 720) fitted with an Elcomatic® 722 preamplifier.

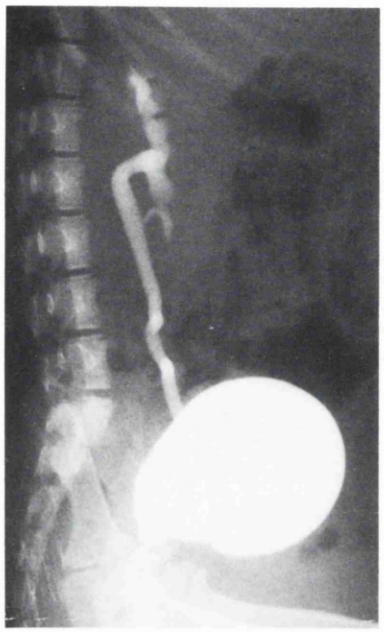


*Fig. 1.* R. 264; cystogram showing unilateral VUR 2 weeks following surgery. Note the position and form of the urethral ring.

All but the first 3 animals studied (R. 208, 217, and 218) had $^{99m}$Tc-DMSA scans performed under general anesthesia with Halothane/$N_2O_2/O_2$. These were carried out at approximately monthly intervals, but in many animals were performed more frequently; at critical periods weekly scans were obtained. Between 1 and 4 mCi $^{99m}$Tc-DMSA were injected intravenously and imaging performed 2–4 h later on a Nuclear Enterprises® (model NE 890C) gamma camera using high resolution and a 6 mm pin hole collimator for a total of $4 \times 10^5$ counts. Posterior and oblique views of each kidney were obtained.

At the end of the experiments the animals received a further dose of $^{99m}$Tc-DMSA 2 h before sacrifice, and the isolated kidneys were scanned on a Scintag-Berthold LFOV gamma camera with high resolution collimation linked to an Informatek Sirius 3 computer. 500K anterior and posterior views of each kidney were obtained in a $64 \times 64$ matrix and the smoothed images displayed with isocontours.

A total of 17 animals have been investigated in this study. Initially 27 animals were entered, but 10 were excluded. These were 4 who died in the immediate postoperative period, 4 who developed spontaneous UTI and 2 who failed to reflux.

First studied were 7 Large White boars (group I). Having evaluated the data from these animals, a further series of 5 male (group II) and 5 female (group III) Gottingen Minipigs were investigated to ascertain if species and gender might influence the results.

*Pathologic Examination.* After each animal was killed the kidneys and urinary tract were removed and examined in the fresh state, the ureteric orifices particularly being examined. The whole urinary tract, the external surfaces and the cut surfaces of each kidney were photographed and close-up views of any macroscopic lesions taken. Representative blocks of parenchyma from macroscopically normal kidneys, and blocks of all lesions in those kidneys where they were present were fixed in buffered formalin (pH 7.0), processed and embedded in paraffin wax. Sections (6μm thick) were stained by HE, van Gieson/elastic, periodic acid-Schiff (PAS) and periodic acid-methenamine silver (PAMS).

Sections of all blocks from parenchymal lesions were examined immunochemically for Tamm-Horsfall protein (THP) using the unlabelled horseradish peroxidase – antihorsradish peroxidase (PAP) method [5].

*Discussion of the Experimental Model.* The animal preparation employed is basically that used in our previous studies [8, 9], but with a number of additional features. Our experience with the model has confirmed that it produces reliable and reproducible VUR which can be demonstrated by cystourethrography, and the presence of an intact vesicoureteral junction on the non-operated side provides a valuable internal control.

Voiding patterns were monitored by observations of the animals in their pens, and serial VCUs were employed to record these data and to follow changes in peak voiding pressures. Placement of the catheters in the bladder and peritoneal cavity for pressure recordings necessitated anesthetizing the animals, but they were allowed to recover full consciousness in a restraining cage before the tracings were made to eliminate the effects of the anesthetic agents on detrusor and sphincter activity.

Ultrasonography was employed to aid localization of the bladder during suprapubic urine sampling for bacteriologic culture. When bladder decompensation or acute obstruction occurred the degree of bladder distension could also be assessed by sonography. This technique was also used to monitor dilatation of the upper urinary tract.

The occurrence of parenchymal lesions could be identified in the intact animal by $^{99m}$Tc-DMSA scanning. The interpretation of these scans was sometimes difficult. In particular, shading of the polar regions due to respiratory excursions of the kidneys was a problem, especially if activity was low and required prolonged screening times to achieve sufficient counts. The examination of sequential scans sometimes allowed correct interpretation in retrospect of an initially equivocal abnormality, but on other occasions changes which were regarded as a possible parenchymal lesion were not confirmed at autopsy. However, the static scans obtained on the isolated kidneys after sacrifice always correlated with the presence or absence of macroscopic and microscopic abnormalities.

In 3 of the female Minipigs in which frequent $^{99m}$Tc-DMSA scans were performed it was possible to pinpoint within a matter of days the precise stage at which lesions developed by observing a definite change from normal to abnormal from one scan to the next.

The emplacement of a ring loosely around the urethra in piglets aged between 2 and 5 weeks was intended to provide gradually increasing bladder outflow obstruction as the animals grew. The degree of obstruction is affected by the growth of the animal and the size of the ring. But in the male animals, both Large White, and Minipigs, the results of using a proximal urethral ring were unsatisfactory.

In most of the latter the ring was so loose that voiding pressures were only moderately elevated, or the relationship between ring size and urethral growth was such that, following

a period of moderately elevated voiding pressure, sudden acute obstruction occurred without a period of bladder decompensation. In only 2 of these animals were the conditions of sustained high pressure voiding leading to a period of bladder decompensation achieved. The importance of a phase of bladder decompensation in the genesis of renal parenchymal lesions only became apparent at a late stage in the study (vide infra).

In the female Minipigs the much less muscular and relatively narrow urethra allowed the use of a smaller diameter ring. Elevation of voiding pressure occurred rapidly and progressed early into a phase of bladder decompensation.

Bladder outflow obstruction following emplacement of the urethral ring produces a progression of changes in bladder function in which 4 phases can be recognized.

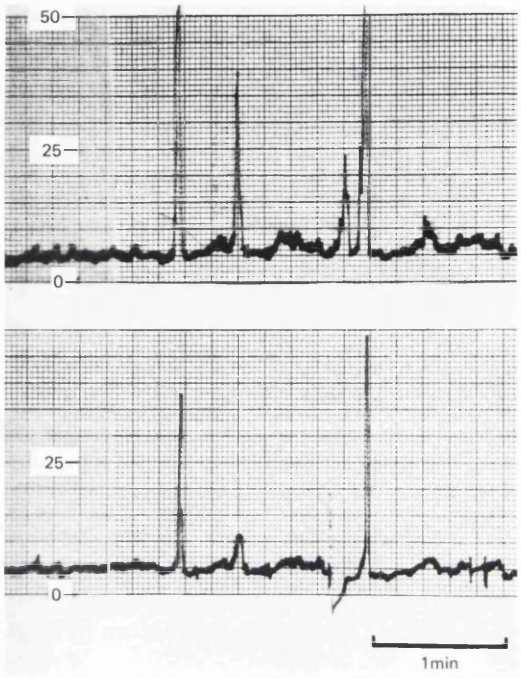
*Phase 1.* An initial stage of acute postoperative obstruction, occurring in some animals only and lasting from 2 to 7 days. In mild cases this phase resolved without intervention becoming necessary. 4 animals were lost from the study of this stage with bladder rupture. Subsequently, the institution of daily, or sometimes twice daily suprapubic aspiration to empty the bladder allowed those animals developing this state to survive and, following this, normal micturition was reestablished.

*Phase 2.* A phase in which peak voiding pressure rose, but the voiding pattern in terms of the frequency and duration of micturition remained essentially normal. Some animals (from the groups of male Large White and Minipigs) did not progress beyond this stage. They developed moderately raised voiding pressures, with normal micturition which remained static even after many weeks.

*Phase 3.* A period in which peak voiding pressure continued to rise slowly or remained stable at an elevated level, while voiding gradually became increasingly prolonged and more frequent, so that eventually an animal might be seen to void for 3 min about every 15 min.

*Phase 4.* A final stage, observed in only the female Minipigs and 2 of the Large White male pigs, in which sustained bladder decompensation occurred. This was characterized by gross bladder distension with dribbling micturition, often virtually continuous. Detrusor activity was sometimes completely absent and expulsion of urine occurred only by abdominal straining (fig. 2). In some animals the detrusor continued to contract to produce high intravesical pressure for long periods. These contractions were completely ineffective and urine was only expelled with superimposed abdominal strains (fig. 3). In 2 of the male Minipigs (R. 247 and 248) *acute* bladder decompensation occurred following a period of sustained high pressure voiding with abnormal micturition (phase 3). It occurred abruptly and was associated with complete obstruction, so that the experiment had to be terminated.

It is apparent from our observations in these animals, together with those from our previous study [8], that the urodynamic consequences of placing a ring around the urethra are varied, complex and change with time; they differ subtly in individual animals apparently prepared in a similar fashion. As we will describe, renal damage in the presence of sterile VUR occurs only under clearly defined conditions for which the term 'high pressure reflux' is clearly an oversimple description.

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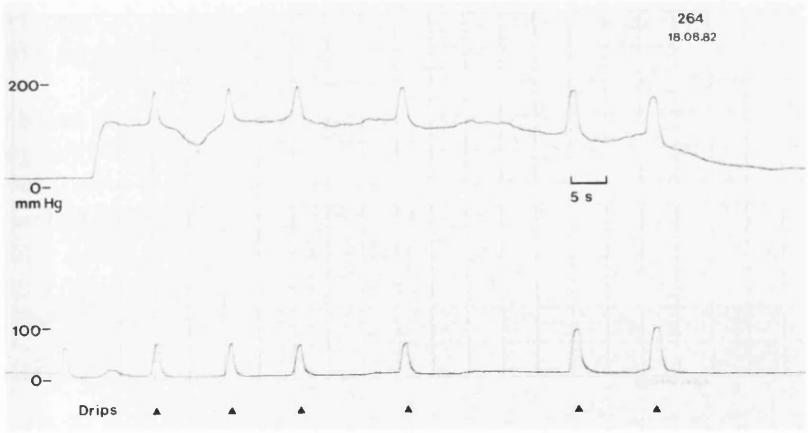



3

*Fig. 2.* Cystometrogram on female Minipig (R. 246) during phase 4 (bladder decompensation see text). The upper trace shows bladder pressure and the lower trace intra-abdominal pressure. Detrusor contractions are absent and expulsion of urine occurred only during abdominal strains. Scale in mm Hg.

*Fig. 3.* Cystometrogram on female Minipig (R. 264) during phase 4. The traces are as in figure 2. There is sustained detrusor contraction, but despite high intravesical pressure, urine is expelled only with superimposed abdominal strains.

*Results*

The urodynamic and gross pathologic findings in all the animals included in the study are summarized in table I. Animals R. 217–240 were Large White Male pigs (group I), R. 243–250 (except 246) were male minipigs (group II) and R. 246 and 261–264 were female Minipigs (group III). Representative VCUs and photomicrographs of the gross and microscopic pathologic findings from the animals in each group, together with illustrative sonograms are depicted in figures 4–11.

Significant segmental changes developed in the kidneys subjected to VUR in 6 animals (R. 208, 240, 246, 261 and 264) and minor abnormalities in 2 others (R. 218 and 223). The pathologic findings, including the examination for THP in these animals are summarized in table II. Kidneys from all the other animals in the study showed no pathologic abnormalities and THP was confined to its normal site in the distal tubules.

In 3 of the female Minipigs (R. 246, 261 and 264) unequivocal differences in consecutive $^{99m}$Tc-DMSA scans were observed in short periods of time allowing precise definition of the circumstances existing at the time of development of parenchymal lesions. These scans are shown in figure 12. In each case the first scan was performed at or near the onset of the phase of bladder decompensation (phase 4). Abnormalities indicating the development of parenchymal lesions were then noted after 7 days (R. 261 and 264) and 14 days (R. 246).

The study shows that in the presence of sterile VUR with raised peak voiding pressures in the range of 41 to 177 cm $H_2O$, significant renal parenchymal lesions develop only when bladder decompensation occurs. In these circumstances acute tubulointerstitial damage ensues after only a few days and progresses quickly to scar formation with interstitial collagen formation and tubular destruction. Extravasation of THP into the interstitium, indicative of tubular rupture was noted in many but not all of these lesions.

*Discussion*

The primary object of this study has been to discover whether VUR of sterile urine in the pig model can initiate renal scarring, and to determine the precise urodynamic conditions under which such lesions might develop. Our difficulties in achieving this aim have been discussed above (see Discussion of Experimental Model) and we have shown that the model has to

*Table I.* Summary of urodynamic and pathologic changes in all the animals in the study. The center columns illustrate the evolution of bladder dysfunction (see text)

| Pig No. | Sex | Type | Max PVP cm $H_2O$ | Post op. obstruction phase 1 | phase 2 | phase 3 | Decompensation + duration (days) phase 4 | Upper tract dilatation | Refluxing side | Parenchymal changes | Duration of experiment, weeks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Group I* | | | | | | | | | | | |
| 217 | M | LW | 41 | | → | | | no | R | none | 11 |
| 218 | M | LW | 54 | | → | | | no | R | puckering ± | 17 |
| 225 | M | LW | 88 | → | | | | no | R + L | none | 21 |
| 226 | M | LW | 48 | | → | | | no | R + L | none | 30 |
| 208 | M | LW | 68 | | → | | 7+ | R | R | scarring + + R | 17 |
| 223 | M | LW | 61 | → | | | | R + L | R | puckering + R | 14 |
| 240 | M | LW | 98 | → | | | 7 | R + L | R + L | scarring + + L & R | 15 |
| *Group II* | | | | | | | | | | | |
| 243 | M | MP | 82 | → | | | | no | R + L | none | 26 |
| 247 | M | MP | 68 | → | | | | L | L | none | 12 |
| 248 | M | MP | 61 | → | | | | R | R | none | 18 |
| 249 | M | MP | 68 | → | | | | no | L | none | 18 |
| 250 | M | MP | 60 | → | | | | no | R + L | none | 22 |
| *Group III* | | | | | | | | | | | |
| 246 | F | MP | – | → | | | 8 | R + L | L | scarring + + L | 6 |
| 261 | F | MP | 98 | | → | | 6 | R < L | R + L | acute lesions L+ R+ | 4 |
| 262 | F | MP | 54 | → | | | 2 | L | L | acute lesions L± | 3½ |
| 263 | F | MP | 109 | → | | | 1 | L | L | none | 6 |
| 264 | F | MP | 177 | → | | | 6 | L | L | acute lesions L+ + | 3 |

LW = Large Whites; MP = Minipigs.

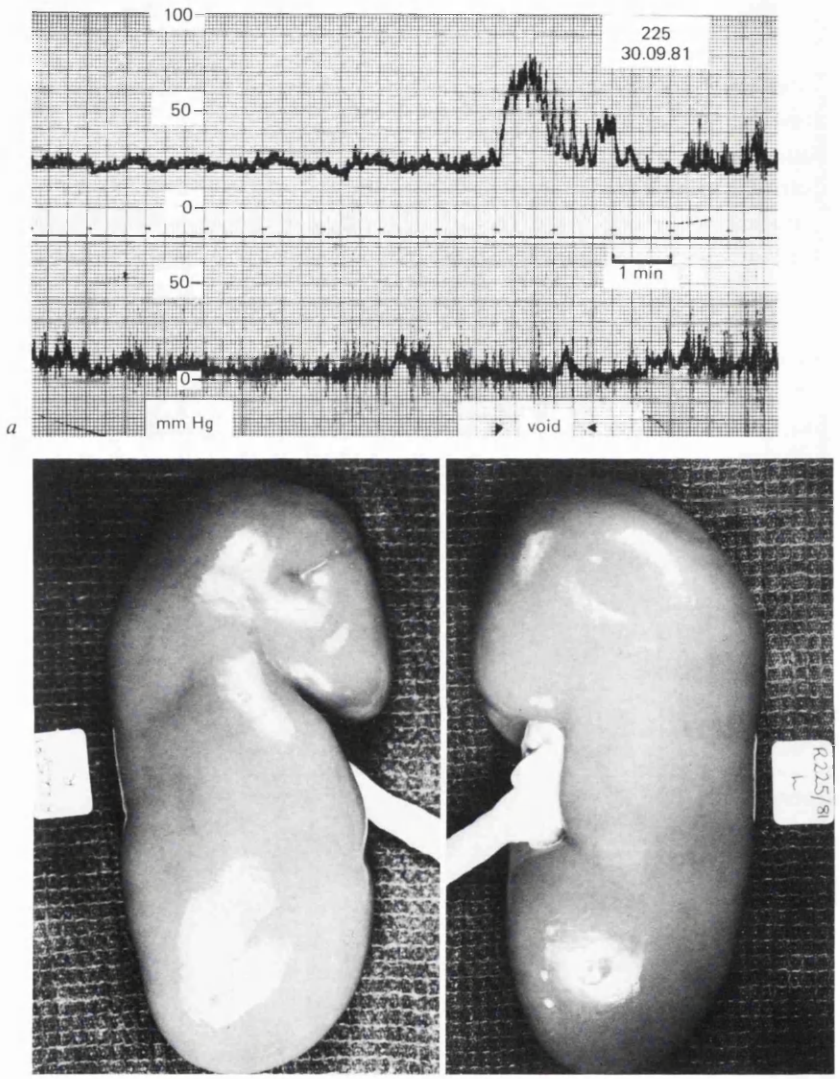



*Fig. 4.* Male Large White (R. 225; group 1). *a* Cystometrogram 19 weeks after surgery (traces as in fig. 2). *b* Kidneys from the same animal showing the absence of parenchymal lesions which was confirmed histologically.

