Vesicoureteric Reflux

Clinical and Laboratory Research
Including
Investigation of the Role and Risks of Plastics

A Thesis for the Degree of Doctor of Philosophy
in the University of Adelaide

PA DEWAN

MD MS BMedSc FRCS FRACS

Dedication

To Brigid, Emily, Rachael and Simon

Motto

A Philosopher is one who doubts

Michel de Montaigne (1580)

Declaration

This manuscript contains no material which has been accepted for any other degree in any University. To the best of my knowledge and belief, this manuscript contains no material previously published or written by any other person, except where due reference is given in the text. I give my consent for this copy of my thesis, when deposited in the University library, being available for loan and photocopying.

PA Dewan MD, MS, BMedSc, FRCS, FRACS

4th May 1999

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Acknowledgements

Interest in the clinical use of plastics stems, at least in part, from the development of injectable, particulate materials for the management of vesicoureteric reflux. However, it would appear the inventors were resistant to pursuing all aspects of their new concept, even when challenged by others. Their dismissive response to concerns about the potential complications has probably limited the propagation of the technique, possibly inappropriately. Consequently, I would like to acknowledge Prem Puri and Barry O'Donnell, in Dublin, Ireland and Ian Aaronson in Charleston, South Carolina for the stimulus to undertake this project. Progress in understanding vesicoureteric reflux has been hastened by the development of ultrasound of the fetal urinary tract, new information which necessitated further research into vesicoureteric reflux and the abnormalities seen in the renal parenchyma. Assistance was received from the Ultrasound Department of the Women's and Children's Hospital, Adelaide for the development of expertise in fetal ultrasound, stimulating the Part III of this manuscript.

Practical assistance for this work was provided by the technical staff of the Animal House and Histopathology Department of the Women's and Children's Hospital, in Adelaide. In particular, I would like to thank Roger Byard for the time and effort he has spent helping me review the histological specimens.

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Also, Brigid Dewan and our children have been generous contributors to this work.

Summary

The understanding of vesicoureteric reflux (VUR) has still only reached the level of "expanding ignorance". This state of affairs is because past workers have often drawn conclusions which, in retrospect, have been based on inadequate evidence. The Ransley and Risdon theory of the "big bang" is one of the best examples, where the renal injury was attributed to the first infection: it has now come to light, with the advent of frequent use of pre-natal ultrasound, that many of those with VUR have abnormal renal parenchyma before infection has occurred [1].

This study was undertaken to review the outcome of the application of new technology for the endoscopic management of VUR and, more importantly, to investigate how the subcutaneous tissue, lung and brain of animals respond to Teflon and silicone. The results, and the literature, indicate that injections under the ureteric orifice can cure VUR and that the tissue response to the plastics becomes quiescent. The question of malignant risk remains open, but the risk would appear low, possibly lower with the smaller Teflon particles than for the larger silicone microspheres.

The research into embolisation from solid implants from intravenous tubing and the possibility of antibody formation to implanted plastics were included, to place the use of injectable plastic in perspective with other clinical uses of plastics: the patient with migration of Teflon to the skin was included to highlight the need for caution in the use of plastic injection techniques. This subgroup of experiments indicates that particles are shed into the blood stream during routine paediatric intravenous infusions, and remain in the patient when the sheath around an implantable device remains *in situ* after removal of the implant.

The operation of open ureteric reimplant for the treatment of VUR is well established; thus there was nothing new in achieving reflux resolution for the patients thus treated

in Adelaide. However, finding improved renal growth after ureteric reimplant for high grade VUR is noteworthy, and the clarification of the debate on the issues surrounding the management of VUR is long over-due.

The next experimental step was to embark on the development of a model of fetal VUR, and the pursuit of information on pre-natal diagnosis of renal anomalies. These studies will hopefully open the door to further research which will help clarify our understanding of the pathophysiology of VUR. It would appear that kidneys do undergo progressive adverse changes *in utero*, possibly secondary to VUR.

The greatest achievement of this study may have been the *further* expansion of my ignorance! I have found more questions than answers and hope to have stimulated others to look for answers, rather than jumping to conclusions.

Introduction

Vesicoureteric reflux is a common disease, the understanding and treatment of which is studied in this work. Although renal abnormalities associated with reflux from the bladder to the kidneys have been alluded to for over fifty years, the development of ultrasound was necessary before a less invasive means of identifying the renal anomalies became available. Subsequently, the frequency of diagnosis of renal anomalies in childhood has become more prevalent and fetal diagnosis has become feasible.

The initial part of this manuscript developed from working in Dublin, Ireland, where the endoscopic treatment of VUR was researched and promoted. Initially, there was a lack of focus on the potential complications of the implants and a lack of questioning of what role the technique should have in the treatment of primary VUR. The finding of a large body of literature on adverse effects of Teflon in workers involved in the manufacturing industry prompted a compilation of the available evidence and conduct of a series of experiments on the risks of plastics. Much of the remainder of the manuscript looks at both the complications of implantable plastics and the success of the treatment of VUR, including studies which place the new technology into context with the potential complications of the use of other plastic devices used in the care of children, including implantable plastic vascular access devices and intravenous fluid infusion sets.

However, it is not sufficient to show that a technique works and that the materials involved are safe. It is also necessary to define the indications for the treatment. Therein lies the greatest problem for those treating primary VUR. The recognition of abnormal kidneys before birth, the increasing acceptance that the disease is different in boys and girls [2], and the poor scientific method of many of the studies aimed at elucidating the appropriate management of this condition has led to an ongoing,

unresolved debate. Therefore, a model for fetal VUR has been developed to clarify some of the uncertainty about reflux disease.

Having outlined the background to the genesis of the study, what of the background to the use of injectable plastics in medicine? Endoscopic augmentation by the injection of foreign substances was first used for the treatment of paralysed vocal cords by Brünings [3]. Subsequently, Arnold [4,5] tested a large number of materials which led to the development of the mixture of Polytetrafluoroethylene (PTFE), glycerin and polysorbate (Polytef) [6]. Since then, hundreds of patients have had vocal cord injections and the histological response has been examined [7,8], although only in small numbers. Studies into the tissue reaction have been more extensive in animals, and have revealed active granuloma formation initially, followed by quiescence and engulfment of the particles by multinucleate giant cells [9,10].

Injectable PTFE since has been widely used for vocal cord augmentation and urinary incontinence management, and non-injectable forms have been used in a large number of patients for sutures, inguinal hernia repair, replacement of the stapes, hip prostheses, cardiac valves and vascular grafts. And, since 1984, many children have been treated with the subureteric injection of Polytef for the management of VUR. This use in young patients has heightened the concern about particle migration and carcinogenesis, particularly as the substance will be in the patient for decades.

What, therefore, is the nature of the substances being injected? Firstly, PTFE: produced in 1938 [11]; the carbon-fluorine bonds enable it to be used for the storage of sulphuric acid, to protect the Statue of Liberty, and to act as a heat shield for craft re-entering earth's atmosphere from outer space [12]. The combination of the stability, plus a low co-efficient of friction, make it a highly effective coating for non-stick frypans!

The pyrolysis used for manufacture of the polymer produces toxic products (difluorophosgene, perfluoroisobutylene and hydrofluoric acid) which are again released when the finished product is heated above 350° Celsius [13]. Thus, rodents housed in a room with heated PTFE die from a combination of haemorrhagic lung disease and coagulative necrosis of the liver and kidneys, caused by the toxic products produced from the heating of Teflon [14-16]. Animal experiments with PTFE and its pyrolysis products have fallen into three different groups: (a) Rodents and larger animals have been exposed to the heated polymer in confined spaces [14,16]; (b) short term histological reaction to Polytef injections has been studied in rabbits [6], dogs [9,10] and monkeys [17]; and (c) rodents have had solid, perforated and powdered forms of PTFE implanted as part of more extensive work looking at the chemical versus mechanical carcinogenic effect of a wide range of metals and polymers. Arnold, who initially developed the use of the PTFE/glycerin mixture for vocal cord augmentation [18], studied the histological response in the rabbit quadriceps before injecting the material into human vocal cords. He examined the rabbit muscle two and four months post injection. Kirschner *et al.* [10] and Toomey and Brown [9] have studied the local response in dog vocal cords after Polytef injection, and Malizia *et al.* demonstrated the local and distant reaction to injected PTFE particles some of which have subsequently migrated [17]. They found no inflammatory reaction in the brain, although, each of these animal studies showed a similar response to the injected material: after two weeks, islands of PTFE were surrounded by histiocytes and foreign body giant cells, with moderate numbers of lymphocytes and plasma cells. The central areas showed marked acute inflammatory changes with lakes of eosinophilic hyaline material and infiltrates of polymorphonuclear leukocytes. A similar reaction was seen at two and three months, with the acute inflammatory changes eventually being replaced with increasing numbers of foreign body multinucleate giant cells, which contained phagocytosed PTFE particles within vacuoles, and there was an increase in the fibroblastic response with time. The longest follow-up in these studies is 18 months [10]. No malignant changes were seen in these animals, and the evolution of

change was similar to that seen in humans. Work from The Institute of Cancer Research at the Colombia University, New York, has found the mechanical effect of the implant probably causes the subsequent malignant transformation, not toxic chemicals in the implant [19-22]. In an elegant set of experiments they produced tumours in eight of 34 rats using solid implants, in six of 32 if perforated materials were used and only one of 387 if the implant was a powder. Other workers have demonstrated malignant transformation in the presence of subcutaneous PTFE implants [23] and other synthetic polymers [24], but not all investigators have been able to produce the same result. In studies by Bryson and Bischoff, sarcomas did not developed over 19 months in a group of 40 rats [25]. The differences between these findings and the results of the New York group may be explained by species, methodology or product variations. Further studies by Oppenheimer found the tumourigenic effect to have a significant latent period, and that tumours developed after the removal of the solid implanted polymer disc, as long as it had remained *in situ* for at least six months. If the capsule around the implant was removed at the time of removing the foreign body no malignancy developed [21]. It should be noted that rats are prone to sarcoma development, which may mean the above results are unnecessarily alarming. However, all these studies were conducted over two years or less (the life-span of a rat), which may not make a non-malignant result an invalid conclusion. Table 1 shows a list of published histological studies where the site of Polytef implantation has been examined, either as a post-mortem specimen or from an excision biopsy: both vocal cord treated and urological patients (adults and children) are represented. There appears to be similar progression of the histological changes to those seen for vocal cord injection in dogs [9,10]. Initially there is an active inflammatory reaction with subsequent invasion by multinucleated giant cells which engulf the Polytef particles and sequester them in vacuoles [5,7,8,12,26-36]. Most of the late follow-up cases show a well circumscribed collection with a significant amount of incorporated collagen, without cellular atypia [7,8].

Author	Site of Implant	Delay to Biopsy	No. Cases
Boedts *et al.*(1967)	Vocal Cords	4 wks	1
Wilson & Gartner(1987)	Vocal Cords	4 mths	1
Marcellin *et al.*(1990)	Bladder	6 mths	3
Stone & Arnold(1967)	Vocal Cords	4-13 mths	2
Lewy(1966)	Vocal Cords	14 mths	1
Stone *et al.*(1970)	Vocal Cords	3-16 mths	3
Goff(1973)	Vocal Cords	4ds-18 mths	2
Stephens *et al.*(1976)	Vocal Cords	11-21 mths	2
Claes *et al.*(1989)	Urethra	24 mths	1
Mittleman & Marraccini(1983)	Urethra	24 mths	1
Harris & Hawk(1969)	Vocal Cords	25 mths	1
Sanfilippo *et al.*(1980)	Vocal Cords	34 mths	1
Stewart *et al.*(1989)	Bladder	7-42 mths	16
Wenig *et al.*(1990)	Vocal Cords	1mth-15 yrs	8
Dedo & Carlsöö (1981)	Vocal Cords	4wks-16 yrs	12

Table 1: Reported histological follow-up of patients who have had Polytef injections.

However, there have been reports of late enlargement of the 'PTFE-oma'; in addition patients have developed urinary obstruction [37], an enlarged vaginal granuloma [38,39], and three reports have documented the development of a neck swelling many months after Polytef injection into paralysed vocal cords [8,28,36]. No malignant change was found in any of these cases and no other reports of significant enlargement of the implants have been published. Three cases have been recorded with a malignancy adjacent to a PTFE implant: a single case of a fibrosarcoma adjacent to a PTFE vascular graft implanted 10 years earlier [40,41]; a chondrosarcoma of the vocal cord had a Polytef implant six years before [42] and one of the patients from Lewy's series (1139 patients) had a carcinoma adjacent to the Polytef [43]. None of these tumours have a definite cause and effect relationship with the PTFE. Arnold and Stephens did not record any cases of malignancy in the 100 cases treated for vocal cord paralysis with up to 10 years follow-up [4], and Politano has not found malignancy in the 1,500 cases he has treated for urinary incontinence with Polytef since 1964 [44]. Unfortunately, in neither of these centres have systematic dissections ever been conducted of patients who have died many years after their injections of Polytef; it may be that an unknown number of these patients may have had undetected subclinical secondary malignancy. The longest follow-up of the human histological response to Polytef is 16 years [7].

Toxic effects of Teflon products have also been seen in the human lung [14,15,45-47]. The two areas of study that have indicated the effect of PTFE and its breakdown products in humans are; (a) the industrial medicine studies of polymer fume fever; and (b) the study of post-mortem or biopsy specimens. Similar to animal experiments on the pyrolysis products of PTFE, an extensive literature exists on the toxic effects on factory workers and others who have been exposed to the fumes of heated PTFE. Workers, who either smoke cigarettes contaminated with Teflon particles, or inhale the toxic products while grinding or heating PTFE, can develop fever, rigours, pulmonary oedema, pulmonary fibrosis or pericarditis [14,45-47]; death has been recorded in one

case [12]. Fortunately, the incidence of workers being affected has decreased since safety in the industry has improved.

It has been suggested that the breakdown products that cause the pulmonary complications are found in small amounts within the finished product and, when released in the tissues, are responsible for a carcinogenic effect on cells [13]. This theory is supported by the finding of particle migration from solid synthetic implants to regional lymph nodes [48,49], but it is refuted by more extensive experiments where malignancies were produced in rats with solid metal and polymer implants [19,20,50] and less frequently with perforated polymers and virtually never with polymer powders [22]. These findings were the stimulus to the testing of injectable Teflon and silicone in animals.

Silicone has been generally accepted as biologically inert, but in recent years immunological responses have been reported in patients with silicone-containing ventriculoperitoneal shunts, catheters for long-term venous access and breast implants [51-55]. Goldblum *et al.* reported two patients with an alleged severe immune-mediated reaction to a silicone ventriculoperitoneal shunt, resulting in inflammation of the tissue surrounding the shunt, which then occluded the shunt [51]. They used an enzyme-linked immuno-sorbent assay (ELISA) and reportedly found that IgG in the sera of these patients bound to silicone tubing at a consistently higher rate than found for sera of control patients, concluding that antibodies to silicone bind specifically via the IgG Fab fragment, adding weight to the evidence of adjuvant disease following the insertion of implantable devices. Further study of this phenomenon constitutes part of the discussion in part three of this manuscript.

The other major concern about the use of particulate plastics is the risk of migration, which was recognised in an animal about the time PTFE was first being used for the treatment of VUR in children [17]. This probably does occur after both small

[17,26,56-58] and large dose injections [32,33,57], but the conclusions of these studies are debated. Polymer particle migration also occurs from solid materials such as heart valves [59], dialysis blood pump tubing [60] and solid implants [49]. The question which must be addressed is, "Does the disseminated material have any adverse effect?" rather than "Does plastic migrate?"; the effects that are of most concern are whether or not malignant transformation and autoimmune disease are stimulated in the long-term.

This study consists of three parts. The first is the analysis of the clinical use of the Polytef paste for the management of VUR; the first four studies were conducted while working in Dublin, Ireland, and learning the technique. The later four prospective clinical studies were conducted in Adelaide, South Australia, after the literature review outlined above. The clinical studies include a report of the skin migration of PTFE particles in a patient treated for urinary incontinence and the review of endoscopic videos to assess factors in the injection technique which may impact on the clinical outcome.

Part two of the study was performed during the clinical application of the *submucosal Teflon injection* (STING) procedure in Adelaide and included a series of investigations looking at the histological response to PTFE and silicone. The studies included; The studies included;

(a) examining the skin and lung of the rat in the short and long-term, to assess migration and malignant potential;
(b) the sheep brain to assess the intermediate term response;
(c) an *in vitro* study assessing embolization from intravenous fluid tubing;
(d) the capsule surrounding implanted venous access devices.

The comparison of the PTFE and the silicone was chosen because of a perceived greater migration risk with the smaller PTFE particles, and a possible increased

malignancy risk with the larger particle size of the silicone alternative. This work may superficially appear to be a repeat of previous studies. However, the manufacture of currently available injectable PTFE and silicone is probably different from that previously tested, differences which do not appear to have been studied; more importantly, the PTFE and silicone particles in the currently available form have not been compared in the same animal models described later in this manuscript.

Part three of this manuscript reviews the debate on the role of surgery in the management of VUR and presents an initial description of a fetal model for VUR. This section also details the outcome for a group of boys with a pre-natal diagnosis of renal deterioration and high grade VUR. Also investigated is the surgical management and renal growth changes of a large group of patients who had surgical treatment of their reflux disease.

PART I

Studies

of the

Endoscopic Management

of

Vesicoureteric Reflux

Introduction

The first urological use of the endoscopic injection technique was with Polytef for the treatment of urinary incontinence. The original proponent, Victor Politano of Miami, has treated over 1,500 adults and children since 1964 [61]. Matouchek of Spain, in 1981, reported the first injection of Polytef paste for the treatment of VUR [62] and Lynne and Politano reported a variation of the technique in 1983 [63]. Matouchek's method has been popularised by Puri and O'Donnell, after they verified its efficacy in a pig model in 1984 [64-66]. In particular, they showed that injection into the floor of the ureteric orifice resulted in resolution of the VUR without causing obstruction, because the roof of the ureteric orifice remains pliable (Fig.1). Many authors have since published encouraging results for all grades of VUR, both primary [67-89] and secondary [77,81,82,90-98]; however, not all authors have reported the same degree of success [99-101].