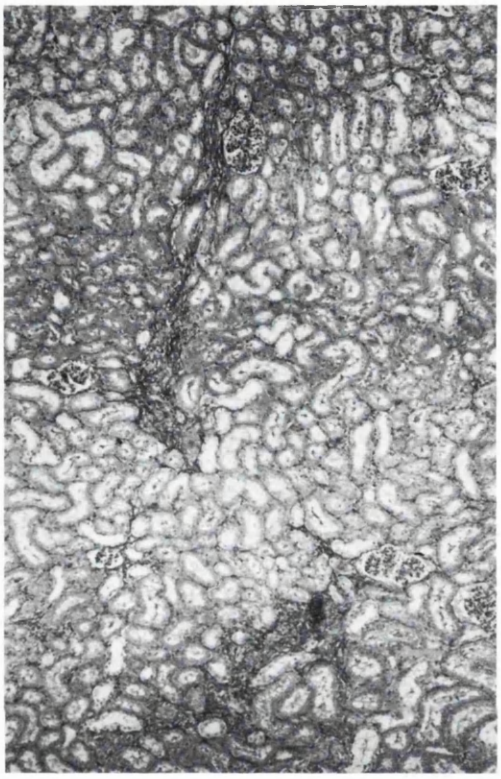
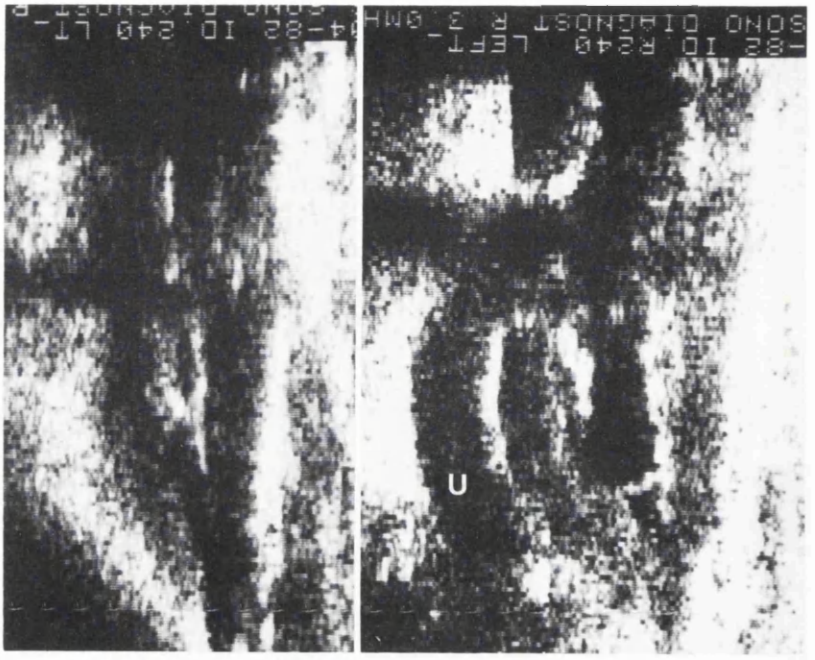
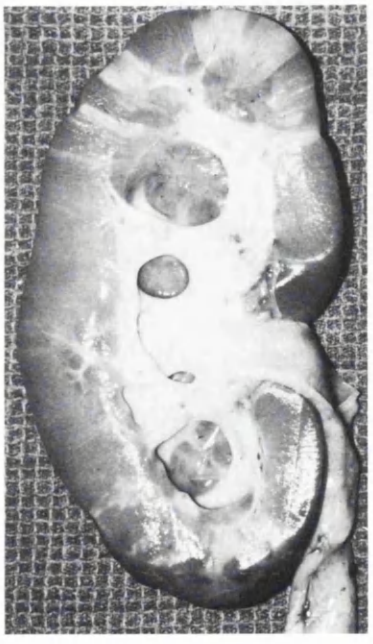


*Fig. 5.* Male Large White (R. 218; group 1). Histological appearances of the minor and insubstantial scarring evident in the right kidney. VG. × 40.

be manipulated to provide sustained bladder decompensation before parenchymal lesions will develop in the kidney. However, once persistent bladder decompensation is established $^{99m}$Tc-DMSA scanning indicates that segmental renal damage can develop quickly in the course of a few days. Conversely, prolonged periods of high pressure voiding without decompensation (that is, where bladder pressures return to normal resting levels between voids and effective bladder emptying occurs – 'compensated bladder outflow obstruction') does not give rise to parenchymal damage. This remained true even for the 2 animals (R. 247 and 248) which progressed to acute obstruction after long periods of high pressure voiding but in which sustained bladder decompensation did not occur.

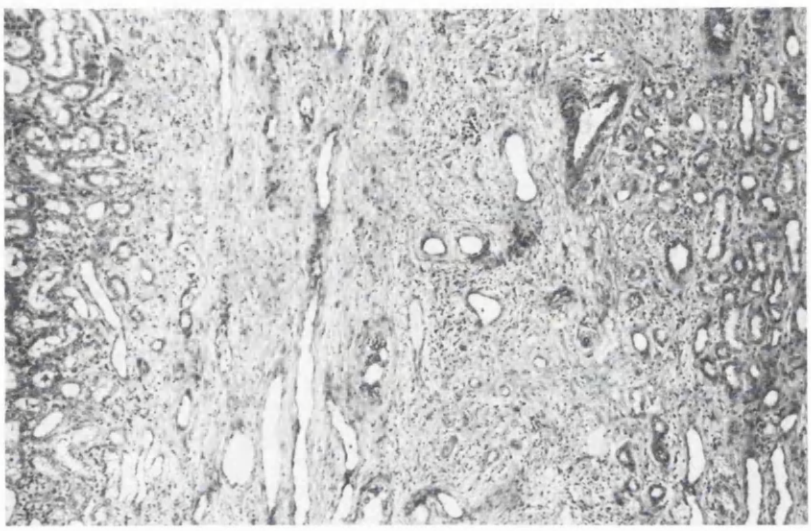



*a* *b*



*c*

*Fig. 6.* Male Large White (R. 240; group 1) which progressed to bladder decompensation (phase 4). Sonograms of the left kidney *(a)* before and *(b)* after bladder decompensation showing the development of severe upper tract dilatation when decompensation occurs. U = Ureter. *c* View of the cut surface of the right kidney showing radiating bars of fibrosis with intervening unscarred parenchyma; note the degree of calyceal dilatation. *d* Histology of a scar showing advanced tubular loss, interstitial fibrosis, scanty inflammation and dilatation of collecting ducts. HE. × 25.

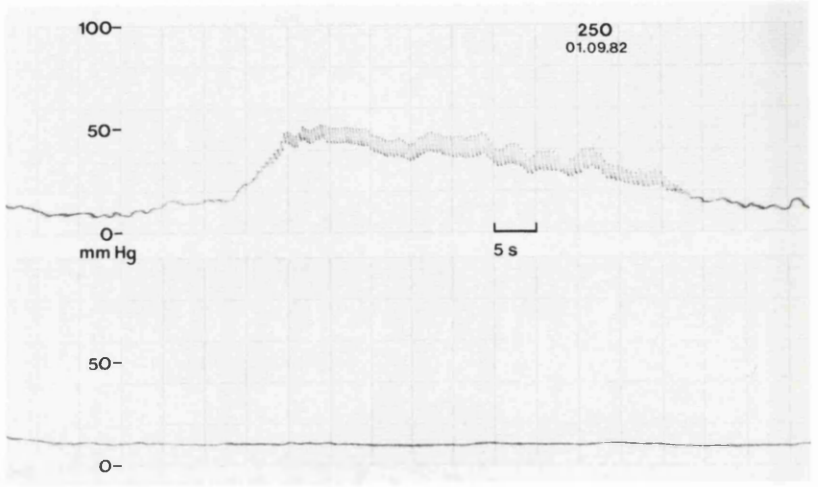


*d*

Acute lesions were observed in 3 of the female Minipigs (R. 261, 262 and 264) and were characterized by areas of parenchymal pallor radiating from the centers of the renal papillae into the overlying cortex. Microscopically, changes were essentially tubulointerstitial and consisted of tubular dilatation and separation with flattening of the lining epithelial cells. The edematous interstitium contained scattered fibroblasts and a generally scanty infiltrate of inflammatory cells – mostly lymphocytes. In some of the lesions deposits of THP were demonstrated, and a proportion of these deposits were associated with localized collections of lymphocytes and plasma cells.

More advanced lesions were seen in 3 animals. 2 were male Large Whites (R. 208 and 240) and 1 a female Minipig (R. 246). In these animals tubular damage was more extensive and interstitial collagen formation was an added feature. Interstitial THP deposits were frequent in the lesions from the female Minipig but were absent in those of the male Large Whites.

In comparison with the infected lesions produced in earlier studies a number of differences were noted. The sterile lesions tended to be smaller and less diffuse; they often consisted of aggregates of linear streaks or bars with bands of uninvolved parenchyma between them. The infected scars, by contrast, tended to be more confluent and usually wedge-shaped. Microscopically the infected lesions were associated with a much more intense interstitial inflammatory infiltrate, frequently with lymphoid follicle formation, and neutrophil polymorphs were seen in some tubules. Interstitial

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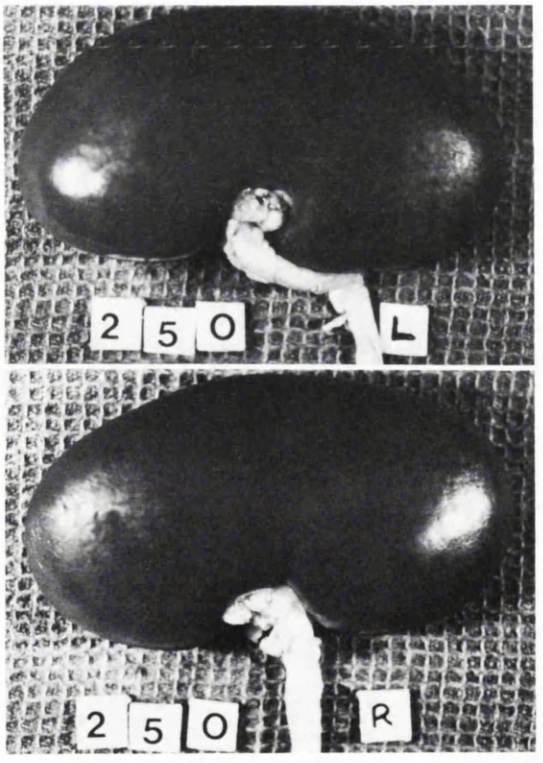


*b*

*c*

*Fig. 7.* Male Minipig (R. 250; group 2). *a* Cystometrogram after 15 weeks in phase 2 (see text). The traces are as in figure 2. *b, c* The kidneys from the same animal showing no macroscopic, nor subsequent microscopic abnormality.

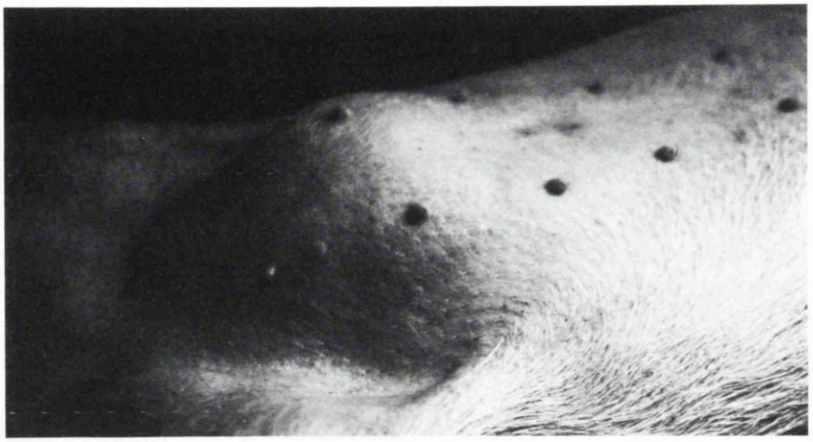

*Fig. 8.* Female Minipig (R. 263; group 3). Abdomen to show the degree of distension of a decompensated bladder.

THP deposits, seen in some of the sterile lesions, were never encountered in infected scars.

The status of the insubstantial scars seen in 2 additional male Large Whites (R. 218 and 223) in the present study is difficult to assess. In 1 of these animals bladder decompensation did not occur. The fine linear scarring and puckering of the surface of the kidneys in these 2 animals is identical to that we have encountered previously in animals treated with antimicrobial agents after a short period of infected VUR [9]. The possibility that both these animals sustained very short periods of UTI undetected by our bacteriologic screening has to be admitted.

Although individual sterile lesions tended to be less extensive than the infected ones, they were distributed more uniformly and occurred in both the polar regions and the midzones of affected kidneys. We have demonstrated previously how in infected lesions access of the organisms which initiate parenchymal scars is mediated by IRR. The predominantly polar distribution of infected scars occurring with VUR at normal voiding pressures is due to the fact that compound papillae which allow free IRR under these conditions are found mainly at the renal poles. When, however, voiding pressure is raised, or an elevated intrapelvic pressure is sustained, deformation of some simple papillae in the midzone of the kidney ensues and IRR then occurs up these normally resistant papillae, and any resulting scar formation becomes more generalized. Clearly, in the present series, in ani-

mals in whom parenchymal lesions developed, intrapelvic pressures were high and diffuse IRR would have occurred. The distribution of individual lesions directly over renal papillae is strong presumptive evidence that IRR is central to the development of sterile as well as infected scars. Under the conditions of bladder decompensation with upper tract dilatation in which the sterile lesions formed, the collecting ducts and tubules where IRR was occurring would be subjected to sustained high intraluminal pressures. Over and above this, there would be transmission of additional peaks of pressure during frequently repeated attempts at voiding. Three possible sequelae are suggested. Firstly the hydrodynamic effects might directly damage the epithelial cells lining the dilated tubules. Secondly tubular distension might inhibit blood flow through adjacent peritubular capillaries, adding an element of ischemic damage. Thirdly, and perhaps during phases of peak pressure transmission into an already maximally dilated system, actual rupture of tubular basement membranes could occur, allowing egress of urine into the interstitium. The demonstration of interstitial THP in at least some of the lesions during the acute phase is evidence in favor of this possibility. Whether THP itself or other urinary constituents, either directly or as a result of humoral [4] or cellular immune mechanisms [6] they may elicit, are responsible for at least some of the parenchymal damage sustained remains to be elucidated.