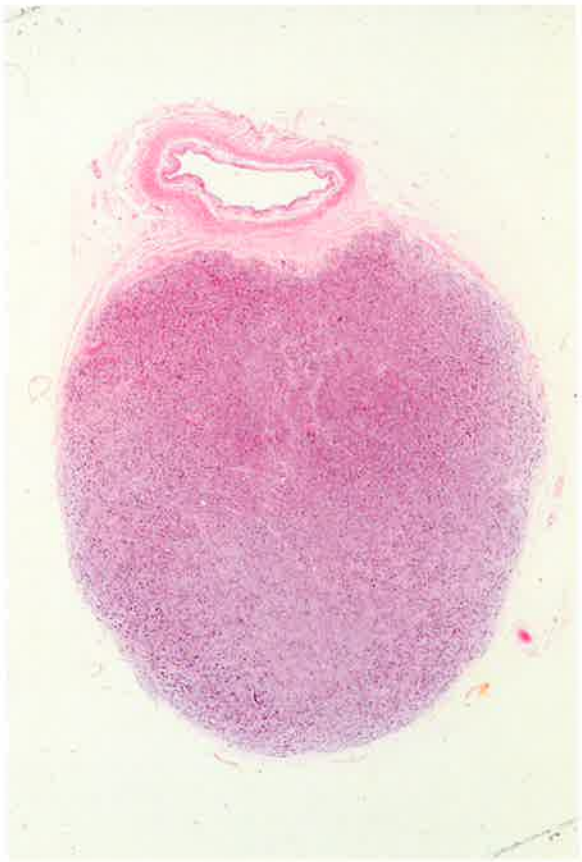


Figure 1: The PTFE and granulomatous reaction seen adjacent to pig ureteric orifice. The pliability of most of the ureteric circumference is suggested by the lack of contact with the 'Teflonoma'.

Concerns about the migration and malignancy risk have led to the development of injectable collagen [76,102-105] and other materials [106-114]. However, the success rate after the injection of collagen is not as great as for Polytef. Bovine collagen is used in the glutaraldehyde crossed-linked form, which increases its resistance to enzymatic degradation, but the handling characteristics and the degradation over time appear to limit the efficacy of collagen. Collagen has the advantage over Polytef that it can be administered through a finer needle, allowing a smaller endoscope to be used; the disadvantages are of a possible allergic reaction, the potential subsequent sensitivity to bovine collagen, concerns about the infiltration of eosinophils and late calcification at the injection site [102]. However, information on the histological response to collagen is limited: only one study has looked at the histological response at the collagen injection site in the urinary tract [102].

Other Related Research

Silicone injectable material has been used by surgeons for many years for breast augmentation [115-121] and the treatment of skin creases [122,123]. More recently the material has been used for the treatment of VUR [124-128]. The silicone used, Bioplastique has a larger particle size (100-150μm) than Polytef (90% <40μm) and when injected into mice did not migrate to the regional nodes as Polytef does [129]. Another polymer, polyvinyl alcohol (150-250μm), has been used for experiments in rabbits [108] and monkeys [114], with good VUR resolution. Similar encouraging results have been obtained with Bioglass, a microparticulate form of glass [130].

Suitable autologous substances have been sought for the endoscopic management of VUR - fat [131], blood [112] and human collagen have all been suggested, but none have yet proven satisfactory. More recently, a product which consists of beads of starch (Deflux^{R}) has been used in both a rat model and in human for the treatment of VUR, with encouraging results. Unfortunately, an extensive study of all the factors of

concern has not been conducted on each of these new substances before they have been used in patients [107].

In contemplating the endoscopic management of VUR it is appropriate to mention the debate on whether VUR should ever be surgically managed; this will be discussed in Part III.

The remainder of Part I of this manuscript is a compilation of results of the endoscopic treatment of reflux, with all data collected prospectively. The four groups of patients in Dublin were those with primary VUR, those with a neurogenic bladder, those with a duplex system and, finally, a group treated at less than two years of age. The patients, in Adelaide, had video recording of their procedures: the group has been analysed for the long and short term outcome of treatment of the VUR, and the correlation between the video appearance of the Polytef mound and resolution of the reflux.

DUBLIN - Studies 1, 2, 3 and 4

Overview

Each of the patients in the first three Dublin studies had cystoscopic examination of their bladder and ureteric orifices followed by Polytef paste injection into the subureteric plane via a rigid needle inserted through an 11.5 FG Wolf 'STINGer'. In the fourth study the Belfast technique [91] was used in those in whom the urethra was too small for the STINGer. The needle was placed into the bladder submucosa at the six o'clock position and advanced approximately 0.5cm under the ureteric orifice and the distal ureter. The rigid needle had a 20 FG tip, which was either 0.9mm or 1.4mm in length; the longer length was used for the older children. Whenever a ureteric orifice was difficult to see, a ureteric catheter was inserted to aid visualisation of the injection, particularly for those with an irregular or inflamed bladder and when a re-injection was being performed.

The ureteric orifice was observed while injecting the Polytef paste, aiming to produce a crescent shaped orifice on the medial slope of a nipple of paste (Fig. 2). Withdrawal of the needle was delayed for 30 seconds after the completion of the injection, to prevent loss of paste through the injection site, enabling stabilisation of the paste within the submucosal space.

In all study-subgroups of endoscopic VUR treatment, the efficacy was assessed in relation to the reflux grade according to the five grade classification defined by the International Reflux Study Group (IRSG) in 1981 (Fig. 3) [132,133].

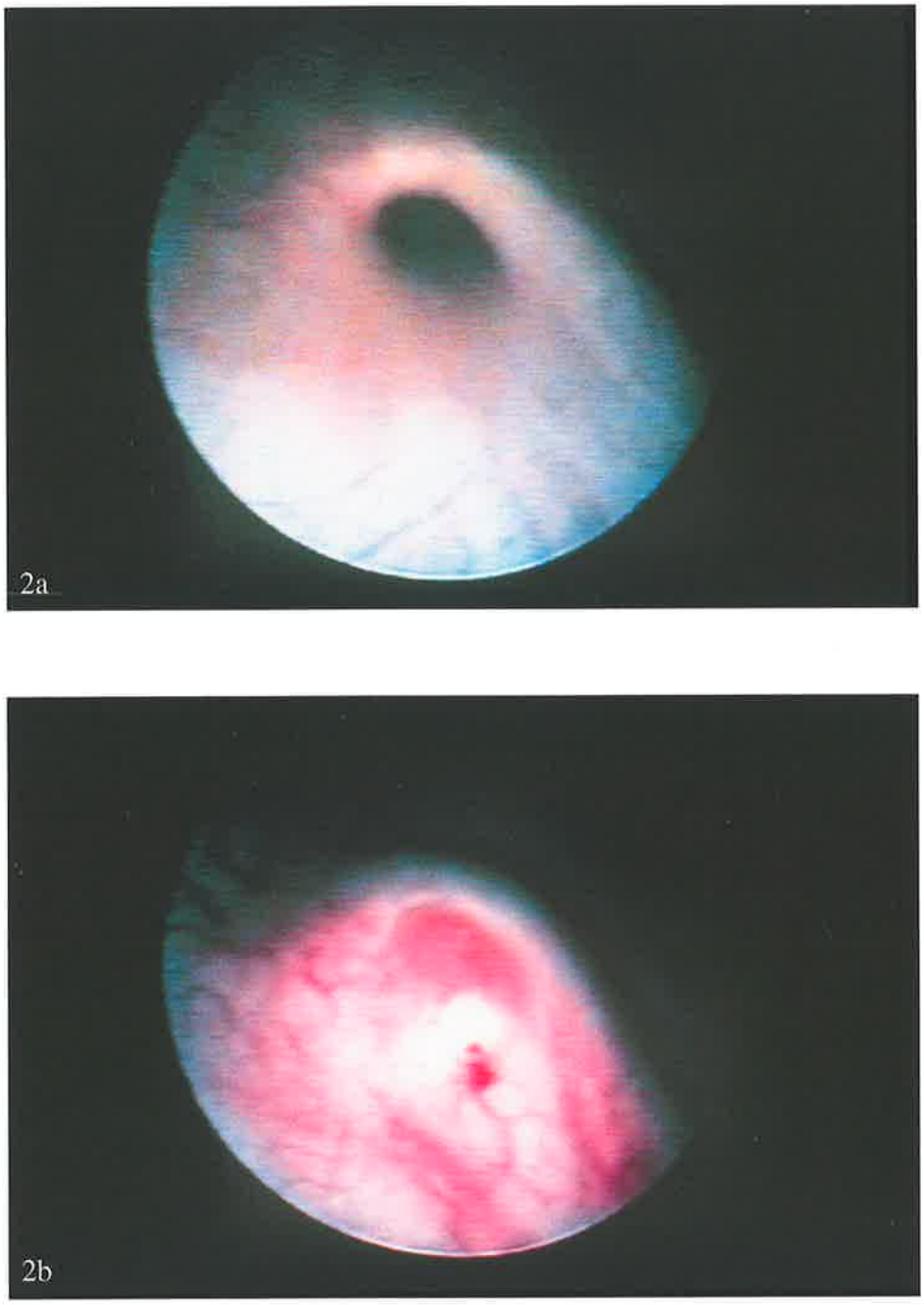


Figure 2: The ureteric orifice before a STING (A) and after injection of 0.3ml of Polytef paste and after removal of the needle (B).

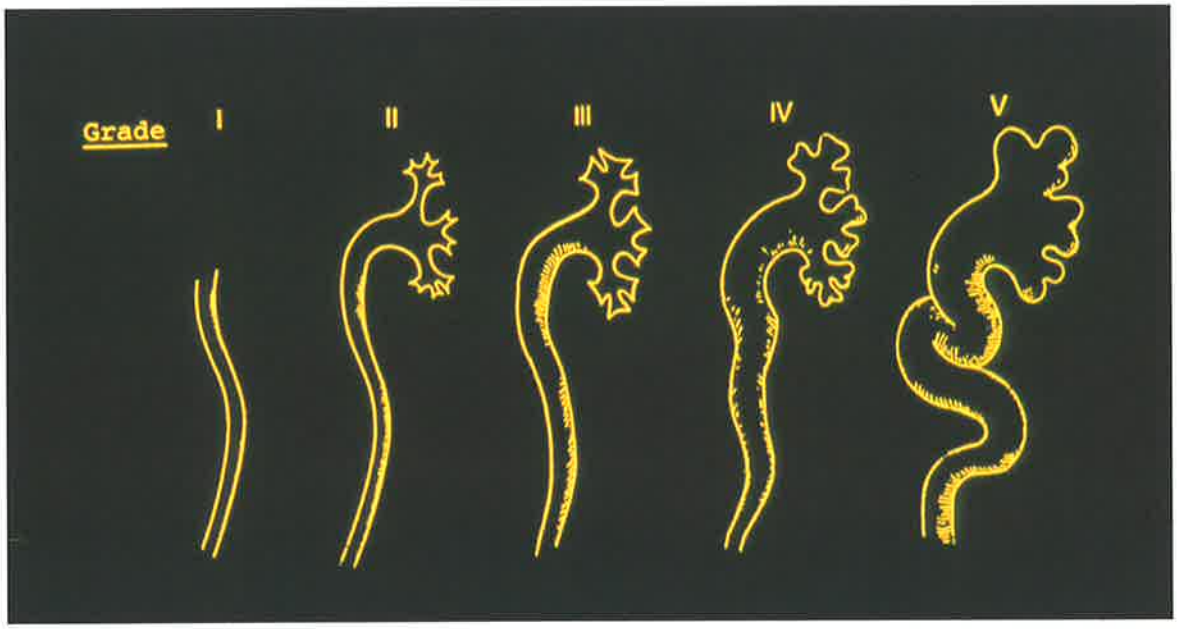


Figure 3: The International Reflux Study Group five part VUR classification [132,133].

STUDY 1

Endoscopic Management of Primary Vesicoureteric Reflux in Irish Children

This series of patients, studied prospectively, had VUR into at least one non-duplex kidney managed in the Hospital for Sick Children, Dublin, by a Paediatric Surgeon who was not involved in the original research (Professor EJ Guiney).

Patients

Seventy-three patients were treated between March 1984 and February 1989, all with grade III VUR or greater. Those ureters with grade I to II VUR were only injected if treating a higher grade contralateral ureter at the same time. Patients with duplex ureters or a neuropathic bladder were excluded from this group.

The 73 patients had 117 refluxing ureters. Four patients with unilateral reflux have not had follow-up, leaving 113 for analysis with the grades of reflux shown in Table 2.

Reflux Grade	Total	No. of ureters Resolved Reflux	Persistent Reflux
I	1	1	-
II	12	6	6
III	46	35	11
IV	42	27	15
V	12	6	6

Table 2: Outcome for each grade of reflux.

The policy was to perform a micturating cystourethrogram (MCU) and ultrasound (US) three months after the procedure and an MCU and intravenous urogram (IVU) 12 months later. Of the 75 ureters where reflux resolved, 44 had one MCU and 31 had more than one MCU after their last STING. These figures were current at the time of completion of the data collection for this study. The MCU was conducted following gravity filling of the bladder, until the child commenced voiding. After the first void, the bladder was refilled until voiding again commenced, at which time the urethral catheter was removed. Screening was conducted during both filling and voiding, and hard copy radiographs were taken as indicated. The 69 patients ranged in age from three months to 13 years (average 58 months). Forty-four children had bilateral VUR and 25 had unilateral reflux.

Results

Sixty-nine children had 161 injections (Table 3) during 105 cystoscopies. The Polytef volume injected per STING was 0.46ml (range 0.1-1.3ml) and the average volume per treated ureter was 0.66ml (range 0.2-1.9ml). The Polytef load per patient ranged from 0.2ml to 3.5ml (average 1.1ml).

Follow-up was performed on 113 ureters; 50% (6/12) of those with grade II, 76% (35/46) of grade III, 64% (27/42) of grade IV and 50% (6/12) of those with grade V VUR stopped refluxing after their last STING (Table 2). As well as 75 of 113 ureters (66%) that had stopped refluxing, a further 21 (19%) have been left with low grade reflux (I and II). Therefore, 96/113 (85%) had either ceased refluxing or showed a lesser degree of reflux following Polytef injection.

The number of STING procedures per ureter are shown in Table 3; 44% of the 113 ureters (50 ureters) no longer refluxed after one injection, and of those that were

injected more than once, 51% (18/35) stopped refluxing after two injections. Only twelve went on to have a third or fourth STING procedure.

Thirty-eight of the total of 113 ureters refluxed after their last treatment, three had spontaneous resolution of their reflux and five were reimplanted. The STING failed to produce reflux improvement in only 11 of these 38 ureters.

STINGs/ Ureter	Total No. of Ureters	Total No. of Injections	No. of Ureters Reflux Resolved	No. of Ureters Reflux Persistent
1	78	78	50	28
2	23	46	18	5
3	11	33	7	4
4	1	4	-	1

Table 3: The number of ureters with resolved reflux compared to the number of injections required per ureter.

Table 4 shows the repeat STINGs matched to the original grade of reflux, indicating the low incidence of repeat STINGs in all but grade V reflux. Table 5 reveals that most of the grade III and grade IV ureters that had either a second or third injection stopped refluxing. Because the VUR has been treated more aggressively in this group, a 72% combined ureter success rate was achieved for grade III/IV VUR.

Recurrence of reflux (not persistence) occurred after 11 of the 161 injections (6.8%). Seven occurred after the initial MCU was performed less than two weeks post operatively and only four when it was carried out at three months: other complications were urinary tract infections and haematuria. Ureteric obstruction and bladder granulomas were not encountered and subsequent ureteric reimplants were not complicated by previous Polytef injection.

Outcome for Patients

One hundred and five procedures resulted in 38 patients (55%) becoming reflux free. An additional 16 patients (25%) had a decrease in the grade of VUR. Five reimplants were necessary in four patients who continued to reflux after their STING procedures; two were reimplanted after a single STING and three ureters in two patients had three Polytef injections each. Eleven patients had either no improvement or a minor change in their reflux, and were awaiting further treatment at the time of data collection.

Reflux Grade	No. of STING Procedures 1	2	3	4	Total
I	1	-	-	-	1
II	11	1	-	-	12
III	33	9	4	-	46
IV	26	12	3	1	42
V	7	1	4	-	12

Table 4: The number of ureters according to grade of reflux and number of STING procedures.

		Grade III-IV No. of Ureters	
No. STING Procedures	**Total**	**Resolved**	**Persistent Reflux**
1	59	42	17
2	21	16	5
3	7	5	2
4	1	-	1

Table 5: Outcome of ureters with grade III and grade IV reflux, matched to the number of STING procedures.

STUDY 2

Endoscopic Management of Vesicoureteric Reflux in Children with Spina Bifida, in Ireland

Patients

Twenty patients (sixteen female and four male) with VUR secondary to a spina-bifida-related neuropathic bladder were studied prospectively by Professor EJ Guiney. The average age at the time of the first STING was 55 months (range of 7-179 months). Reflux was unilateral in nine and bilateral in 11. None had duplex ureters. Of the 31 refluxing units, 29 had at least one follow-up MCU. Table 6 shows the initial reflux grade of the 29 kidneys. Grade I reflux was treated in one patient in whom a higher grade was present on the contralateral side. Persistent reflux after a STING resulted in reinjection on seven occasions.

All patients were treated from March 1984 to February 1989. Some of those treated early in the series had an MCU on the first post-operative day. This practice was later replaced by performing an MCU and US at three months and an MCU and an IVU at twelve months. Patients were usually discharged on the same day and maintained on antibiotic prophylaxis for two weeks.

Results

Complete resolution of reflux occurred in 16 of 29 ureters. Table 6 shows the reflux grades for those ureters where reflux was either resolved by the STING or where reflux persisted. Notably five of seven ureters with grade V reflux ceased refluxing (Fig. 4). However, nine of 15 grade IV refluxing ureters had persistent reflux, three of which were subsequently recorded as grade V reflux. Figure 5 indicates the grade changes in those ureters with persistent reflux.