Comparison of the macroscopic appearances of our sterile lesions with the illustrations of those produced by *Hodson et al.* [3] is of considerable interest. *Hodson* [1] has drawn attention to the flat polar and deep cleft-like midzonal scars occurring directly over flattened papillae which he regards as characteristic of RN. We were struck by the fact that *Hodson et al.* [3] often removed the urethral ring in the course of their experiments. Judging by our own experience this would be essential, since the state of bladder decompensation necessary to initiate scar formation would be incompatible with continued survival. If, however, the ring were removed, contraction of the scarred area coupled with growth of the adjacent unaffected parenchyma would ultimately result in the deep cleft scars that *Hodson et al.* [3]

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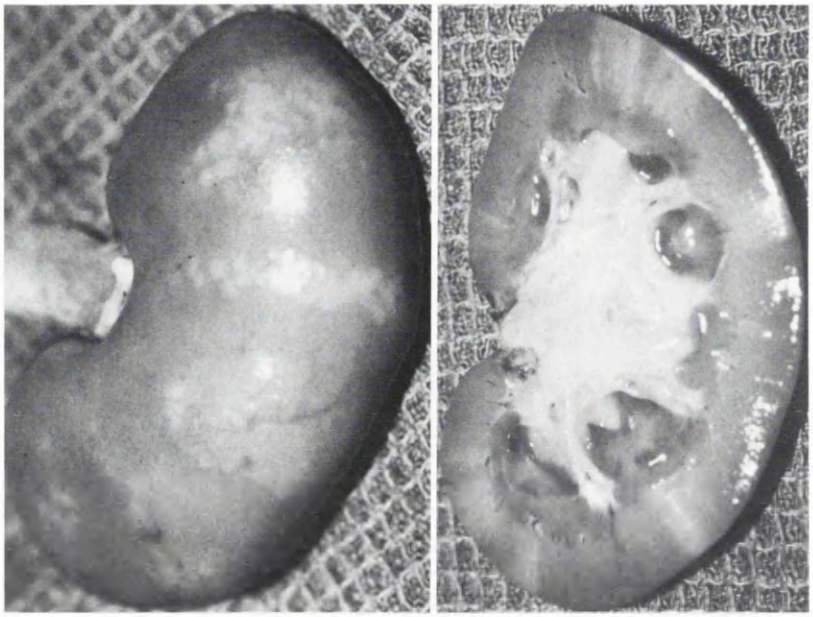
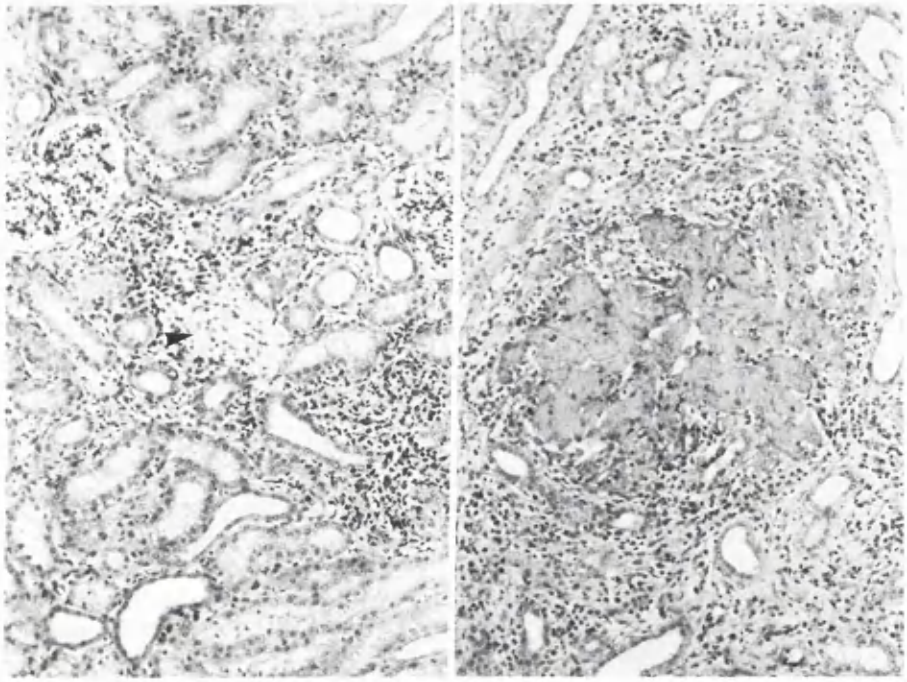
*Fig. 9.* Female Minipig (R. 246; group 3). The cystometrogram from this animal during phase 4 is shown in figure 2. *a*, *b* Macroscopic appearances of the left kidney showing multiple bar-like parenchymal lesions (see also fig. 12). *c* Histologic appearances of a scarred area showing interstitial lymphocytic and plasmacytic infiltration and a deposit of THP (arrowed). HE. × 250. *d* A large interstitial deposit of THP with associated interstitial inflammation and adjacent fibrous scarring. PAP technic for THP. × 250.



*a* *b*



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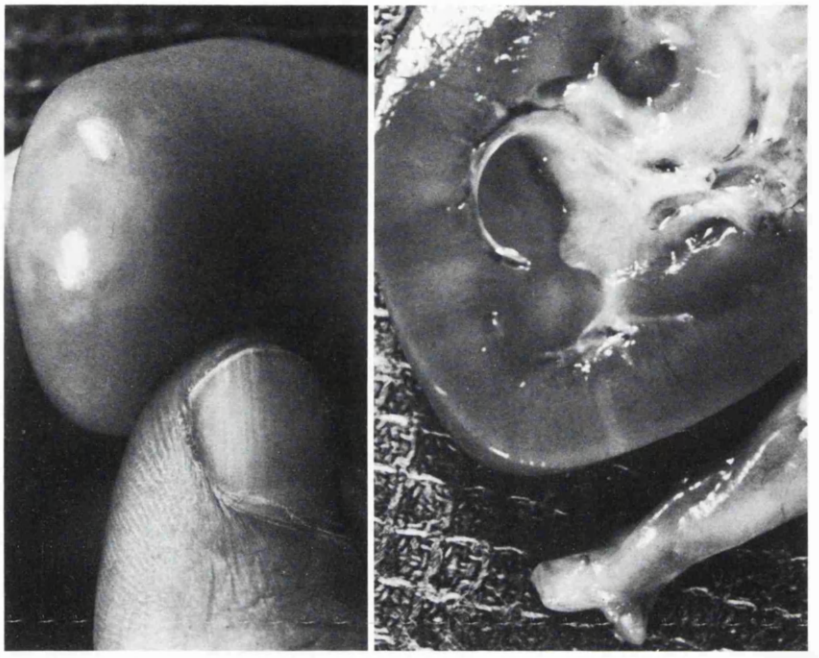
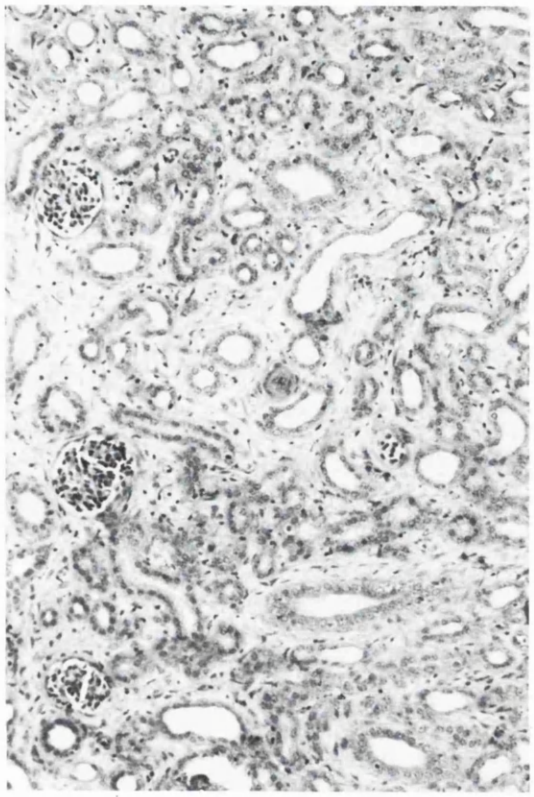


*a* *b*

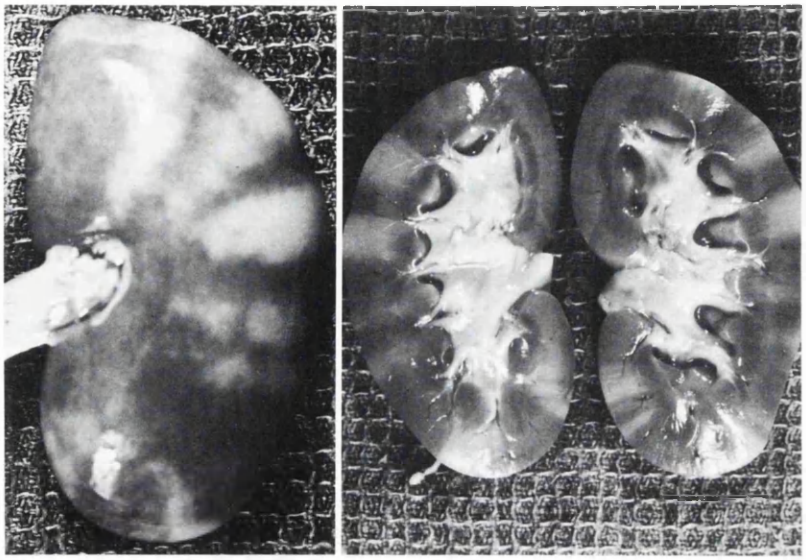
*Fig. 10.* Female Minipig (R. 261; group 3). *a*, *b* Views of the right kidney showing very early parenchymal lesions after 6 days of bladder decompensation (see also fig. 12). *c* Histologic appearances showing tubular dilatation and separation with interstitial fibroblastic proliferation but little inflammation. HE. × 100.

demonstrated. However, it is likely that the parenchymal damage leading to this scarring occurred in the period before the ring was removed. Their work, as well as our study reported here, demonstrates that in the pig model of VUR under conditions of sustained bladder decompensation and upper tract dilatation it is possible to produce segmental renal parenchymal lesions in the absence of UTI. We are, however, unwilling to ascribe great clinical significance to this observation. Our work indicates that sustained bladder decompensation with VUR rather than high pressure VUR per se is the essential prerequisite for the development of sterile scars and clinical parallels for this are few. It is possible that such a mechanism might operate, for example, in some infant boys with obstructive posterior urethral valves, or in some children with a neuropathic bladder. However, complicating factors cloud the issue, even in these situations. Firstly, UTI is



c

extremely common under these circumstances, and all workers using this model have demonstrated that the combination of infected VUR with bladder outflow obstruction produces devastating and rapid destruction of the kidney even in the absence of bladder decompensation [9]. Secondly, it is very difficult to ascertain in many of the clinical examples cited whether the renal abnormalities observed are due to maldifferentiation of the kidney during intrauterine life or lesions acquired after birth. Not uncommonly the kidneys of children with obstructive posterior urethral valves show histologic evidence of renal dysplasia, giving an unequivocal indication of maldifferentiation. It is also true that occasional examples of severe bilateral renal dysplasia associated with widely dilated refluxing ureters and narrowing of the posterior urethra are encountered. It may be possible to distinguish whether the lesions present in these kidneys are due to anomalous



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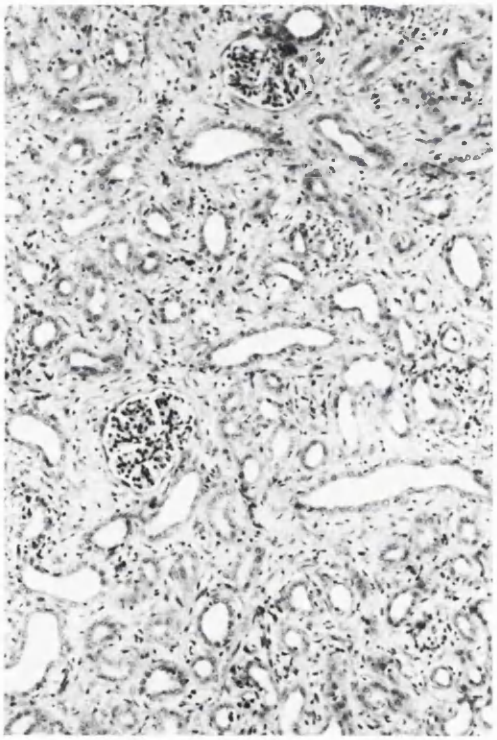

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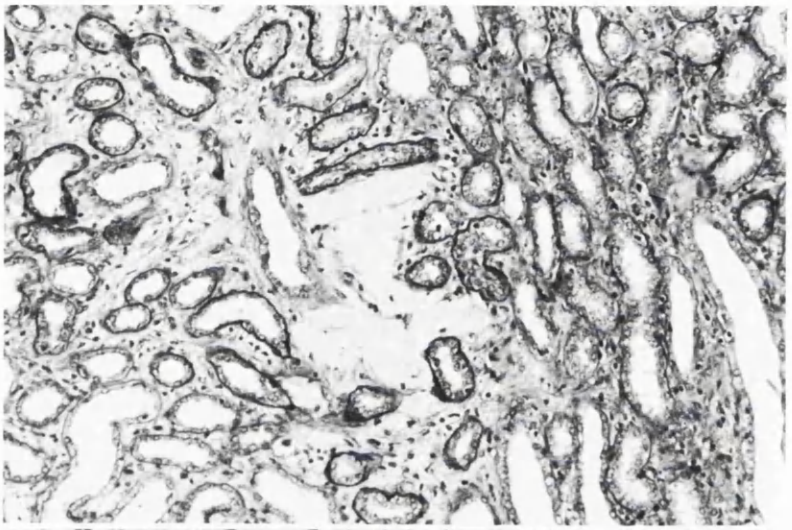

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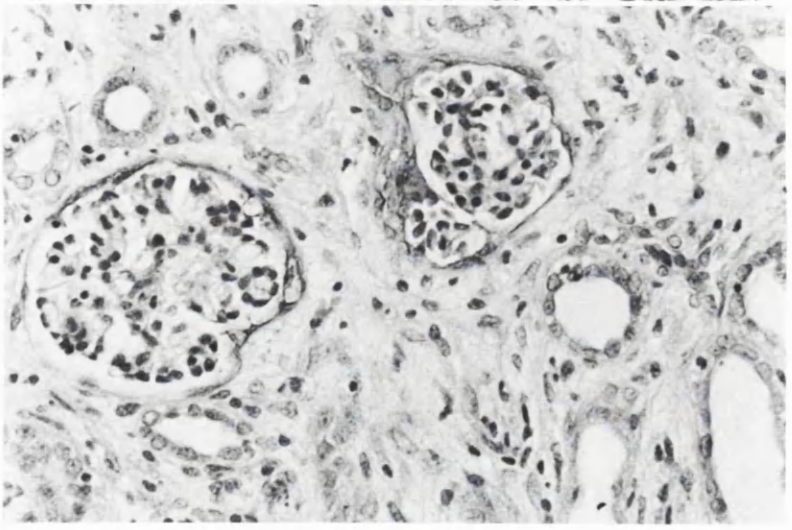

*e*

*Fig. 11.* Female Minipig (R. 264; group 3). The cystometrogram from this animal during phase 4 is shown in figure 3. *a*, *b* Macroscopic appearances of the left kidney showing prominent bar-like lesions (see also fig. 12). *c* Histologically these are similar to those in R. 261 (see fig. 10c). HE. × 100. *d* An interstitial deposit of THP with no associated inflammation. PAMS. × 100. *e* Deposition of THP in the glomerular urinary space along Bowman's capsule. PAP technic for THP. × 400.

*Table II.* Summary of parenchymal lesions in the kidney

| Animal | Reflux | Affected side(s) | Pathologic lesions | | Localization of THP in lesions | |
|---|---|---|---|---|---|---|
| | | | macroscopic | microscopic | in glomerular urinary spaces | deposits in interstitium |
| *Group I* | | | | | | |
| 208 | R | R | coalescent contracted scars over papillae throughout kidney | dense interstitial fibrosis and widespread tubular loss; sparse interstitial inflammation | P | A |
| 240 | R + L | R + L | linear and bar-like scars with intervening bands of unaffected parenchyma over papillae throughout the kidney | dense interstitial fibrosis with tubular loss; focal interstitial inflammation; dilatation of residual tubules; generalized tubular and glomerular dilatation in unscarred parenchyma | P | A |
| 218 | R | R | focal pitting and puckering of kidney surface at poles; very fine linear cortical scarring | small foci of fine linear fibrosis with localized tubular loss and glomerular scarring | A | A |
| 223 | R + L | R | focal pitting and puckering of kidney surface at poles; very fine linear cortical scarring | small foci of fine linear fibrosis with localized tubular loss and glomerular scarring | A | A |

metanephric differentiation, or to acquired UTI, or sterile scarring, particularly by radiologic or other imaging techniques alone.