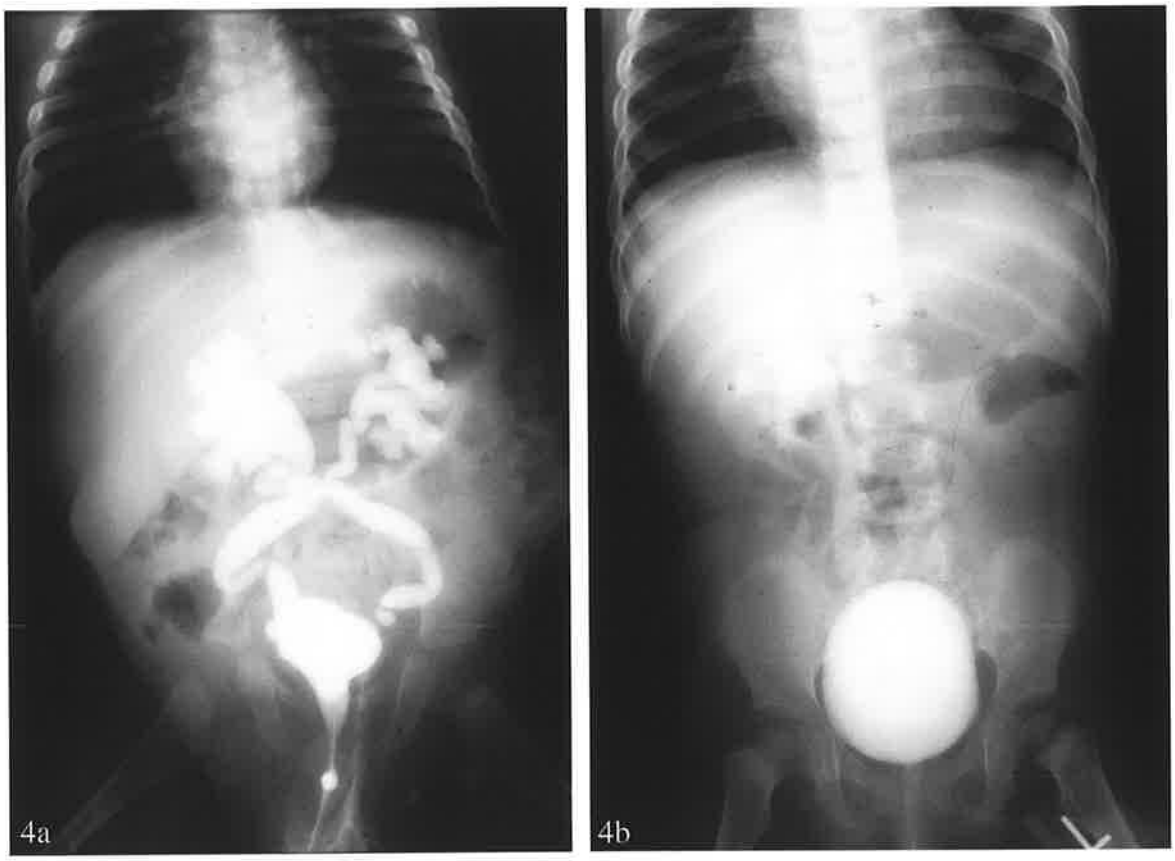


Figure 4: A. Bilateral grade V reflux in a six month old boy. B. An MCU two months after 0.5ml of paste injected below each ureter. No reflux was seen at 14 months or 26 months post STING.

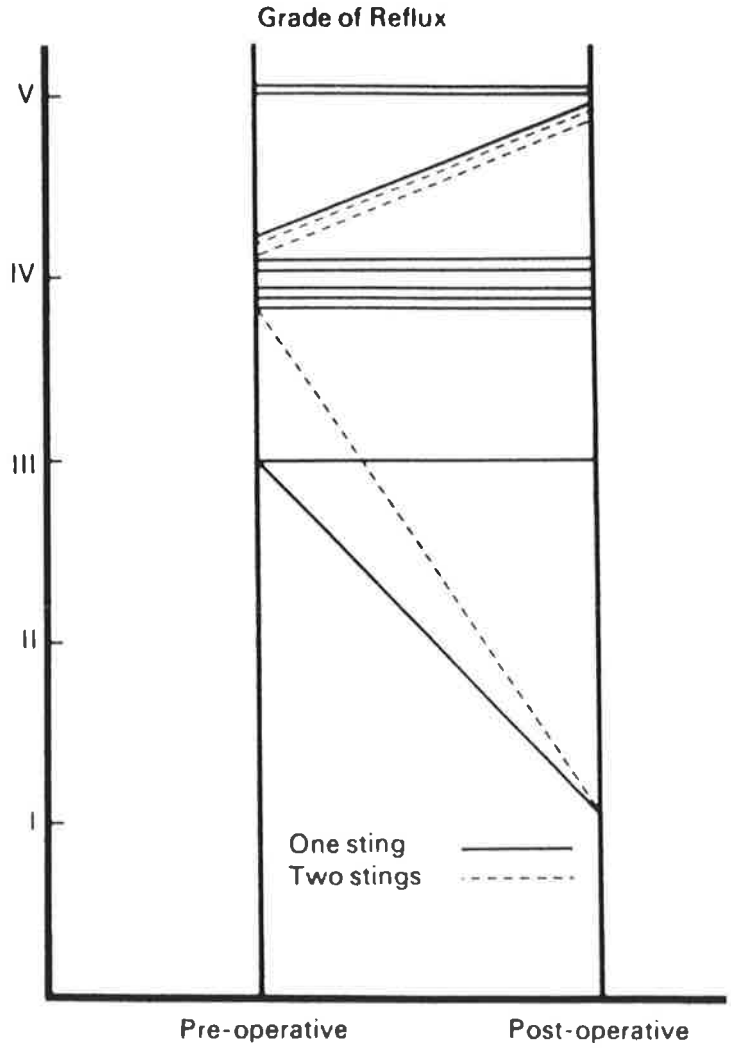


Figure 5: Vesicoureteric reflux grade changes for the 13 ureters with persistent reflux.

Grade	Resolved Reflux No. of Ureters	Persistent Reflux No. of Ureters	Total
I	1	0	1
II	0	0	0
III	4	2	6
IV	6	9	15
V	5	2	7
Total	16	13	29

Table 6: Original reflux grade and outcome of the twenty-nine ureters followed post STING.

The number of Polytef injections to achieve cessation of reflux are given in Table 7. Twelve ureters had one STING and one ureter required three injections before reflux resolved. Half of those that had a second STING no longer refluxed.

No. of STINGs/ per Ureter	Resolved Reflux No. of Ureters	Persistent Reflux No. of Ureters	Total No. of Ureters
1	12	10	22
2	3	3	6
3	1	0	1
Total	16	13	29

Table 7: The number of injections of Polytef per ureter (STING) for those with resolved and persistent reflux.

No patient underwent a ureteric reimplant in this group and none of the 29 ureters were obstructed. A number of other features are relevant to these patients; two patients had aborted attempts at Polytef injection, because their bladder was markedly oedematous and inflamed on cystoscopic examination, preventing identification of the ureteric orifice. On four occasions reflux recurred after treatment was initially successful - all the initial MCUs were performed the day after the STING; one patient had no change in grade V reflux after one STING but eventually underwent spontaneous improvement to grade II disease.

STUDY 3

Endoscopic Management of Vesicoureteric Reflux into Duplex Ureters

Patients and Methods

Between March 1984 and May 1989, 34 patients were treated by Polytef paste injection for VUR into a duplex system. The ureter distribution according to grade of VUR is shown in Table 8. All kidneys had lower pole VUR and 17 also had reflux into the upper pole. The reflux was bilateral in one patient, left sided in 19 and right sided in 14. Eight of the 34 children had contralateral single-system VUR.

VUR Grade	No. of Ureters
I	1
II	0
III	19
IV	13
V	2
Total	35

Table 8: Highest reflux grade for each of the 35 duplex systems ***ureters***.

The 28 females and six males were aged three months to 12 years (mean 5.2±4.1 years). Injections were usually carried out on a day-case basis and the patients were followed by an MCU and US at three months and an MCU and an IVU at 12 months. Where VUR was again identified, a repeat Polytef injection was usually performed: antibiotics were continued for two weeks after the procedure.

With reference to the single-system refluxing ureters, each STING was performed with the 11.5 FG Wolf 'STINGer', a glass syringe and a long, 20 FG needle. The needle was introduced into the submucosal plane at the six o'clock position of each refluxing ureter of each ipsilateral pair, and Polytef paste injected. If both ureters refluxed, a third injection site was occasionally used, located between the two initial injection positions. Ultimately, a single mound of paste was produced with two surmounted crescent shaped orifices. When the duplex ureters were immediately adjacent and were cranio/caudally related within the bladder wall, paste was injected beneath the caudal ureter only, producing two crescent shaped orifices, one on top of the other, on a single mound of paste. Further paste, if required to supplement the effect on the cranial of the two orifices (i.e. the roof of the caudal ureter), was placed beneath the caudal orifice, thus avoiding obstruction of the caudal ureter.

Two ureteric catheters were often inserted to assist with visualisation of the orifice during the injection. When the ureteric tunnel was markedly short, a Fogarty balloon catheter was used to pull the ureter into the bladder, thus giving better orifice delineation and subsequent improved Polytef placement.

Sixty-eight duplex system and 11 single-system paste injections were performed during 70 cystoscopies. The volume of paste on the 34 used for the duplex ureters, ranged from 0.15ml to 1.4ml (mean 0.59±0.31ml) per injection and up to 2.47ml per side (average 1.14±0.65ml). In comparison, the eight single-system ureters required 11 STINGs which used an average of 0.34ml per injection and 0.47ml per side.

Results

The 11 ureters with reflux into a single-system were corrected with a single Polytef injection.

In the thirty-five duplex ***systems*** treated, 22 had the reflux resolved, 10 had persistent VUR, one was obstructed by the Polytef and two were lost to follow-up. The number of STINGs for the 35 ureters are shown in Table 9. A high proportion of the successfully treated systems required only one injection (13/22), and beyond two STINGs the success ratio was lower (Table 9). Where both duplex segments refluxed, the number of Polytef injections needed was not increased: more injections were required for grade IV reflux, but not for grade V disease (Table 10).

	Number of *DUPLEX SYSTEMS*				
Number of STINGs	**Resolved Reflux**	**Unresolved Reflux**	**Obstructed**	**Unknown**	**Total**
1	13	2	-	1	16
2	6	3	1	-	10
3	2	2	-	1	5
4	1	2	-		3
5	-	1	-		1
	22	10	1	2	35

Table 9: Number of duplex ***Systems*** requiring up to five Polytef injections for the management of reflux, and their current reflux status.

Number of STINGs	Highest Grade of Reflux for each Duplex system		
	III	IV	V
1	10	4	1
2	6	3	1
3	2	3	-
4	-	3	-
5	1	-	-
	19	13	2

Table 10: The numbers of Duplex systems with grade III-V reflux matched to the number of Polytef injections required.

The number of cystoscopies for the 34 ***patients*** is shown in Table 11. The total of 70 sessions included three patients who had asynchronous bilateral reflux. Thirty-two were followed after their last STING, of whom 21 (66%) are reflux free (Table 11). Of the remainder of the 32 patients, one patient had an obstructed ureter after two injections, three had no change in their grade of reflux and seven had some improvement. Therefore, 28 of the 32 children (87%) had either improvement or resolution of their VUR, without any treatment complication. The outcome of those with persistent reflux is shown in Table 12.

	Number of *PATIENTS*				
Theatre Trips	**Resolved Reflux**	**Unresolved Reflux**	**Obstructed**	**Unknown**	**Total**
1	11	1	-	1	13
2	7	3	1	-	11
3	2	3	-	1	6
4	1	2	-	-	3
5	-	1	-	-	1
	21	10	1	2	34

Table 11: Number of cystoscopies matched to the outcome for the 34 ***patients***.

Reflux Grade Change	**Number of Patients**	**Number of STINGs per Patient**
No Change	3	5*,2,1
Down one	2	4*,3
Down two	1	2
Upper pole Resolved	2	3^{+},2
Lower pole Resolved	2	4,1^{+}
Total	10	27

Table 12: Shift of reflux grades in ***patients*** with persistence of reflux. (*) patients with a ureteric reimplant after their last STING. (+) heminephrectomy performed post STING.

STUDY 4

Endoscopic Correction of Vesicoureteric Reflux in Infants

Patients

Forty-seven patients, under two years of age, were diagnosed as having VUR on an MCU and 41 had follow-up for at least three months after a STING procedure. Their upper tracts were assessed with US and IVU; some cases were further investigated with a nuclear medicine scan. Information on the procedures and investigation results was collected prospectively.

The 41 patients with adequate post treatment investigation had 72 refluxing ureters and a total of 105 STING procedures during 71 cystoscopies. There were 24 males (40 kidneys) and 17 females (32 kidneys) with an average age of 10.6 months (range 3-23). All were treated by Professor O'Donnell and were selected for this study if less than two years old at the time of their first Polytef injection.

Of the 60 single-systems, the degree of reflux was grade II in nine, grade III in 21, grade IV in 23 and grade V in seven; of the 12 duplex refluxing systems, the degree of reflux was grade III in four, grade IV in six and grade V in two.

A STING procedure was performed on ureters with grade III or more reflux at the time of diagnosis, or where grade II reflux was present on the contralateral side of a higher grade reflux, or in one patient, for bilateral grade II reflux where several break-through infections, while on prophylaxis, had occurred.

The Belfast technique [91], in which a 10 FG cystoscope, a long fine rigid needle and a high pressure injection gun is used, was required in two younger males in whom the 11.5 FG Wolf 'STINGer' was too large.

The patients were maintained on low dose cotrimoxazole (based on 2mg/kg of the Trimethoprim component of this compound drug) for two weeks post-operatively and had an MCU and an US three months after the procedure. A further MCU and IVU were performed 12 months after the Polytef injection. Patients showing persistent reflux were usually re-injected, and then re-entered the investigation cycle.

Results

Of the 47 patients (83 refluxing ureters and 117 STINGs), 41 (72 ureters and 105 injections) were available for analysis; the others had not had sufficient follow-up at the time of compilation of the data. In the 41 patients, 55 of the 60 single-system ureters (92%) no longer refluxed. Four of the five that did not resolve had an improvement in the grade of reflux and remained infection free. Forty-four of these 60 (73%) ureters required one STING, the other 16 had a combined total of 37 (Table 13).

Number of *Single-system Ureters*

No. of		**Persistent Reflux**	**Resolved Reflux**	**Total**
STINGS	1	3	41	44
	2	2	10	12
	3	0	3	3
	4	0	1	1
Total No. Ureters		5	55	60
Total No. STINGs		7	74	81

Table 13: Number of STINGs administered to the single-system ureters.

The STING was more successful with lower reflux grades (Grade II-IV = 94%), but was also successful in five of seven single-system grade V ureters (Table 14). Duplex system ureters required more injections and more Polytef paste per injection and did not respond as well to the treatment, although seven of 12 did cease refluxing (Table 15).

Reflux Grade	**Resolution Rate**	**Percentage**
2	9/9	100
3	19/21	90
4	22/23	96
5	5/7	71
Total	55/60	92

Table 14: Resolution of reflux for single-system kidneys.

		Number of *Duplex Ureters*		
		Persistent Reflux	**Resolved Reflux**	**Total**
No. of STINGS	1	2	2	4
	2	1	4	5
	3	1	1	2
	4	1	0	1
Total No. Ureters		5	7	12
Total No. STINGs		11	13	24

Table 15: Number of STINGs administered to the duplex system ureters.

The average Polytef paste volume used was 0.43ml per injection and 0.60ml per ***ureter***. The single-system ureters required 0.52ml per ureter, whereas the duplex needed an average of 1.15ml.

Comparing the results of the ***patients*** rather than their ***ureters***; the 41 infants included 31 with only single-system reflux (22 bilateral and nine unilateral), seven with bilateral reflux of which one side was duplex, two with reflux into bilateral duplex kidneys and one with unilateral duplex VUR. Thirty-one of the 41 infants became reflux free, 27 following only one or two cystoscopies. Twenty-six of the 31 patients with only single-system reflux (84%) and half those with duplex reflux on one or both sides were subsequently reflux-free (Table 16).

One patient had an obstructed ureter; the two injections of her left sided duplex ureters were made difficult by chronic inflammatory bladder changes and, despite the presence of the Teflon paste, a subsequent reimplantation was carried out without difficulty - this is the same patient as mentioned in the duplex series in Study 3.

		No. of Patients		
		Persistent Reflux	**Resolved Reflux**	**Total**
	1	3	17(1)	20(1)
Trips	2	4(2)	10(3)	14(5)
to	3	2(2)	4(1)	6(3)
Theatre	4	0	0	0
	5	1(1)	0	1(1)
Total		10(5)	31(5)	41(10)

Table 16: Number of visits to the operating theatre. Six further patients have not completed follow-up investigations. The ten infants with duplex system reflux are in parentheses ().

ADELAIDE - Studies 5, 6 and 7

Overview

The endoscopic treatment of VUR was introduced to the Adelaide Children's Hospital in May 1991. All cases in this study were managed by the author; all endoscopic procedures were video recorded, and a prospective, database of the VUR, the technique used and the outcome of the reflux treatment was accrued.

Patients were assessed pre-operatively with an MCU or an indirect radionuclide cystogram (IRC), within three months of the procedure. Three months post operatively, assessment was with an MCU, a direct isotope cystogram (DIC) or an IRC according to the age of the patient and the state of bladder function: an indirect cystogram was only used in patients with normal bladder function, who were at least four and a half years old at the time of the investigation. A further cystogram was performed 12 months after the STING procedure. The upper tracts were investigated with ultrasound and nuclear medicine studies at the same intervals. The results of these tests were also entered into the database prospectively.

Other features of these three studies were: (a) reflux was defined according to the International Reflux Study Group five grade classification (Fig.3: Page 26) [132,133]; (b) injections were usually performed when VUR of grade III or greater was present; and (c) all patients had a UTI as part of their presenting illness; Gentamicin and Ampicillin were administered intra-operatively and oral Cotrimoxazole was continued until resolution of the VUR was confirmed.

Unlike the Dublin patients, the STING procedure was performed with the smaller 9.3 FG Wolf, purpose-built cystoscope and only a short length to the needle bevel was available. The needle was introduced into the submucosal plane at the six o'clock

position of the ureteric orifice and Polytef paste was injected under vision, to produce a crescentic orifice on a mound of paste as described by O'Donnell and Puri [64,66]. A significant advantage for the Adelaide patients was the ability of both the assistant and the surgeon to see the procedure on a video monitor.

Authors presenting the early follow-up results of Polytef injection have demonstrated the effectiveness of the treatment of VUR; however, few investigators have assessed the long-term efficacy of the procedure, therefore data was specifically collected on those who were free of reflux both at three months and again at 12 months.

The endoscopic appearance of the STING procedure has not previously been correlated with prospectively collected clinical information on the management of the VUR. This information was collected by an assistant, unaware of the VUR management outcome, who scored the endoscopic appearance of the procedure from prospective video recordings; later the video scores were correlated with VUR outcome.

STUDY 5

Endoscopic Management of Vesicoureteric Reflux in South Australian Children - Short-Term Outcome

Patients

Eighty-six patients were treated for VUR by the submucosal injection of Teflon paste, between May 1991 and October 1996. Three were excluded from the follow-up analysis; two because of a lack of available data and one because she only had a ureteric stump injected.

Informed consent was obtained from the parents after full discussion of open ureteric reimplantation and the STING procedure, particularly the reported potential risks of plastics; the parents made the final choice. One third of parents of children offered surgical treatment of VUR elected to have their child's reflux treated by Polytef injection rather than by open operation. Ureters with grade I or II VUR were injected only while injecting a contralateral ureter with a higher grade VUR; the thirteen ureters had contralateral injection for the reasons shown in (Table 17).