It could be argued that any parenchymal maldevelopment is itself due to VUR and/or obstruction operating in utero, but equally, as suggested by *Stephens* [11], the association of grossly dilated ureters, renal dysplasia and VUR may together reflect a common developmental abnormality of the ureteric bud. It seems probable that a whole spectrum of possible

*Table II.* (continued)

| Animal | Reflux | Affected side(s) | Pathologic lesions | | Localization of THP in lesions | |
|---|---|---|---|---|---|---|
| | | | macroscopic | microscopic | in glomerular urinary spaces | deposits in interstitium |
| *Group III* | | | | | | |
| 261 | R + L | R + L | focal streaks and bars of parenchymal pallor most marked at poles, radiating from papillae | focal areas of tubular dilatation and separation; interstitial edema with fibroblastic proliferation and sparse interstitial inflammation | P | P (single deposit in one lesion) |
| 262 | L | L | focal streaks and bars of parenchymal pallor most marked at poles, radiating from papillae | focal areas of tubular dilatation and separation; interstitial edema with fibroblastic proliferation and sparse interstitial inflammation | P | P in many one lesion) |
| 264 | L | L | bars of parenchymal pallor and swelling over papillae throughout the kidney | focal areas of tubular dilatation and separation; interstitial edema with fibroblastic proliferation and sparse interstitial inflammation | P | P (in some lesions) |
| 246 | L | L | bars of parenchymal pallor and swelling over papillae throughout the kidney | interstitial edema with some fibrosis; some tubular destruction; interstitial lymphocytic and plasma cell infiltration often associated with deposits of THP | P | P (in all lesions) |

pathogenic mechanisms may account for the renal parenchymal lesions seen in association with VUR.

We would restate our view that the majority of parenchymal scars acquired postnatally in children with VUR are wholly or predominantly initiated by parenchymal infection. A mechanism for sterile scarring has been demonstrated and could explain at least some of the renal damage occurring in children with a combination of VUR and bladder outflow obstruc-

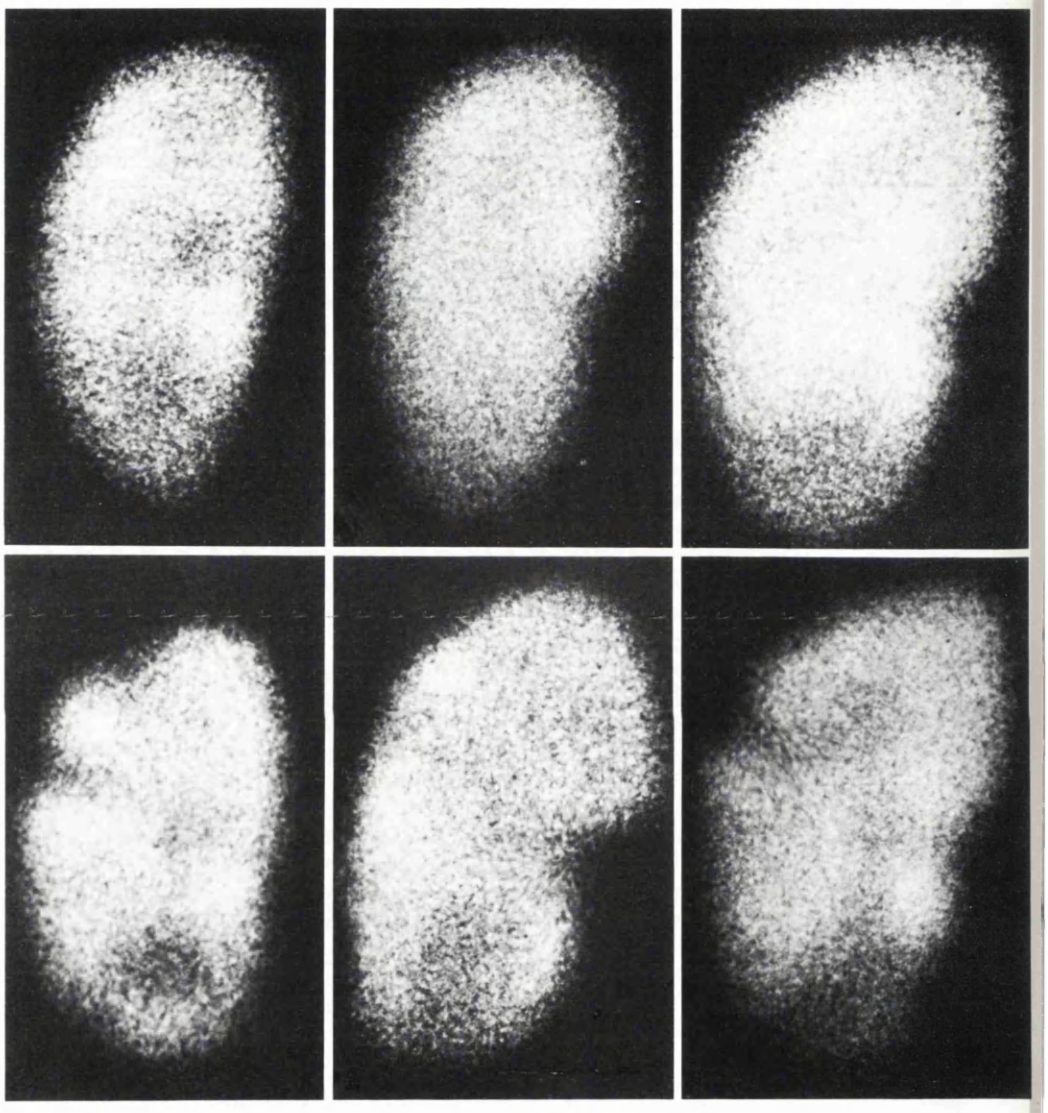

*Fig. 12.* Pairs of $^{99m}$Tc-DMSA scans in which unequivocal parenchymal changes from normal (1) to abnormal (2) occurred with bladder decompensation (see text). These are from the female Minipigs depicted in figures 9–11. *a1, 2* R. 246 (interval between scans 14 days). *b1, 2* R. 261 (7 days). *c1, 2* R. 264 (7 days). In R. 246 calyceal dilatation without parenchymal lesions is evident. Lower pole shading (see text) is seen in some extent in all three preliminary scans.

tion. Even in these cases, however, intrauterine renal maldevelopment or coexistent UTI are probably more important factors.

## *Acknowledgements*

This work has been supported by a grant from the Stiftung Volkswagenwerk. We wish to thank Drs. *I. Gordon* and *G. Vivian* and Mrs. *C. Haskell* of the Department of Diagnostic Imaging, The Hospital for Sick Children; Dr. *M.W. Turner*, Department of Immunology, Institute of Child Health; Mr. *R.E. Bartholomew* and the staff of the Department of Medical Illustration, Institute of Urology; Miss *Carole Nichols*, Department of Morbid Anatomy, The London Hospital Medical College and Mr. *B. Gregory* and the staff of the Animal House, Institute of Child Health for their generous help throughout the project.

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# Effects of Vesicoureteric Reflux on Renal Growth and Function as Measured by GFR, Plasma Creatinine and Urinary Concentrating Ability

## An Experimental Study in the Minipig

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Summary—The influence of vesicoureteric reflux (VUR) on renal growth and function measured by glomerular filtration rate (GFR), plasma creatinine concentration and urinary concentrating ability has been examined in a simple one-kidney model in the growing minipig over a period of approximately 5 months. Animals with reflux in association with low voiding pressures and normal bladder function (n = 6), as well as those with raised voiding pressures and abnormal bladder function (n = 5), were investigated together with appropriate non-refluxing controls (n = 12). Urinary infection and renal scarring were avoided since these factors may affect kidney function and growth independently.

Statistical tests of difference failed to demonstrate any effect of VUR on $^{51}$Cr EDTA GFR or renal growth even in the presence of elevated voiding pressures and abnormal detrusor function. However, a significant association between VUR and reduced urinary concentrating ability was shown.

The association between vesicoureteric reflux (VUR) and renal scarring (reflux nephropathy) is well established and previous clinical and experimental studies have focused in detail on the various mechanisms involved in this process (Hodson and Edwards, 1960; Rolleston *et al.*, 1974; Hodson *et al.*, 1975b; Ransley and Risdon, 1978; Ransley *et al.*, 1984).

Whilst segmental renal scarring and its sequelae have been regarded as the most serious clinical consequences, it has also been suggested that in young subjects with VUR, kidney growth may be retarded and renal function progressively impaired (Redman *et al.*, 1974; Ibsen *et al.*, 1977; Orikasa *et al.*, 1978; Aperia *et al.*, 1976; Piepsz *et al.*, 1981). However, the clinical investigation of these aspects of VUR is fraught with difficulties. The presence of urinary infection, abnormal bladder function or associated renal scarring may in themselves affect kidney growth and function irrespective of the presence of VUR *per se*. There are considerable problems inherent in the radiological assessment of renal size (Griffiths *et al.*, 1975) and thus of kidney growth from serial examinations, and for a variety of reasons it is inappropriate to compare the growth and functional performance of scarred with normal kidneys (Ransley, 1978). However, these factors have seldom been considered in clinical studies and in particular the complication of renal scarring has been ignored or incompletely documented.

In this experimental study in the growing pig we have examined the effects of VUR in the presence of normal bladder function and bladder outflow obstruction: (1) on renal growth, and (2) on renal function as measured by GFR, plasma creatinine concentration and urinary concentrating ability. We have sought to avoid radiological assessment of renal size and to eliminate the complicating factors of urinary infection and renal scarring so that the effects of VUR alone can be evaluated. A single kidney model has been used to provide a more sensitive means of determining the influence of

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VUR on renal growth; additionally, the effect of VUR on renal function is simplified.

## Material and Methods

### *Experimental Design*

Twenty-three pigs submitted to unilateral nephrectomy soon after birth were followed for approximately 5 months. The animals were assigned to refluxing and non-refluxing groups as shown in Figure 1A. The investigation protocol is outlined in Figure 1B. Animals were weighed weekly and the first determinations of GFR (in both groups A and B) and bladder voiding pressures by cystometry (CMG: group B) were undertaken 4 weeks after the animals had achieved a weight of 7.5 kg (study week 0). At the end of the study period (weeks 15–19) all animals had determinations of GFR, bladder voiding pressure, plasma creatinine and urinary concentrating ability. In addition to the early (week 4) and final investigations, most animals had at least one interim determination of GFR (at study week 8, 10 or 12). This was followed by a CMG in animals of group B. At the end of the study period all animals were killed, the urinary tract removed and the kidneys weighed.

### *Choice of Experimental Animal*

As in previous experimental work (Ransley and Risdon, 1978), the pig was chosen for this study because of the similarities between porcine and human kidneys. The Gottingen minipig was used for the convenience of its smaller size.

### *Surgery*

Weanling piglets underwent unilateral nephrectomy. VUR into the solitary remaining kidney was induced during the same anaesthetic in animals in group A1 and B1 by resecting the roof of the intravesical tunnel as described previously (Ransley and Risdon, 1978).

Animals in group B (both refluxing and non-refluxing) had a ring placed around the urethra well below the bladder neck. This "ring" was in the form of a horseshoe of silver wire 5 to 7 mm in diameter which was completed by tying the ends together with silk. Obstruction was produced in male pigs only because of the difficulty in achieving the right degree of bladder outflow obstruction in female pigs (Ransley *et al.*, 1984).

### *Radiology*

Cystography to confirm the presence of VUR was performed as described previously (Ransley *et al.*, 1984) approximately 2 weeks after surgery in animals from groups A1 and B1.

### *Microbiology*

All animals received oral Macrodantin (50–200 mg/day) for the duration of the experiment. Urine samples for

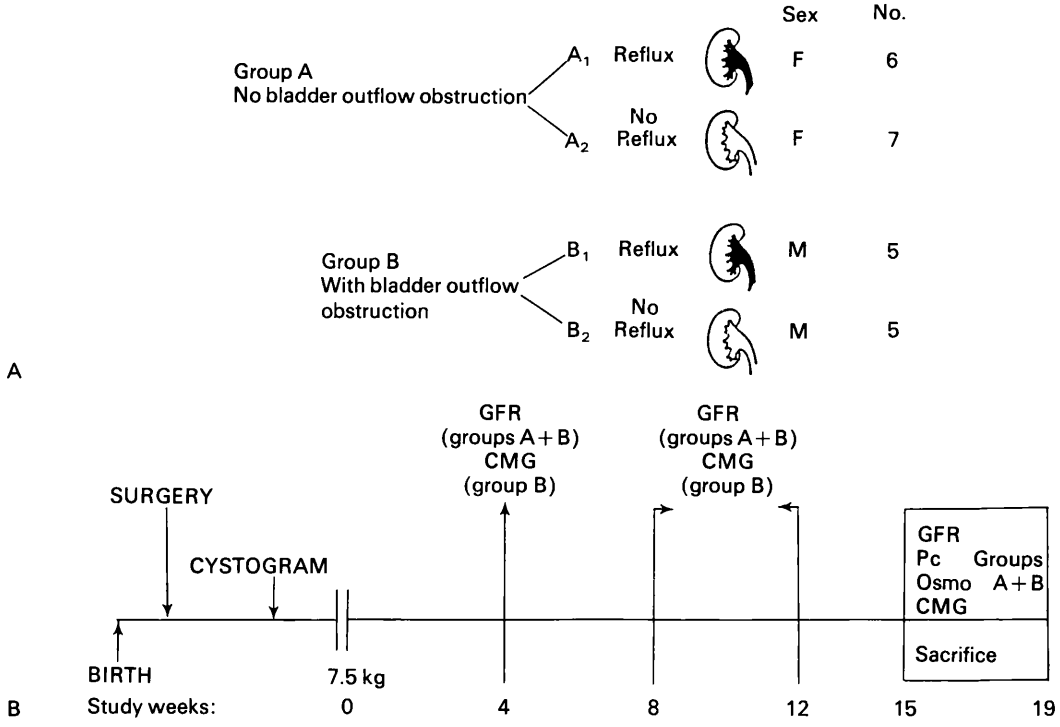



**Fig. 1A** Experimental design. **(B)** Experimental protocol. Surgery: Group A—unilateral nephrectomy; creation of VUR (A1). Group B—unilateral nephrectomy; creation of VUR (B1). GFR: $^{51}$Cr EDTA glomerular filtration rate. Pc: plasma creatinine concentration. Osmo: urinary concentrating ability. CMG: cystometry.

microscopy and microbiological culture were obtained by suprapubic puncture at approximately 2-weekly intervals throughout the study. All surgery and manipulations involving suprapubic puncture were covered by a single intravenous injection of gentamicin (2 mg/kg). Rectal temperatures were taken daily throughout the study.

*Ultrasonography*

All animals had regular ultrasound examination of the urinary tract daily after surgery for a week and then at the times of urine sampling using a Philips SonoDiagnost R with a linear array transducer.

*Urodynamics*

Conscious filling and voiding cystometry was performed as described previously (Ransley *et al.*, 1984) at the intervals detailed earlier (Fig. 1B).

*Glomerular Filtration Rate*

GFR was estimated by the method of Chantler and Barratt (1972). $^{51}$Cr EDTA (5 μCi in 0.5 ml 0.9% NaCl/kg body weight) was injected intravenously into an ear vein. Accurately timed blood samples (5 ml) were taken 100 min and 200 min after the initial injection. For injections and samplings the animals were given transient light Halothane $N_2O/O_2$ anaesthesia. Centrifuged aliquots of plasma, together with a standard prepared from the original dose injected, were counted in a well counter (LKB 1282 Compugamma). Each GFR determination was a mean of two estimations which were always performed at a time when the animals had not been subject to other investigative procedures for at least 3 days.

*Plasma Creatinine*

Plasma samples collected at the termination of the experiment were analysed for creatinine concentration by an auto-analytical method (Rank-Hilger Chemispek).

*Urinary Concentrating Ability*

Urinary concentrating ability was measured on two occasions separated by 1 week at the end of the study period.

Drinking water was withdrawn in the evening (from 17.00 h) and on the following morning urine samples were collected after 16 and 18 h of dehydration, emptying the bladder on each occasion (confirmed by ultrasound). The osmolality of the urine samples was measured immediately by freezing-point determination. If the osmolality of the second sample was greater than the first by more than 10% a further sample was collected between 19 and 20 h of dehydration. The urinary concentrating ability was assessed as the mean of the highest osmolality achieved during each of the two tests. Macrodantin was not given for 30 h prior to collection of these urine samples.

*Statistics*

Growth and function data were analysed by mean and standard deviation. Statistical tests of difference were applied to the small numbers of animals in the groups of this study by Wilcoxon's rank sum test where appropriate.

## Results

*Microbiology*

Of the 226 suprapubic urine samples examined a positive (*Klebsiella*) culture was obtained on only one occasion, from pig 287; group B2. This culture was taken in the last week of the study and all previous samples from this animal were negative.

*Ultrasound*

No dilatation of the upper urinary tracts was seen by ultrasound examination during the early post-operative period. Later in the study, splitting of the caliceal echoes was seen in one animal in group A1 (pig 297) and in all of the animals in group B2. However, progressive dilatation was seen in only one animal (pig 412; group B1), which developed evidence of more marked pelvicaliceal dilatation between weeks 16 and 19.

*Post Mortem Appearances*

No bladder wall hypertrophy was present in any of the animals without bladder outflow obstruction (group A). The ureters from these animals were of normal calibre, with the exception of one animal with VUR (pig 297; group A1) in which marginal ureteric dilatation was present.

In all of the animals with bladder outflow obstruction (group B) there was bladder wall hypertrophy. This was most marked in two animals (pig 412; group B1 and pig 416; group B2) and was also detected by ultrasound examination towards the end of the study. In group B animals, ureteric dilatation was present only in those with VUR (group B1). Dilatation was moderate in two animals (pigs 412 and 423) and marginal in three (pigs 286, 401 and 413).

With one exception, the kidneys from all animals were macroscopically normal and showed no evidence of hydronephrosis. In one animal from group B1 (pig 412) extensive segmental scarring was present. The terminal data from this animal were excluded from the statistical analysis of results.

*Urodynamic Findings*

The female animals (group A) were observed to void normally throughout the study. Conscious

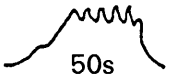
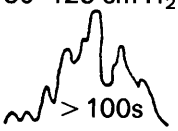
| *Category* | *Urodynamics* | | *Flow residual* |
|---|---|---|---|
| 1 | 20 cm $H_2O$  20s | Normal female voiding | |
| 2 | 35 cm $H_2O$  25s | Normal male voiding | Normal stream<br>No residual |
| 3 | 45 cm $H_2O$  50s | Prolonged void, raised end filling pressure | Slow stream<br>Small residual |
| 4 | 60 cm $H_2O$  60s | As 3, further increases in pressure and duration. Instability during filling | Slow/weak stream<br>Small residual |
| 5 | 27 cm $H_2O$  30s | Frequent low pressure voids of small volume | Weak stream<br>Small residual |
| 6 | 68 cm $H_2O$  50s | Prolonged void with phasic high pressure detrusor contractions | Barely continuous weak stream<br>Small residual |
| 7 | 80–125 cm $H_2O$  > 100s | As 6, with increases in duration of void, phasic detrusor contraction at high pressures | Intermittent, weak streams<br>Large residual volume |

**Fig. 2** Urodynamic parameters. Categories 1 and 2 represent normal voiding patterns. Categories 3 and 4 indicate degrees of dysfunction in male animals as the result of urethral obstruction. The patterns in categories 5–7 were seen in only three male animals. Pig 412 progressed through categories 5–7 and developed renal scarring. Pigs 410 and 416 suddenly developed category 7, changes interpreted as incipient complete obstruction. After enlargement of the urethral rings the urodynamic patterns reverted to category 3.

voiding cystometrograms obtained at the end of the study in this group showed a simple bladder contraction with a peak voiding pressure (PVP) of up to 25 cm $H_2O$ (median 20). The duration of voiding varied between 10 and 30 s.

By the end of the study period the male animals with a urethral ring (group B) all voided at higher PVPs than the females. Voiding was prolonged and often associated with raised end filling pressures and sustained detrusor contractions (for up to 120 s). In some animals unstable detrusor activity was observed with pronounced pre- and post-void contractions. In two animals (pigs 410 and 416) in group B2 it was necessary to enlarge the urethral ring latterly because of the development of incipient detrusor decompensation characterised by a large residual volume. In Figure 2 the urodynamic parameters are ranked to provide an index of the functional disturbance to which the kidney is subjected in the presence of VUR. No relationship exists between individual GFR determinations (standardised to 30 kg body weight) and the

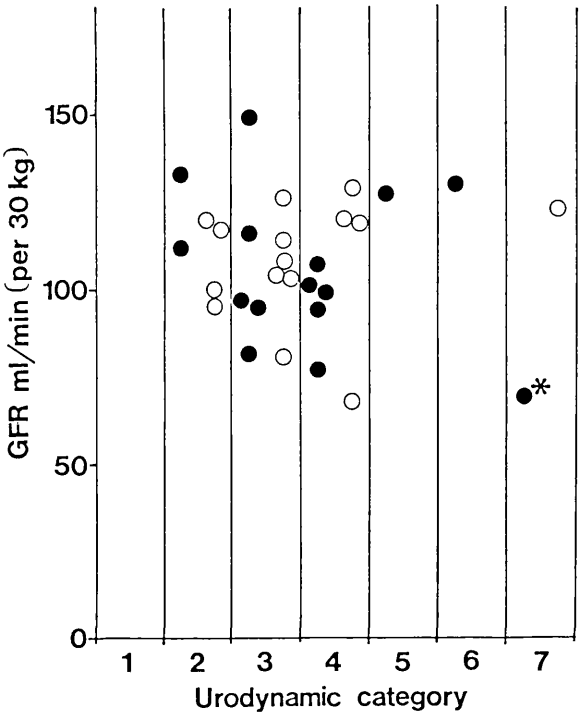



**Fig. 3** Urodynamic categories (see Fig. 2) in male animals plotted against GFR. (●—group B1; ○—group B2). No relationship is demonstrated. (*pig 412—final study. See text).

prevailing urodynamic status in animals from group B with bladder outflow obstruction (Fig. 3).

*Growth*

Renal growth was assessed by measurement of post mortem kidney weight. Table 1 shows the age, body weight and kidney weight (actual kidney weight and kidney weight/30 kg body weight) of individual pigs at the end of the study.

*Kidney Weight*

*Group A (females—no bladder outflow obstruction).* The values for renal weight at the end of the study were almost the same in the animals of the refluxing group A1 and the non-refluxing group A2. To eliminate the influence of body weight on kidney weight the latter was also expressed as g/30 kg body weight; the difference between groups A1 and A2 was not statistically significant.

*Group B (males—with bladder outflow obstruction).* The mean values for kidney weight and kidney weight/30 kg were slightly lower in the refluxing animals of group B1 compared with those in the non-refluxing group B2, but the differences between the two were not statistically significant.

**Table 1** Details of Individual Animals

| | *Females—normal bladder function* | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Group A1 (Reflux)* | | | | | | | | | *Group A2 (No reflux)* | | | | | | |
| *Pig No:* | *279* | *291* | *296* | *297* | *408* | *419* | | | | *278* | *281* | *290* | *295* | *299* | *407* | *418* |
| Age (weeks) | 26 | 26 | 28 | 29 | 26 | 28 | 27.2 | Mean | 26.1 | 26 | 27 | 25 | 26 | 26 | 25 | 28 |
| | | | | | | | 1.3 | SD | 1.1 | | | | | | | |
| Pig weight (kg) | 36 | 31 | 35 | 34 | 31 | 41 | 34.5 | Mean | 32.4 | 33 | 31 | 33 | 30 | 33 | 32 | 35 |
| | | | | | | | 3.4 | SD | 1.6 | | | | | | | |
| Kidney weight (g) | 101 | 108 | 110 | 90 | 101 | 117 | 104.5 | Mean | 110.0 | 114 | 105 | 109 | 97 | 101 | 129 | 115 |
| | | | | | | | 9.3 | SD | 10.6 | | | | | | | |
| Kidney weight (g/30 kg pig weight) | 84 | 105 | 94 | 79 | 98 | 88 | 91.3 | Mean | 102.0 | 104 | 102 | 99 | 97 | 92 | 121 | 99 |
| | | | | | | | 9.5 | SD | 9.2 | | | | | | | |
| | *Males—abnormal bladder function* | | | | | | | | | | | | | | |
| | *Group B1 (Reflux)* | | | | | | | | | *Group B2 (No reflux)* | | | | | | |
| *Pig No:* | *286* | *401* | *412* | *413* | *423* | | | | | *285* | *287* | *298* | *410* | *416* | | |
| Age (weeks) | 25 | 25 | 29 | 24 | 29 | | 26.4 | Mean | 25.4 | 25 | 27 | 23 | 26 | 26 | | |
| | | | | | | | 2.4 | SD | 1.5 | | | | | | | |
| Pig weight (kg) | 36 | 34 | 35 | 33 | 37 | | 34.0 | Mean | 34.0 | 38 | 35 | 32 | 30 | 35 | | |
| | | | | | | | 1.6 | SD | 3.1 | | | | | | | |
| Kidney weight (g) | 144 | 123 | 140 | 120 | 121 | | *127.0 | Mean | 135.8 | 123 | 164 | 172 | 93 | 127 | | |
| | | | | | | | 11.4 | SD | 32.3 | | | | | | | |
| Kidney weight (g/30 kg pig weight) | 120 | 109 | 120 | 109 | 98 | | *109.0 | Mean | 122.6 | 97 | 141 | 161 | 95 | 119 | | |
| | | | | | | | 9.0 | SD | 28.5 | | | | | | | |

* Excludes data from pig 412

**Table 2** Glomerular Filtration Rate for Each Animal at Each Time Point and Plasma Creatinine and Maximum Urinary Concentrating Ability at the End of the Study

| | *Females—normal bladder function* | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Group A1 (Reflux)* | | | | | | | *Group A2 (No reflux)* | | | | | | |
| *Pig No:* | *279* | *291* | *296* | *297* | *408* | *419* | | *278* | *281* | *290* | *295* | *299* | *407* | *418* |
| Glomerular filtration rate (ml/min) | | | | | | | | | | | | | | |
| Weeks 4–5 | 38 | 64 | 62 | 49 | 43 | 57 | 52 Mean<br>10.5 SD | 48<br>7.1 | 58 | 45 | 53 | 44 | 37 | 44 | 52 |
| Weeks 8–10 | 45 | — | — | 60 | 88 | 72 | 66 Mean<br>18.2 SD | 68<br>15.4 | 71 | 55 | 91 | 59 | 53 | — | 81 |
| Weeks 12–13 | 53 | — | — | — | — | 90 | — Mean<br>— SD | 89<br>20.9 | 109 | 64 | 107 | 65 | 71 | 103 | 102 |
| Weeks 15–19 | 94 | 102 | 100 | 87 | 101 | 108 | 99 Mean<br>7.3 SD | 101<br>11.6 | 116 | 83 | 101 | 103 | 88 | 106 | 109 |
| Plasma creatinine (μmol/l) | 89 | 80 | 79 | 101 | 89 | 72 | 87 Mean<br>13.6 SD | 82<br>8.5 | 79 | — | 77 | 79 | 95 | 89 | 72 |
| Osmolality (mosmol/kg) | 946 | 910 | 842 | 859 | 933 | 856 | 891 Mean<br>44 SD | 1079<br>111 | 1069 | 1065 | 1205 | 1215 | 1073 | 927 | 950 |

| | *Males—abnormal bladder function* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Group B1 (Reflux)* | | | | | | *Group B2 (No reflux)* | | | | | |
| *Pig No.* | *286* | *401* | *412* | *413* | *423* | | *285* | *287* | *298* | *410* | *416* | |
| Glomerular filtration rate (ml/min) | | | | | | | | | | | | |
| Weeks 4–5 | 36 | 41 | 49 | 53 | 62 | −48 Mean<br>10.4 SD | 51<br>9.7 | 50 | 45 | 43 | 47 | 67 |
| Weeks 8–10 | 71 | 63 | 91 | 83 | 75 | 77 Mean<br>10.7 SD | 78<br>15.3 | 56 | — | 84 | 83 | 91 |
| Weeks 12–13 | — | — | — | — | — | | | 79 | 98 | — | — | — |
| Weeks 15–19 | 90 | 91 | 72 | 105 | 116 | 101* Mean<br>12.4 SD | 110<br>21.6 | 83 | 119 | 127 | 92 | 130 |
| Plasma creatinine (μmol/l) | — | 134 | 136 | 67 | 72 | 91* Mean<br>37.3 SD | 84<br>13.3 | 101 | — | 84 | 80 | 69 |
| Osmolality (mosmol/kg) | 962 | 887 | 629 | 1058 | 812 | 930* Mean<br>105 SD | 1089<br>151 | 1014 | 1321 | 1148 | 934 | 1029 |

*Excludes data from pig 412

*Renal Function*

The GFR determinations for each animal at each time point and the plasma creatinine concentrations and maximum urinary osmolalities at the end of the study are shown in Table 2.

*Glomerular Filtration Rate and Plasma Creatinine*

All GFR values for individual pigs are plotted sequentially against pig weight with separate plots for groups A1, A2 (Fig. 4) and B1, B2 (Fig. 5). Figure 6 shows the variation in GFR with time.

*Group A (females—no bladder outflow obstruction).* There was no significant difference in the GFR values between the refluxing group (A1) and the non-refluxing group (A2) either at the beginning or at the end of the study period.

There was no significant difference in the plasma creatinine values between groups A1 and A2.

*Group B (males—with bladder outflow obstruction).* There was no significant difference in the GFR values between the refluxing group (B1) and the non-refluxing group (B2) either at the beginning or at the end of the study.

The mean plasma creatinine concentration for the refluxing group (B1) was 91 μmol/l (SD 37.3). This excludes a value of 136 μmol/l obtained for the one pig which developed scars (412) and one sample which was lost. The mean value for group B2 was

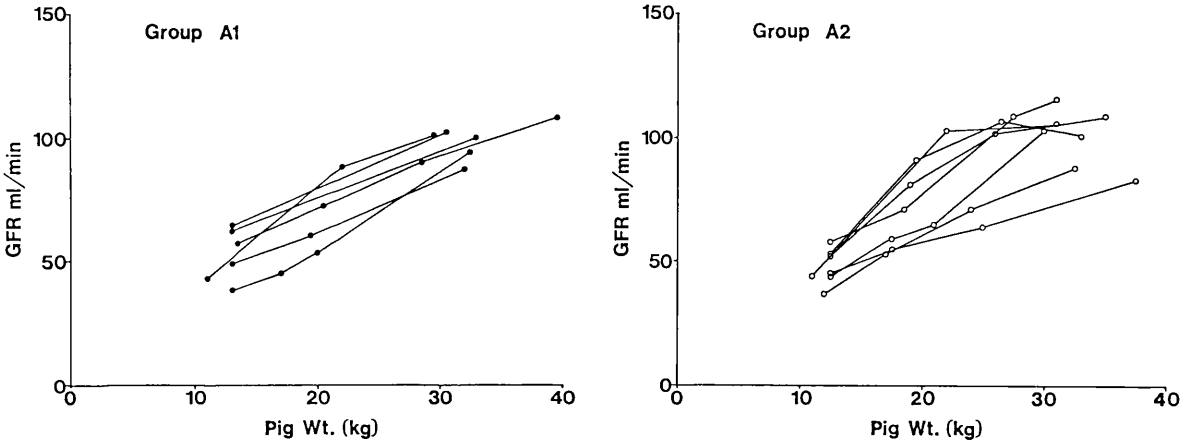



**Fig. 4** Glomerular filtration rate plotted against pig weight in female animals with (A1) and without (A2) VUR. Lines represent individual animals.

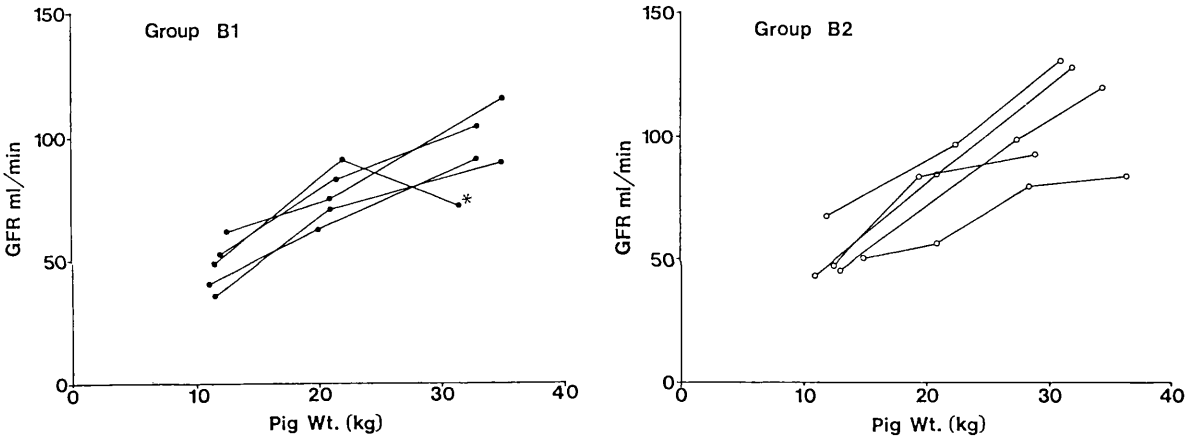



**Fig. 5** Glomerular filtration rate plotted against pig weight in male animals with urethral rings (B1 with and B2 without VUR). Lines represent individual animals. (*pig 412).