1. Persistent vesicoureteric reflux, renal scarring, hypertension.
2. Recurrent 'breakthrough' UTIs.
3. Large, gaping ureteric orifice on cystoscopy.
4. Poor renal growth.
5. Reflux into duplex kidney with single ureteric orifice.

Table 17: Relative indications for anti-reflux therapy in patients with unilateral grade II vesicoureteric reflux.

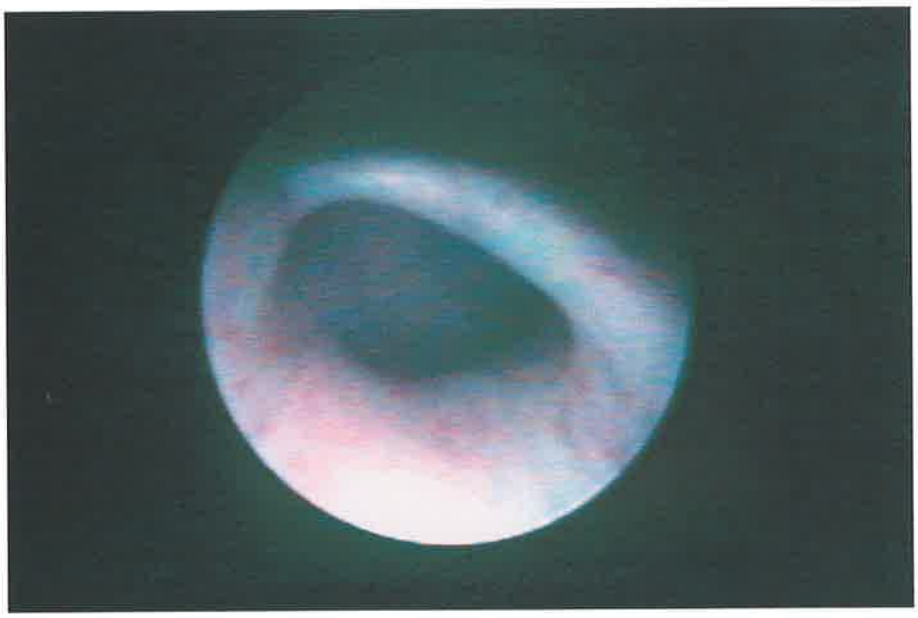


Figure 6: The grade V refluxing orifice is seen prior to the injection of Teflon paste. The post injection view was similar to that seen in Figure 7B, which shows an adequate crescent-shaped ureteric orifice.

In 80 of 83 patients, the procedure was performed with a 9.3 FG Wolf 'STINGer' cystoscope. Three patients had their injection as an open procedure; one had a congenital urethral stricture associated with an imperforate anus and left grade IV VUR into a ureteric orifice which could not be visualised endoscopically; the second had bilateral grade V VUR and a neuropathic bladder, in a boy who had previously undergone a renal transplant. Ureterocystoplasty, using the grossly dilated left ureter, was performed and the right ureteric orifice was injected, to allow for formation of an intubatable stoma subsequently, if required. The third had the right ureteric orifice injected when a left ureteric reimplant was performed as an open procedure, because of a larger paraureteric diverticulum. In these patients the paste was injected through a 21 gauge needle attached to a 2ml leuer-lock syringe; the positioning of the needle and the desired visual response was similar to the endoscopic procedure (Fig. 6).

All patients were followed-up at three months with an US and a direct, or indirect, radioisotope cystogram, depending on age.

Results

Eighty-three (116 ureters) patients underwent the STING procedure, ranging in age from 9.3 to 186.6 months (78.6±36.9 months). The 59 females (21.6-151 months; median 78.6): the males (9.3-186.6; median 78.9). The volume of Polytef paste required was 0.08-0.60ml (0.24±0.18ml) on the left and 0.05-1.05ml (0.19±0.18ml) on the right.

Fifty patients had unilateral VUR and 33 had bilateral VUR. The grade of VUR in the refluxing ureters is summarised in Table 18. Ninety of the 116 refluxing ureters (78%) were grade III or higher.

VUR Grade	Right	Left	Total
0	1	3	4
I	2	3	5
II	7	10	17
III	28	34	62
IV	10	14	24
V	2	2	4
Total	50	66	116

Table 18: Numbers of ureters per grade of reflux.

Following a single injection, VUR was abolished in 93 (80%) ureters. Of the 23 (13 left and 10 right ureters) treatment failures after a single injection, of which 14 ureters showed a reduction of the grade of VUR while two remained unchanged and seven got worse (Table 19). A repeat injection was performed in 11 left and 10 right ureters (15 patients) and VUR was abolished in 19. Of the two ureters with persistent VUR following the repeat injection, one was followed expectantly and the other underwent ureteric reimplantation. One patient with bilateral VUR proceeded to open ureteric reimplantation after failure of the first injection; the parents preferred not to opt for a re-injection.

Initial VUR	Final VUR	No. cases
III	II	7
IV	III	2
IV	II	4
V	III	1
II	II	2
0	I	1
0	II	3
0	III	1
I	IV	1
III	IV	1

Table 19: Outcome in 23 ureters with failure of single injection to abolish vesicoureteric reflux.

No patient in this series had ureteric obstruction following the STING procedure, and all patients were discharged on the day of the procedure if the STING was the only procedure performed.

Eight patients had a duplex system, four on the left, three on the right and one had bilateral duplex VUR. Of the nine duplex ureters injected, VUR was abolished in six. Persistent VUR after a second injection occurred in two, each of which was treated by ureteric reimplantation (see above).

Five ureters (three left and two right) had grade V reflux, and a further 16 ureters (10 left and six right) had a ureteric orifice sufficiently large to allow easy passage of the cystoscope, ie ureteroscope (one had no VUR, three had grade II VUR, eight had grade III VUR and four had grade IV reflux). All but five of the total of 21 of the ureteric orifices which were able to be entered with the cystoscope were successfully treated with one injection; a further two had a second injection followed by reflux resolution. The persistent reflux in the remaining three was grade I or II in asymptomatic patients who initially had grade V reflux.

STUDY 6

Endoscopic Management of Vesicoureteric Reflux in South Australian Children - Long-Term Follow-up

Patients and Methods

Between May 1991 and December 1993, an endoscopic STING procedure was performed on 58 patients and a further three children had the Polytef paste injected at open operation. Two ureters with grade I and five ureters with grade II reflux were injected, only whilst injecting the contralateral ureter with a higher grade VUR, with the exception of six cases of unilateral grade II VUR which were injected for the reasons given in Table 20. In addition, six non-refluxing ureters were injected during the contralateral procedure for the reasons indicated in Table 21. All patients had a UTI as part of their presenting illness.

1. Persistent VUR, renal scarring, hypertension.
2. Recurrent UTIs.
3. Large, gaping ureteric orifice.
4. Poor renal growth.
5. Duplex kidney with open single ureteric orifice.

Table 20: Indications for Teflon injection in patients with unilateral grade II vesicoureteric reflux.

Of the 87 ureters treated during this period, 26 have not been included in this series as they have not followed the protocol of a 12 month study after showing no VUR at three months. Of these 26, nine ureters had persistent reflux on the three month cystogram. Two ureters had inadequate IRC studies at three months and reflux was present on repeat investigations MCU or DIC soon after. A second injection was

performed in 10 of these ureters, one of which had a ureteric reimplantation at the request of the parents. Six ureters did not show reflux pre-operatively but had either an open orifice, previous reflux or reflux nephropathy, in patients who were undergoing an injection for contralateral reflux. A further eight ureters had not been investigated 12 months after the STING at the time of data collection, and one ureter did not have a three month cystogram.

1. Previous reflux
2. Reflux nephropathy
3. Duplex system
4. Hypertension
5. Large, gaping ureteric orifice

Table 21: Reasons for injection of non-refluxing ureters in patients with contralateral reflux.

Results

Forty-nine patients (61 ureters) had the STING procedure, showed no reflux three months after injection and were then reviewed 12 months post-STING. Their ages ranged from 1 to 189 months (mean 75 months). The volume of Polytef paste injected ranged from 0.07 to 1.05ml (mean 0.31ml). Forty-nine (80%) of the ureters had grade III or higher reflux (Table 22).

Twelve months after a single injection, VUR was resolved in 59 (97%) ureters, with reflux into only two ureters. One patient showed no reflux on an IRC at three and 12 months (both were reported as technically good studies) but a DIC at 12 months demonstrated reflux, probably recurrent, in one ureter. In a second patient, reflux was

not detected during an IRC at three and 12 months. However, the false negative IRCs were probably due to the patient not voiding to completion, a feature recognised in hind-sight. Subsequently, an MCU performed following a UTI, 31 months after the STING, demonstrated persistent reflux in a single ureter. A repeat injection was performed in each of these two cases, with resolution of the reflux.

Fifteen ureters had grade IV or V reflux, eleven had large, gaping orifices on cystoscopy and six were duplex systems. All were free of reflux at the 12 month study.

Grade of VUR	**No. of Ureters**
I	2
II	10
III	34
IV	13
V	2
Total	61

Table 22: Numbers of ureters per grade of reflux in 49 patients.

STUDY 7

Correlation of the Endoscopic Appearance with Clinical Outcome of Submucosal Polytef Paste Injection in Vesicoureteric Reflux

Patients

The 46 patients (33 female, 13 male) included in this study had a video-recording of their first STING procedure between 1991 and 1993. Table 23 shows the VUR grade of the cases involved. This group of patients were also included in the studies on the outcome of VUR management.