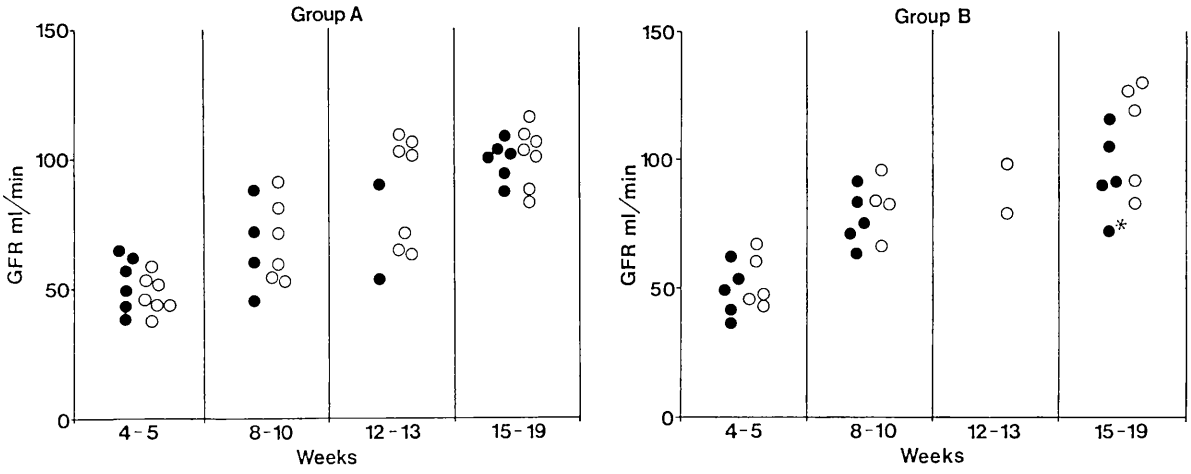



**Fig. 6** Glomerular filtration rates at the various time points for animals in groups A and B with (●) and without (○) VUR are compared (*pig 412—final study. See text).

84 μmol/l (SD 13.3). One sample was also lost from this group.

*Urinary Concentrating Ability*

*Group A (females—no bladder outflow obstruction).* At the end of the study there was a significant difference ($P<0.01$) in the maximum urinary osmolality between the refluxing group A1 and the non-refluxing group A2.

*Group B (males—with bladder outflow obstruction).* The mean maximum urinary osmolality was lower in the refluxing group B1 (mean 930, SD 105 mosmol/kg*) than in the non-refluxing group B2 (mean 1089, SD 151 mosmol/kg). This difference failed to reach statistical significance.

*Group A and Group B*

When all of the refluxing (A1 and B1) and the non-refluxing (A2 and B2) animals were compared, the mean maximum urinary osmolalities were significantly lower in the refluxing animals ($P<0.01$).

* Excludes data from pig 412.

## Discussion

This experimental study using the growing minipig aimed to investigate the renal consequences of VUR in terms of growth and function. The female animals provided a model of VUR with normal bladder storage and low voiding pressures. The male piglets had urethral rings, the object of which was to produce stable bladder outflow obstruction with prolonged voiding, raised detrusor pressure and abnormal storage dynamics. On the whole we were successful in achieving the desired stable urodynamic state in the group B animals, but there were occasional difficulties stemming from the tendency for the degree of outflow obstruction produced by the ring to increase as the animals grew. In two of the animals it was necessary to enlarge the ring because of the development of incipient complete obstruction. Further, in one other animal (pig 412), which was excluded from the statistical analysis, there was late detrusor decompensation with renal scarring, a pattern observed in a previous study (Ransley *et al.*, 1984). Thus group B exemplifies the most severe conditions in which the effects of VUR alone over a long period of time can be assessed experimentally in the pig model.

Male pigs have larger kidneys than females both as an absolute measure and when expressed for 30 kg body weight. As kidney weight may conceivably relate to GFR, the respective data for groups A and B were treated as two separate populations and it was considered inappropriate to pool these data (*i.e.* comparing all refluxing animals with all non-refluxing animals) for the statistical tests. We were unable to detect any effect of VUR on GFR over the study period, the results of GFR estimations showing no significant differences between refluxing and non-refluxing control groups after approximately 5 months of rapid growth even in the presence of elevated voiding pressures and abnormal bladder function.

Other experimental studies using various canine models have similarly failed to demonstrate any effects of VUR on renal function measured by clearance studies (King and Idriss, 1967; King and Sellards, 1971; Lenaghan *et al.*, 1972a and b; Danforth *et al.*, 1980; James *et al.*, 1981). Roberts *et al.* (1982) performed individual kidney function studies using $^{131}$I Hippuran in monkeys subjected to unilateral VUR. After 3 to 17 months there was no difference in effective renal plasma flow (ERPF) between the refluxing and contralateral normal kidney.

In a further primate model of unilateral reflux with bladder outflow obstruction, Mendoza and Roberts (1983) observed that when resting bladder pressures were less than 10 cm $H_2O$ there was no change in ERPF after 6 to 14 months of VUR. However, in four monkeys in which resting bladder pressures rose above 16 cm $H_2O$ the ERPF fell during the same period. In these animals there was both diffuse interstitial scarring and ureteric dilatation indicative of functional obstruction. It is interesting to note that the mean voiding pressures were the same in the two groups of animals and the urodynamic factor which separates them is the resting bladder pressure. This work underlines the importance of bladder storage pressures and instability occurring during filling compared with the brief exposure to high pressures during voiding.

Helin (1975) obtained quite different results in a study using six Landrace pigs. Following 3 to 4 months of uninfected unilateral VUR at normal bladder pressures, individual kidney GFR estimations were obtained by an invasive technique involving catheterisation of both renal arteries and veins in anaesthetised animals. A reduction in GFR was demonstrated in the refluxing kidneys compared with the contralateral non-refluxing controls. It is difficult to interpret this result since

there is no discussion of the size or morphology of the refluxing kidneys or ureters, or mention of any possible renal scarring or ureteric obstruction.

Clinical studies of reflux and renal function are fraught with problems which are difficult to overcome. The major difficulties are the presence of renal scarring in many children with gross reflux and the heterogeneous nature of the refluxing population. Once a kidney is scarred it is clearly invalid to compare its function with a non-refluxing kidney. In addition, radioisotope scintigraphy with $^{99m}$Tc dimercaptosuccinic acid (DMSA) has shown that the intravenous urogram on which many clinical studies are based is an insensitive measure of the extent of parenchymal loss with scarring. No conclusions with regard to the effects of VUR on renal function can be deduced from studies which fail to take renal scarring into account (Aperia *et al.*, 1976; Piepsz *et al.*, 1981). The study by Fritjofssen and Sundin (1966) is the closest parallel with the experimental model. In a follow-up study of nine adults with iatrogenic reflux, individual kidney inulin and PAH clearances were estimated. In six patients who maintained a sterile urine, renal function remained intact for up to 11 years.

In a prospective study of children with and without reflux investigated as a result of asymptomatic bacteriuria, Verrier Jones *et al.* (1984) documented that individual kidney GFR is not influenced by VUR alone. Individual kidney function remained stable over the 4-year study period for both scarred and unscarred kidneys irrespective of the presence of vesicoureteric reflux.

A deleterious effect of VUR on kidney function has been inferred from the demonstration of an improvement in GFR following successful anti-reflux surgery in children (Scott *et al.*, 1986). However, this study was retrospective, there was no documentation of urinary infection and there were no conservatively managed controls.

The Birmingham Reflux Study Group (1983) performed an excellent prospective and controlled clinical trial comparing surgical and conservative management of VUR. They found no significant difference in overall GFR after 2 years between the two groups. However, as they did not analyse separately the patients with bilateral reflux, this study does not provide information on the effects of reflux on individual kidney function. A clinical trial of successful surgery against successful conservative management (*i.e.* no break-through infection) for infants (less than 1 year of age) with gross bilateral reflux, which is designed to detect any effect of reflux alone on renal function, is in progress at our own institution. The GFR data show the normal increase in GFR with age and no significant difference between the two groups after 2 years. The 5-year data are awaited with interest.

The information presently available regarding reflux and renal function, both experimental and clinical, is very limited. The experimental studies, including our own, indicate that when reflux occurs in the absence of infection and under urodynamic conditions which are insufficient to initiate the scarring process, renal function measured by clearance studies is sustained even through a period of rapid renal growth. The clinical investigations which allow an assessment of the effects of VUR in the absence of renal scarring strongly support the contention that VUR alone does not adversely affect renal function.

### *Urinary Concentrating Ability*

In the present study, maximum urinary concentrating ability was reduced in the animals with VUR compared with the non-refluxing controls. The difference was statistically significant when all of the refluxing animals (groups A1 and B1) were compared with the non-refluxing animals (groups A2 and B2). Since there is no evidence that concentrating ability is related to renal mass, the objections to pooling these data cited in the discussion of GFR (*vide supra*) need not apply. Considering only the animals with normal bladder function (groups A1 and A2), the difference was significant. However, in those with abnormal bladder function (groups B1 and B2), although the concentrating ability was reduced in the presence of reflux, the difference failed to reach statistical significance. It is noteworthy that even in the refluxing animals (with the exclusion of pig 412) the individual values for maximum concentrating ability were above the level of 800 mosmol/kg, taken in clinical practice as the lower limit of normal.

Walker *et al.* (1973) reported a similar reduction in renal concentrating ability in children with VUR. However, measurements of urinary concentrating ability were performed only 6 weeks after the elimination of urinary infection, which alone may reduce concentrating ability for up to 12 weeks after its eradication (Winberg, 1958). In a study of 115 children with urinary tract infections with and without reflux, Uehling (1971) demonstrated that an improvement in concentrating ability is related to the control of infection and not to the control of reflux.

*Renal Growth*

In this study kidney growth was assessed in terms of its result, *i.e.* the weight of the kidney at the end of the experiment. That rapid growth had occurred was confirmed by the approximately eight-fold increase in kidney weights at the end of the study compared with the weights of the nephrectomy specimens at the beginning. The use of kidney weight as an index of growth is validated by the observation of Sands *et al.* (1979) that in the rat kidney weight gain is proportional to the increase in cell number. Previous unpublished data from this laboratory indicate that the same is true for the pig with regard to kidneys undergoing normal growth and compensatory growth after unilateral nephrectomy. Despite rapid kidney growth during the course of this experiment, there was no significant effect of reflux on growth even in the presence of abnormal bladder function.

Other experimental studies relating VUR with renal growth are few. King and Sellards (1971) and Danforth *et al.* (1980), using canine models, appear to support our findings that VUR does not affect renal growth. On the other hand, a number of clinical studies have suggested different conclusions. The problems encountered in clinical studies of reflux and renal function also apply to those of reflux and renal growth. There are additional difficulties concerning the choice and use of parameters of radiographic renal size as an index of growth and their comparison with "normal" standards, of which there are a number (Hodson *et al.*, 1962; Hodson *et al.*, 1975a; Eklof and Ringertz, 1976; Claesson *et al.*, 1981).

Lenaghan *et al.* (1976) associated impaired renal growth in patients with VUR with the presence of renal scarring or urinary tract infection. These important factors are ignored in the retrospective analyses of Ibsen *et al.* (1977) and Orikasa *et al.* (1978), who nevertheless infer that VUR has a deleterious effect on renal growth. Willscher *et al.* (1976) reported results which appear to show that renal growth is accelerated after anti-reflux surgery whether or not pyelonephritic scarring is present. This study is frequently quoted to substantiate the hypothesis that VUR impairs renal growth. However, it is incorrect to imply this from a study limited to the examination of renal growth after anti-reflux surgery which does not claim to consider renal growth in the presence of VUR. Indeed these authors reported that the pre-operative lengths of 103 refluxing kidneys without scarring was not significantly different from normal. The children studied ranged in age between 6 months and 14 years at the time of operation and were followed for 6 months to 7 years. The results representing renal length are presented graphically as a mean (without standard error) plotted against the mean for age. It is unacceptable to use mean values in this way when growth is non-linear up to 5 years of age. The mean age at operation was 3.4 years and the majority of renal length measurements both pre- and post-operatively were therefore obtained during a non-linear phase of renal growth. The results of Atwell and Cox (1981) in a study on post-operative renal growth suffer from similar problems. Their conclusions that observed mean growth was greater than the expected "normal" growth are affected by including kidneys from children as young as 1 month, and comparing the growth with the linear growth expected in older children. However, this study is interesting in that there was no difference between the lengths of the refluxing and non-refluxing kidneys pre-operatively, even including scarred kidneys.

The Birmingham Reflux Study Group (1983) did stratify patients by age when analysing the length (Eklof and Ringertz, 1976) of 135 kidneys in patients with severe unilateral or bilateral reflux. After 2 years of surgical or medical management, renal growth was retarded irrespective of treatment. However, approximately half of the kidneys followed had radiological evidence of scarring. In a further trial of operative versus conservative treatment, De Gracia and Brueziere (1984) showed that in 40 kidneys with reflux but without dilated ureters or renal scarring, growth was normal in both groups after 10 to 18 years. The discrepancy between these latter two studies could be attributed to the severity of reflux. However, in a prospective study of 111 kidneys with proven VUR in children managed conservatively for 2 to 22 years, Smellie *et al.* (1981) showed that impaired renal growth was associated independently with infection and renal scarring, but not the severity of reflux. These results were supported by those of Riebel *et al.* (1982), who followed 46 kidneys of conservatively managed patients for 1 to 6 years. These latter studies corroborate the results of this investigation—that in the presence of reflux without urinary tract infection or scarring, renal growth is normal.

It is clear that the evaluation of the renal consequences of VUR have been confounded by repetitive inaccurate interpretation of the literature. Clinical data are often difficult to analyse due to the common association and therefore the combined effects of infection and reflux. There is a

frequent tendency to ignore the infection problem when quoting reflux data and such errors are reinforced by repeated citation.

## Acknowledgements

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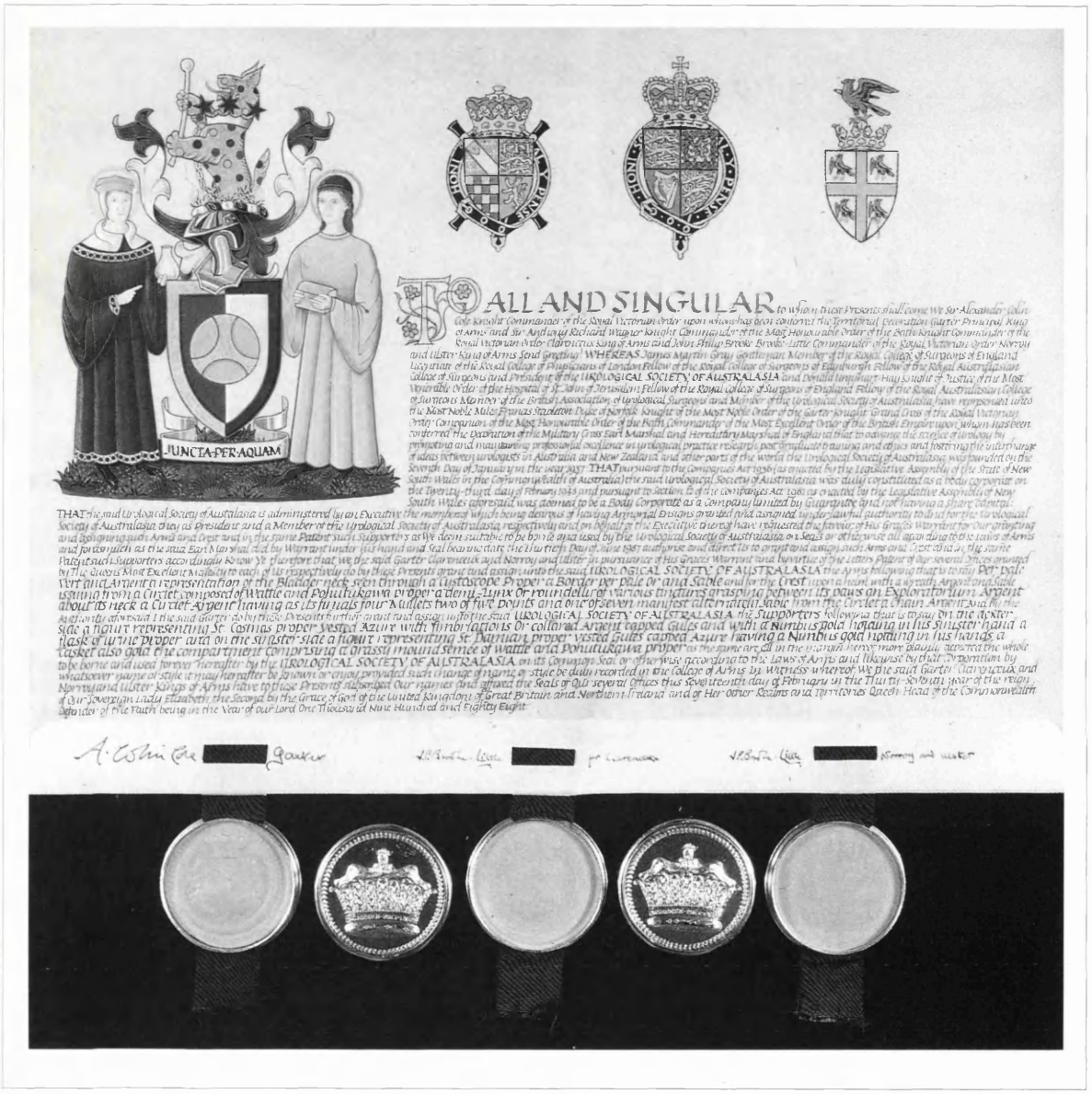


**Fig. 2** Letters Patent.

of St Cosmos proper vested azure with fimbriations or collared argent capped gules with a nimbus gold holding in his sinster hand a flask of urine proper and on the sinster a representation of St Damian proper vested gules capped azure with a nimbus gold holding in his hands a casket gold.

Motto: *juncta per aquam.*

The Society takes great pride in this royal recognition and honour.

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# Effect of Unilateral Vesicoureteric Reflux on Renal Growth and the Uptake of $^{99m}$Tc DMSA by the Kidney. An Experimental Study in the Minipig

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Summary—The influence of unilateral vesicoureteric reflux (VUR) on renal growth and the uptake of $^{99m}$Tc DMSA by the kidney has been investigated in a 2-kidney model in the growing minipig over a period of approximately 5 months. Animals with reflux in association with low voiding pressures and normal bladder function (n = 5), as well as those with raised voiding pressures and abnormal bladder function (n = 7), were investigated with appropriate non-refluxing controls (n = 12). Urinary infection and renal scarring were avoided since these factors may affect kidney function and growth independently.

Statistical tests of difference failed to demonstrate any effect of VUR on renal growth or renal uptake of $^{99m}$Tc DMSA even in the presence of elevated voiding pressures and abnormal detrusor function.

In a recent experimental study using a 1-kidney pig model (Ransley *et al.*, 1987) we were unable to demonstrate any effect of vesicoureteric reflux on renal growth or function measured by glomerular filtration rate (GFR). VUR was, however, associated with a small decrease in urinary concentrating ability.

We now report an investigation which was carried out in parallel with and mostly employed litter mates of the animals used in the work cited above. For this study both kidneys were retained and VUR induced on one side only. The 2-kidney model allows the performance of one kidney to be compared with that of the other, so minimising any effects of variation between animals on the results. The model is suitable for examining the influence of VUR on the renal uptake of $^{99m}$Tc dimercaptosuccinic acid (DMSA), using the contralateral kidney for purposes of comparison. It also provides information on the effects of unilateral VUR on renal growth in the presence of a non-refluxing contralateral system.

As in the first series, the effects of VUR in the presence of both normal bladder function and bladder outflow obstruction were considered and we sought to eliminate the complicating factors of urinary infection and renal scarring so that the influence of VUR alone could be evaluated.

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## Material and Methods

### *Experimental design*

A series of 27 piglets were assigned to refluxing or non-refluxing groups (Fig. 1A) and followed for approximately 5 months. The investigation protocol is outlined in Figure 1B. The animals were weighed weekly and the first determinations of DMSA uptake (in both groups A and B) and bladder voiding pressures by cystometry (CMG: group B) were undertaken 4 weeks after the animals had achieved a weight of approximately 7.5 kg (study week 0). At the end of the study period (weeks 15–19) all animals had determinations of GFR, bladder voiding pressures, urinary concentrating ability and renal uptake of DMSA. The animals in group B had an additional CMG, generally at week 10. At the end of the study period all animals were killed, the urinary tracts removed and the kidneys weighed.

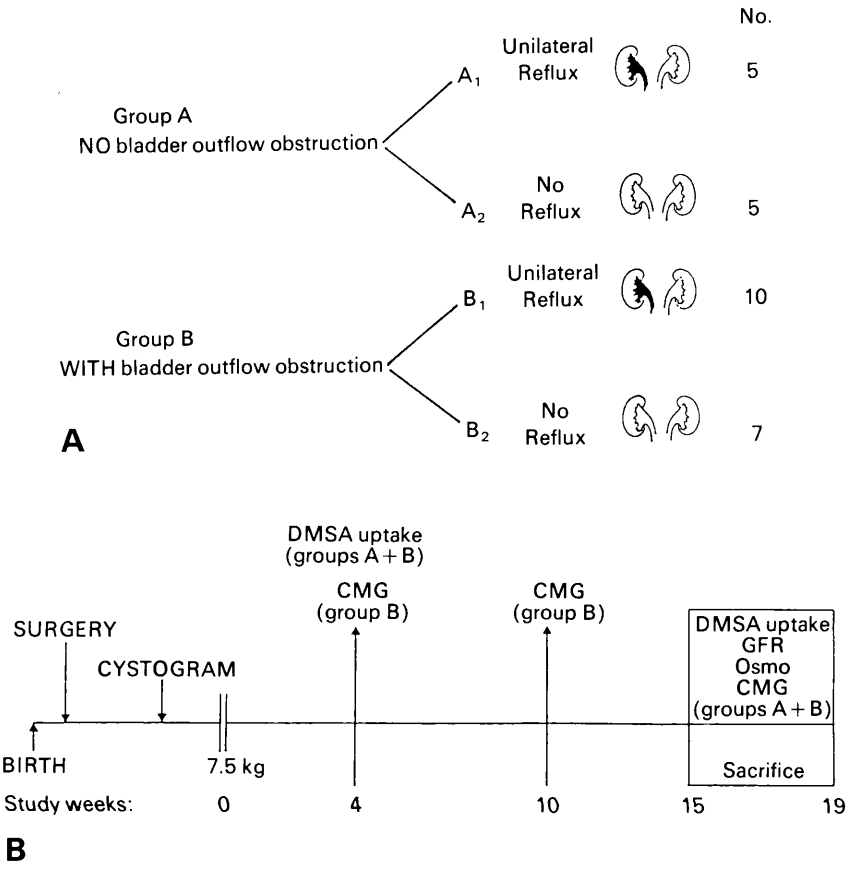


**Fig. 1** (A) Experimental design. (B) Experimental protocol. Surgery: Group A—creation of VUR (A1); Group B—application of urethral ring (B1, B2); creation of VUR (B1). DMSA uptake: quantification of renal uptake of DMSA. CMG: cystometry. GFR: $^{51}$Cr EDTA glomeruler filtration rate. Osmo: urinary concentrating ability.

*Experimental model*

In weanling animals of group A1 and B1, left unilateral VUR was induced under halothane anaesthesia, by resecting the roof of the intravesical tunnel as described previously (Ransley and Risdon, 1978). In addition, animals in group B (both refluxing and non-refluxing) had a ring of silver wire (diameter 5–7 mm) placed around the urethra well below the bladder neck.

The animals had frequent ultrasound examination of the urinary tract for a week post-operatively and subsequently at the times of urine sampling (*vide infra*), using a Phillips SonoDiagnost R with linear array transducer.

Cystography to confirm the presence of VUR was performed as described previously (Ransley *et al.*, 1984) approximately 2 weeks after surgery in animals from groups A1 and B1.

Rectal temperatures were taken daily and urine samples were obtained by suprapubic puncture at approximately 2-weekly intervals for microbiological culture and microscopy of the centrifuged deposit. All animals received oral Macrodantin (50–200 mg/day) for the duration of the experiment, in addition to a single intravenous injection of gentamicin (2 mg/kg body weight) following surgery and manipulations involving suprapubic puncture.

Conscious filling and voiding cystometry, $^{51}$Cr EDTA GFR and urinary concentrating ability were estimated as described previously (Ransley *et al.*, 1984, 1987) at the intervals detailed (Fig. 1B).

*Renal growth*

Renal growth was assessed by the weight of the kidneys at the end of the study and the relative weight calculated from the percentage ratio of the weight of the left kidney to the total renal weight (100*L/L+R).

*Renal uptake of DMSA*

DMSA (CisR) was radiolabelled using a pertechnetate generator (Amersham International plc) to provide a 5 ml solution containing 750MBq of activity. Precautions were taken to reduce pertechnetate contamination and to preserve the optimal DMSA-Tc complex as described previously (Godley *et al.*, 1985).

A weighed dose (approximately 25 µl/kg body weight) of DMSA was injected intravenously using a butterfly needle. At accurately timed intervals approximately 6 and 24 h later the renal activity was recorded (Nuclear Enterprises Mark V, high sensitivity collimation) for a pre-set time of 100 s in the posterior projection with the animal lying prone and in contact with the gamma camera face. The animals were lightly anaesthetised for each procedure. The dual area selection facility permitted 2 superimposed regions of interest of different sizes to be positioned over each kidney image to determine renal activity with background subtraction. The mean renal depth for each kidney was assessed ultrasonically and a correction made for attenuation of activity by tissue. The correction factor was derived from a graph obtained independently using a perspex phantom.

The renal uptake of DMSA was calculated as the percentage of the administered dose (uptake %/dose) as described previously (Godley *et al.*, 1985). The activity of the dose was calculated from the weight of the dose and the activity of a known weight of a standard drawn from the same preparation of DMSA and subtracting the activity of the residue in the dose syringe and butterfly. The activity of the standard, the residue and each kidney in turn was recorded from the same marked area of the gamma camera face.

The relative renal uptake of DMSA was calculated from the percentage ratio of the uptake by the left kidney to the total uptake by both kidneys (100* L/L+R).

At the end of the study period each renal uptake was a mean of 2 estimations separated by 1 week. The animals were usually killed following acquisition of the 24-h renal activity in the final uptake study. The activity of the isolated kidneys taken from 23 animals was recorded and the uptake calculated from these *ex situ* kidneys was compared with that obtained from the 24-h acquisition *in situ*.

*Statistics*

Regression analysis was applied to the log transformed ratio values and the interaction between bladder outflow obstruction and VUR was tested. If there was no interaction ($P > 0.1$), then separate estimates of the effects of bladder outflow obstruction and VUR were calculated, in each case taking the other variable into account.

The method of Altman and Bland (1983) was used to assess the agreement between the DMSA uptake calculated from the activity of the kidneys *in situ* and that calculated from the *ex situ* kidneys of the same animal.

## Results

*Experimental model*

The data from 3 animals were excluded from the analysis because these animals did not conform with the otherwise homogeneous study group.

One of these animals developed incipient bladder decompensation (urodynamic category 7, *vide infra*) with a large residual urine volume. There was marked bladder wall hypertrophy, unilateral ureteric dilatation and the kidney showed some limited scarring.

In the second animal excluded, the left (refluxing) kidney was seen to be smaller than the contralateral kidney on the initial DMSA renal image at study week 4. Post mortem examination showed a dilated left ureter and fibrosis around the vesicoureteric junction; these appearances suggested that some early post-operative obstruction had occurred despite the fact that we had failed to detect this by ultrasound during this period.

The third animal was excluded because 2 positive urine cultures were obtained between study weeks 10 and 12. The relative kidney weight value for this animal was 3.7 standard deviations from the expected mean and thus appeared to be an outlier.

For the animals included in the experimental analysis no positive cultures were obtained from any of the urine samples and all kidneys were macroscopically normal, with no evidence of hydronephrosis. Bladder wall hypertrophy and minimal ureteric dilatation associated with VUR was present only in the animals from group B (with abnormal bladder function).

*Renal function*

At the end of the study the values for GFR/30 kg body weight and maximum urinary osmolality were similar for all 4 groups (A1, A2, B1, B2). These parameters were not the subject of primary investigation in this study but demonstrate the functional uniformity between the groups; the means and ranges of these values are shown in Table 1, together

**Table 1** Age, Weight, Glomerular Filtration Rate and Urinary Concentrating Ability for Each Group at End of Study

| | *Normal bladder function* | | | | *Abnormal bladder function* | | | |
|---|---|---|---|---|---|---|---|---|
| | *Group A1* *Unilateral reflux* | | *Group A2* *No reflux* | | *Group B1* *Unilateral reflux* | | *Group B2* *No reflux* | |
| No. of animals | 5 | | 5 | | 7 | | 7 | |
| | Mean | Range | Mean | Range | Mean | Range | Mean | Range |
| Age (weeks) | 25.8 | 22–27 | 24.8 | 22–30 | 27.7 | 25–29 | 26.1 | 21–31 |
| Weight (kg) | 34.4 | 31–42 | 33.6 | 32–35 | 31.4 | 27–39 | 34.6 | 32–39 |
| GFR (ml/min/30 kg body weight) | 105 | 91–127 | 111 | 100–138 | 103 | 89–133 | 109 | 86–130 |
| Osmolality (mosmol/kg) | 1137 | 964–1258 | 1139 | 1000–1267 | 1125 | 934–1249 | 1094 | 938–1206 |

with those for age and body weight for each group of animals at the end of the study.

*Urodynamic findings*

As in the previous study (Ransley *et al.*, 1987), each urodynamic study was ranked (1–7) to provide an index of the functional disturbance to which the kidney was subjected in the presence of VUR.

The group A animals were observed to void normally throughout the study. Voiding cystometrograms at the end of the study showed a simple bladder contraction with a peak voiding pressure of up to 30 cm $H_2O$ (median 25; females category 1, males category 2).

At the end of the study period the animals with a urethral ring (group B) all voided at higher detrusor pressures (50–75 cm $H_2O$, median 60) than the group A animals. Voiding was slow (up to 90s), incomplete and sometimes associated with a raised end filling pressure (category 3). In some animals unstable detrusor activity was observed with pronounced pre- and post-void contractions (category 4). The category for the 3 urodynamic studies in each animal of group B is shown in Figure 2.

*Growth*

The individual and relative kidney weights for each animal are shown in Table 2. There was no interaction between bladder function and reflux ($t_{20}=0.037$) and no significant effect of reflux on relative kidney weight ($t_{20}=0.77$).

*Renal uptake of DMSA*

The results of the differences between the *in situ* and *ex situ* estimates of DMSA renal uptake are shown in Table 3. There was good agreement for both the uptake %/dose and the relative uptake.

The uptake %/dose for individual kidneys and the relative uptake values for each animal at study week 4 and at the end of the study period are shown in Table 4. There was no interaction between reflux and bladder function either at week 4 ($t_{19}=0.88$) or at the end of study period ($t_{20}=0.62$) and no significant effect of reflux on relative uptake values either at study week 4 ($t_{19}=0.14$) or at the end of the study period ($t_{20}=0.13$).

## Discussion

This experimental study employed a 2-kidney model in the growing pig in which unilateral VUR (consistently left-sided) was induced in the test groups. The effects of VUR were examined in animals with normal bladder function and low voiding pressures as well as in animals with abnormal bladder function induced by the application of a urethral ring and characterised by prolonged voiding at elevated detrusor pressures and sometimes by unstable contractions during bladder filling.

The urine was kept sterile and the size of the

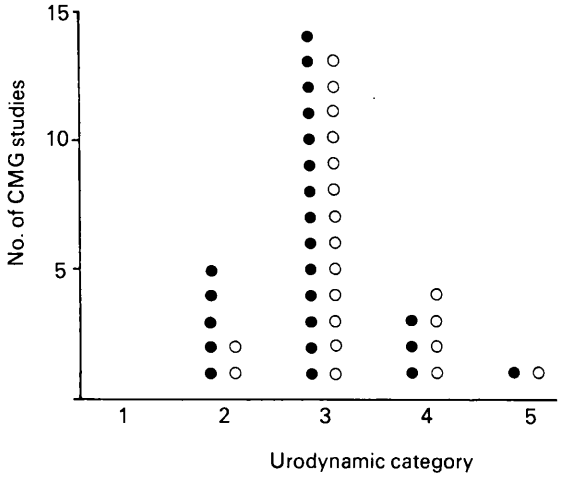


**Fig. 2** Urodynamic categories (see text) for each of the urodynamic studies in animals of group B. (●: B1; ○: B2)

**Table 2** Kidney Weights and Left Relative Kidney Weights (100* L/L+R) for each Animal

| | *Normal bladder function* | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Group A1 (Unilateral reflux)* | | | | | | | | | | *Group A2 (No reflux)* | | | | | | |
| *Pig. no.* | — | — | *280* | *289* | *293* | *403* | *411* | | | | *409* | *405* | *294* | *282* | *277* | — | — |
| Kidney weight (g) | | | | | | | | | | | | | | | | | |
| Left | — | — | 82 | 57 | 75 | 58 | 61 | 66.6 | Mean | 67.8 | 68 | 63 | 66 | 56 | 86 | — | — |
| | | | | | | | | 11.2 | SD | 11.1 | | | | | | | |
| Right | — | — | 82 | 55 | 73 | 52 | 61 | 64.6 | Mean | 64.4 | 69 | 57 | 61 | 52 | 83 | — | — |
| | | | | | | | | 12.6 | SD | 12.1 | | | | | | | |
| Relative kidney weight % | | | | | | | | | | | | | | | | | |
| Left | — | — | 50.0 | 50.8 | 50.7 | 52.7 | 50.0 | 50.8 | Mean | 51.4 | 49.6 | 52.5 | 52.0 | 51.9 | 50.8 | — | — |
| | | | | | | | | 1.0 | SD | 1.0 | | | | | | | |
| | *Abnormal bladder function* | | | | | | | | | | | | | | | | |
| | *Group B1 (Unilateral reflux)* | | | | | | | | | | *Group B2 (No reflux)* | | | | | | |
| *Pig. no.* | *283* | *421* | *425* | *426* | *427* | *428* | *429* | | | | *430* | *424* | *422* | *420* | *415* | *400* | *292* |
| Kidney weight (g) | | | | | | | | | | | | | | | | | |
| Left | 72 | 63 | 69 | 72 | 54 | 52 | 61 | 63.3 | Mean | 75.6 | 57 | 100 | 66 | 73 | 69 | 78 | 86 |
| | | | | | | | | 8.2 | SD | 14.1 | | | | | | | |
| Right | 71 | 59 | 68 | 71 | 56 | 51 | 64 | 62.9 | Mean | 73.1 | 57 | 91 | 66 | 74 | 65 | 74 | 86 |
| | | | | | | | | 7.8 | SD | 12.0 | | | | | | | |
| Relative kidney weight % | | | | | | | | | | | | | | | | | |
| Left | 50.6 | 51.6 | 50.4 | 50.3 | 49.1 | 50.5 | 48.8 | 50.1 | Mean | 50.7 | 50.0 | 52.4 | 50.0 | 49.6 | 51.5 | 51.3 | 50.0 |
| | | | | | | | | 1.0 | SD | 1.0 | | | | | | | |

**Table 3** Comparison between DMSA Uptake Calculated from the Kidneys within the Animal (*in situ*) with that Calculated from the same Kidneys Isolated after *post mortem* (*ex situ*)

| | *In situ—ex situ difference* | | *Confidence limits 95% (+/−2SE)* |
|---|---|---|---|
| | *Mean* | *SD* | |
| Uptake %/dose | | | |
| Left | −0.64 | 1.46 | −1.24 to +0.04 |
| Right | +0.73 | 1.63 | −0.02 to +1.44 |
| Relative uptake % | | | |
| Left | −1.30 | 1.26 | −1.85 to +0.75 |

urethral ring in the high pressure groups (B1 and B2) was tailored to provide stable bladder outflow obstruction without hydronephrosis. This was because we wished to avoid complicating factors such as infection, renal scarring and upper tract obstruction in examining the effects of VUR on the kidney. For these reasons, 3 animals (1 with upper tract obstruction, 1 with renal scarring and 1 with urinary tract infection) were excluded from the study. The present investigation complements a parallel one already published (Ransley *et al.*, 1987) which examined the effcts of sterile VUR in a 1-kidney model. The use of the 2-kidney model has allowed us to examine the effcts of VUR on renal growth in the presence of a non-refluxing contralateral system and to extend the functional studies to include the influence of VUR on the renal uptake of DMSA.

### *Renal growth*

The effect of left unilateral VUR on the growth of the kidneys was assessed from consideration of the weight of the left kidney relative to the total renal weight, in comparison with non-refluxing control animals. The validity of using weight as an index of growth was discussed previously (Ransley *et al.*, 1987).

By the end of the experimental period the individual kidneys had achieved a weight of between 60 and 80 g. This implies that in the approximately 5-month period from the induction of reflux to the end of the study, the kidney weights had increased 4- to 5-fold when compared with the weights of the kidneys (mean 16.5 g) removed at unilateral nephrectomy in the parallel study from animals of the same age and body weight. Despite

**Table 4** Uptake of DMSA by Individual Kidneys (uptake %/dose) and Left Relative Uptake (100*L/L+R) for each Animal at Week 4 and at End of Study Period

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Normal bladder function* | | | | | | | | | | | | | | | | |
| | *Group A1 (Unilateral reflux)—pig no.* | | | | | | | | | | *Group A2 (No reflux)—pig no.* | | | | | | |
| | — | — | *280* | *289* | *293* | *403* | *411* | | | | *409* | *405* | *294* | *282* | *277* | — | — |
| Uptake %/dose | | | | | | | | | | | | | | | | | |
| Week 4 | | | | | | | | | | | | | | | | | |
| Left | — | — | —* | 21.3 | 17.8 | 25.0 | 23.9 | 22.0 | Mean | 20.7 | 18.7 | 23.3 | 21.3 | 19.9 | 20.3 | — | — |
| | | | | | | | | 3.2 | SD | 1.7 | | | | | | | |
| Right | — | — | —* | 22.8 | 17.2 | 24.1 | 19.4 | 20.9 | Mean | 20.5 | 22.0 | 20.5 | 22.1 | 19.9 | 18.1 | — | — |
| | | | | | | | | 3.2 | SD | 1.7 | | | | | | | |
| Weeks 15–19 | | | | | | | | | | | | | | | | | |
| Left | — | — | 18.8 | 17.8 | 20.1 | 21.2 | 21.2 | 19.8 | Mean | 20.8 | 19.5 | 23.0 | 19.8 | 20.2 | 21.4 | — | — |
| | | | | | | | | 1.5 | SD | 1.4 | | | | | | | |
| Right | — | — | 20.8 | 19.6 | 19.9 | 20.2 | 22.7 | 20.6 | Mean | 21.6 | 19.5 | 22.8 | 22.0 | 20.8 | 22.7 | — | — |
| | | | | | | | | 1.2 | SD | 1.4 | | | | | | | |
| Relative uptake % | | | | | | | | | | | | | | | | | |
| Week 4 | | | | | | | | | | | | | | | | | |
| Left | — | — | —* | 48.3 | 50.9 | 50.9 | 55.2 | 51.3 | Mean | 50.2 | 45.9 | 53.2 | 49.1 | 50.5 | 52.9 | — | — |
| | | | | | | | | 1.1 | SD | 1.1 | | | | | | | |
| Weeks 15–19 | | | | | | | | | | | | | | | | | |
| Left | — | — | 47.5 | 46.9 | 49.9 | 48.7 | 48.1 | 49.0 | Mean | 49.1 | 50.4 | 47.9 | 45.6 | 47.4 | 47.6 | — | — |
| | | | | | | | | 1.0 | SD | 1.0 | | | | | | | |
| | *Abnormal bladder function* | | | | | | | | | | | | | | | | |
| | *Group B1 (unilateral reflux)—pig no.* | | | | | | | | | | *Group B2 (No reflux)—pig no.* | | | | | | |
| | *283* | *421* | *425* | *426* | *427* | *428* | *429* | | | | *430* | *424* | *422* | *420* | *415* | *400* | *292* |
| Uptake %/dose | | | | | | | | | | | | | | | | | |
| Week 4 | | | | | | | | | | | | | | | | | |
| Left | 20.9 | 24.0 | 21.5 | 20.5 | 23.3 | 25.5 | 22.5 | 22.6 | Mean | 21.9 | 24.4 | 20.8 | 23.4 | 19.0 | 24.6 | 24.0 | 17.2 |
| | | | | | | | | 1.8 | SD | 2.9 | | | | | | | |
| Right | 21.3 | 24.0 | 22.0 | 20.2 | 26.8 | 23.2 | 24.9 | 23.2 | Mean | 22.1 | 24.3 | 20.5 | 24.9 | 19.2 | 24.7 | 23.3 | 17.5 |
| | | | | | | | | 2.3 | SD | 3.0 | | | | | | | |
| Weeks 15–19 | | | | | | | | | | | | | | | | | |
| Left | 21.4 | 18.9 | 26.1 | 23.5 | 20.1 | 27.3 | 24.4 | 23.1 | Mean | 21.3 | 25.5 | 22.7 | 21.7 | 17.7 | 20.5 | 22.3 | 18.7 |
| | | | | | | | | 3.1 | SD | 2.6 | | | | | | | |
| Right | 22.6 | 18.3 | 25.9 | 22.8 | 23.4 | 27.4 | 24.9 | 23.6 | Mean | 22.4 | 28.9 | 22.4 | 22.2 | 19.3 | 22.0 | 21.9 | 20.0 |
| | | | | | | | | 2.9 | SD | 3.1 | | | | | | | |
| Relative uptake % | | | | | | | | | | | | | | | | | |
| Week 4 | | | | | | | | | | | | | | | | | |
| Left | 49.5 | 50.0 | 49.4 | 50.4 | 46.5 | 52.4 | 47.5 | 49.4 | Mean | 49.9 | 50.1 | 50.4 | 48.4 | 49.7 | 49.9 | 50.7 | 49.6 |
| | | | | | | | | 1.0 | SD | 1.0 | | | | | | | |
| Weeks 15–19 | | | | | | | | | | | | | | | | | |
| Left | 48.6 | 50.8 | 50.2 | 50.8 | 46.2 | 49.8 | 49.5 | 49.4 | Mean | 48.8 | 46.9 | 50.3 | 49.4 | 47.8 | 48.2 | 50.5 | 48.3 |
| | | | | | | | | 1.0 | SD | 1.0 | | | | | | | |

* These values were not recorded because of technical problems.

this rapid growth, the ratio of left to combined (left+right) renal weights was no different in animals with reflux than in non-refluxing controls even when reflux was occurring in the presence of abnormal bladder function and elevated detrusor pressures. Thus in this series of experiments, as in the parallel study employing the 1-kidney model, VUR had no influence on renal growth. Furthermore, these results corroborate the few clinical and animal investigations which consider the effects of VUR on renal growth in the absence of urinary tract infection and renal scarring (King and Sellards, 1971; Danforth *et al.*, 1980; Smellie *et al.*, 1981; Riebel *et al.*, 1982; De Gracia and Breuzière, 1984).

### *Renal uptake of DMSA*

The uptake of DMSA by the left kidney relative to the total (left+right) renal uptake was calculated from separate estimations of the percentage of the

administered dose taken up by each kidney (*i.e.* uptake %/dose). No effect of unilateral VUR on relative uptake was demonstrated even in the presence of abnormal bladder function and elevated detrusor pressures.

Care was taken to minimise errors associated with the preparation of DMSA, low renal counts, background activity and (most significantly) attenuation of activity by tissue depth. This last factor is of crucial importance even when estimating relative renal function. It cannot be assumed that the 2 kidneys are equidistant from the skin surface and an error of 1 cm in depth is equivalent to 13% error in renal activity (Gruenewald *et al.*, 1985; van Poppel *et al.*, 1985). In order to evaluate these errors the uptake values calculated from the kidneys *in situ* at 24 h were compared with those of the same kidney immediately after removal from the animal. Although the *in situ* and *ex situ* values were not indentical, the mean of the difference (*in situ—ex situ*) between the two was less than 1% (uptake %/dose) for both the right and left kidneys and the variation in these differences was small, indicating a good agreement between the two values. The results show that DMSA uptake by the left kidney was slightly underestimated whilst that of the right was slightly overestimated, producing a small negative bias in the left relative uptake.

There have been very few studies of the influence of VUR on renal uptake of DMSA. In a study measuring the absolute uptake as a functional index, Goldraich *et al.* (1984) found that renal uptake was normal in children with low grade VUR but was significantly lower than normal in those with high grade VUR; however, diminished uptake was never found in the absence of scarring and indeed a normal uptake was frequently observed in scarred kidneys. These results support those of our investigation, but whilst we can state that under the conditions of our experiment VUR *per se* has no effect on the renal uptake of DMSA, there are difficulties in interpreting these findings in the context of renal function. Despite the clinical use of DMSA in this respect, the precise mechanisms of its localisation in the cortical tubular cells are obscure, although a recent theoretical analysis suggests that DMSA uptake occurs predominantly by glomerular filtration and tubular reabsorption (Peters *et al.*, 1988). A number of clinical and experimental studies indicate that renal uptake of DMSA reflects various interrelated parameters. These include functional cortical mass (Kawamura *et al.*, 1979), renal blood flow (Daly *et al.*, 1979), effective renal plasma flow (Holten and Storm, 1979; Taylor, 1980; Daly and Henry, 1981), $^{99m}$Tc DTPA glomerular filtration rate (Bingham and Maisey, 1978; Taylor *et al.*, 1986), creatinine clearance (Powers *et al.*, 1981; Baillet *et al.*, 1986) and tubular function (van Luijk *et al.*, 1983, 1984; Provoost and Aken, 1985). It follows that renal uptake of DMSA reflects the amalgamation of various parameters which together determine overall renal performance.

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Errata: (individual relative uptake values %)

| | Group A1 | | | | Group A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 289 | 293 | 403 | 411 | 409 | 405 | 294 | 282 | 277 |
| week 4 | | | | | | | | 50.0 | |
| weeks 15-19 | 47.6 | 50.3 | 51.2 | 48.3 | 50.0 | 50.2 | 47.4 | 49.2 | 48.5 |

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# Effect of Animal and Vegetable Protein Intake on Oxalate Excretion in Idiopathic Calcium Stone Disease

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Summary—Oxalate excretion was measured in healthy subjects and idiopathic calcium stone-formers on dietary regimens which differed in the type and amount of protein allowed; 24-h urine collections were obtained from 41 practising vegetarians and 40 normal persons on a free, mixed, "mediterranean" diet. Twenty idiopathic calcium stone-formers were also studied while on two low calcium, low oxalate diets which differed in that animal protein was high in one and restricted in the other.

Vegetarians had higher urinary oxalate levels than controls and although the calcium levels were markedly lower, urinary saturation with calcium/oxalate was significantly higher. This mild hypercalciuria was interpreted as being secondary to both a higher intake and increased fractional intestinal absorption of oxalate.

Changing calcium stone-formers from a high to a low animal protein intake produced a significant decrease in calcium excretion but there was no variation in urinary oxalate. As a result, the decrease in calcium oxalate saturation was only marginal and not significant.

It was concluded that dietary animal protein has a minimal effect on oxalate excretion. Mild hyperoxaluria of idiopathic calcium stone disease is likely to be intestinal in origin. Calcium stone-formers should be advised to avoid an excess of animal protein but the risks of a vegetable-rich diet should also be borne in mind.

The dietary manipulation of proteins has been found to induce changes in urinary biochemistry, but agreement has so far not been reached as to the effects on oxalate excretion in man. Robertson *et al.* (1979a) have shown that the consumption of large amounts of animal protein can markedly increase oxaluria and, conversely, that low animal protein intake can significantly decrease it (Robertson *et al.*, 1979b). Others have reported that dietary variation in animal protein has no effect on urinary oxalate (Butz *et al.*, 1980; Brockis *et al.*, 1982). Thus, Robertson *et al.* (1979a, b) advised stone-formers to become vegetarians, but others warned about the hazards of excessive amounts of vegetables in the diet.

We have studied urinary oxalate excretion in relation to variations in the amount and type of ingested protein.

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## Patients and Methods

The study was performed in two parts. In the first part, 81 subjects with no history of renal calculus or other renal disease were studied. Renal function, assessed by creatinine clearance, was normal in all subjects. Of these, 41 were vegetarians (22 males and 19 females; mean age $35.2 \pm 12.1$ SD years). The protein content of the diet in these subjects, derived from a retrospective enquiry, was approximately 59 g/day (range 40–80) ($\approx$ 1.1 g/kg bw/day).

While milk and diary products were eaten regularly by 15 subjects, all abstained from eating flesh protein. All of the vegetarians consumed wholemeal or unprocessed bread, rice and pasta. They weighed slightly (but significantly) less than normal subjects ($57.6 \pm 9.5$ *vs* $65.3 \pm 10.2$ kg; $P < 0.01$).

The control group comprised 40 subjects (20 males and 20 females, mean age $36.9 \pm 12.1$ years) eating a free mixed "mediterranean" diet. The