Only the first STING for any one ureter was considered for correlation with the outcome shown by the cystogram at three months. Thirty-seven left and 27 right ureters were injected and 17 patients had both ureters treated at the one endoscopy. Three patients with unilateral VUR on cystogram had a bilateral STING because the non-refluxing side had renal scarring and a wide open ureteric orifice at cystoscopy.

Reflux Grade	Continued VUR	Resolved VUR	Total
0	0	3	3
I	0	1	1
II	1	10	11
III	4	28	32
IV	2	13	15
V	2	0	2
Total	9	55	64

Table 23: Results of treatment by grade of reflux.

Methods

An independent observer, unaware of the treatment outcome, reviewed the video recordings after all patients had undergone their STING procedure and three months follow-up. The following information was recorded; the general appearance of the bladder and ureteric orifices, including whether they were ureteroscopable (large enough to admit the 9.3 FG cystoscope); the site of injection in relation to the ureteric orifice and number of injections required to complete each STING; the presence and effect of any extravasation of Polytef paste, the visibility of paste through the bladder mucosa and any haemorrhage from the injection site; the shape of the resulting ureteric crescent and its position on the mound of paste in the coronal and sagittal planes. Each STING was given a score from one to 10, based on an overall assessment. A score of 10 was given if the final appearance was of a crescent shaped orifice on the near side of the crest of the mound of paste, with the mound consisting of one rounded bulge. A point was given for each of the following 10 observations: i) the position of the mound of paste; ii) the size of the mound of paste; iii) single mound of paste; iv) position of the needle; v) single needle hole; vi) position of the needle hole at centre-front of the mound; vii) early forward bulging of the back wall of the orifice during the Teflon injection; viii) paste not too superficial; ix) no paste oozing from the ureteric orifice; x) no paste oozing from the needle hole.

The information on the status of the VUR disease, the volume of Polytef paste used and the outcome of the reflux treatment were recorded prospectively and initially assessed independent of the information from the endoscopic review.

Results

Forty-three STINGs had a good overall video score and had the VUR successfully treated. The overall scores given to the post STING video appearance ranged from four to nine (Table 24).

Nine ureters continued to reflux after endoscopic treatment, including three of the thirteen that were ureteroscoped (Table 24). The failed group had a smaller average volume of Polytef paste injected of 0.23±0.12ml compared to 0.33±0.19ml in the successfully treated group (Table 24). In the four with an average amount of paste, but persistent VUR, the following factors were observed and may have been significant; submucosal tracking of the paste, resulting in an additional mound in front of the ureteric orifice, in one case; the ureteric orifice situated between two mounds of paste in another; penetration of the adjacent ureter in a child with a duplex system; and significant bladder instability in a girl, resulting in recurrent VUR secondary to bladder disease.

Video Score	**Continued VUR**	**Resolved VUR**	**Total**
4	2	4	6
5	1	8	9
6	1	8	9
7	3	17	20
8	1	13	14
9	1	5	6
Total	9	55	64

Table 23: Scores of STING appearance compared with the VUR outcome.

		Continued VUR	Resolved VUR	Total
Needle insertions /STING*	=1	6 (14)	37 (86)	43 (100)
	>1	3 (14)	18 (86)	21 (100)
Ureteroscopy		3 (23)	10 (77)	13 (100)
Av Vol/STING (ml)		0.23±0.12	0.33±0.19	0.32±0.18

Table 24: Comparison of failed and successful STINGs. *An inadequate initial mound was augmented by re-inserting the needle medial or lateral to the first passage. Numbers in parentheses are percentages.

Twelve patients who had submucosal injections received a poor video score (i.e. four or five), but the ureters were successfully treated (Table 24). Four were large ureteric orifices, three of which had a sufficiently dilated ureteric orifice to allow the cystoscope to be easily passed into the ureter [ureteroscopy] (Fig. 7). A primary megaureter with a small orifice also scored poorly, but was successfully treated. Six of these 12 ureters required more than one injection to complete the STING, ie, during the one anaesthetic, the needle was inserted under the same ureter more than once; the second injection site was deemed necessary when the initial site of injection appeared to be either too medial or lateral during the formation of the mound of Polytef. Two had more than one mound created, five ureteric orifices were not on top of the mound of paste and the crescent shape was barely adequate, or poor, in all 12. The average volume of paste injected in the poor score for the STING, but with successful treatment, was 0.32ml.

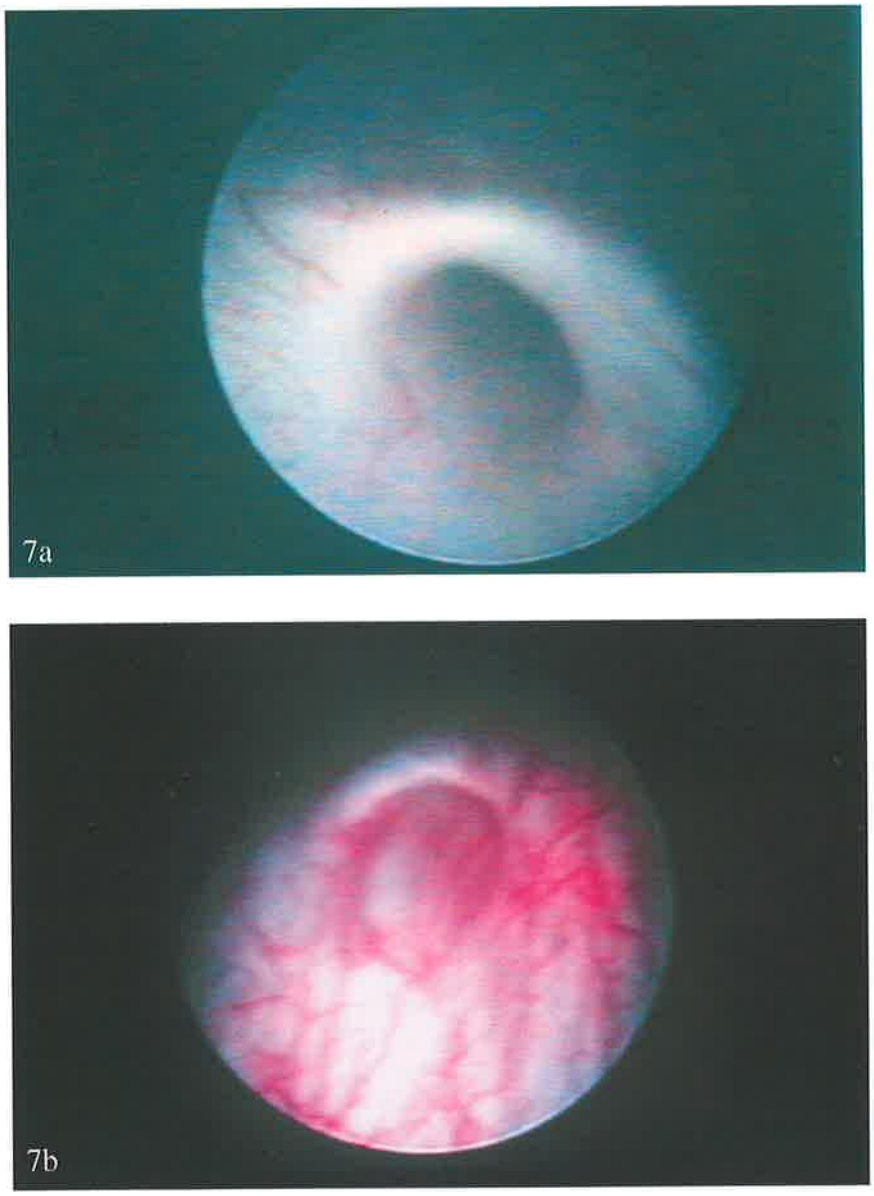


Figure 7: (A) A large orifice which after 1.05ml of Polytef paste (B) was cured of reflux but has a less neat appearance than that seen in Fig. 2.

Discussion

Polytetrafluoroethylene has been used for vocal cord injection since 1963 [134] and has been used extensively in the management of urinary incontinence in adults and children [135,136]. Sporadic reports of its use in VUR appeared up to 1984 [62,63] when Puri and O'Donnell [65] reported the successful management of refluxing pig ureters by Polytef injection, following which the technique has been widely adopted. Several authors have now reported favourable Polytef injection results in the treatment of primary reflux, reflux from neuropathic bladders [68,94,137], urethral diverticula [138] and deroofed ureteroceles [139].

The STING technique is simple and prevents the need for ongoing antibiotic prophylaxis without the need for open operation, with the advantage that most patients are injected on a day-case basis. When the Polytef paste is injected under the distal ureter it produces an elevated crescent shaped ureteric orifice, which is thought to limit the lateral migration of the distal ureter during detrusor contraction; both the change in shape of the orifice and its fixation are thought to help resolve reflux. The paste consists of PTFE particles suspended in glycerin, which makes up half the weight of the paste. The glycerin dissolves, leaving PTFE to become incorporated in fibrous tissue at the site of injection: it is likely that the presence of the glycerin explains the recurrences seen if an MCU is performed, intra-operatively or within two weeks.

The results for complete cessation of reflux in the Dublin single-system study were less favourable than reported by many [68-70,72,89,140,141], but are explained by an acceptance of a reduced grade of reflux if the patient is rendered symptom-free without infections. For instance, grade II reflux was only treated where it was coincident with higher grade contralateral reflux, and only one patient had a second injection into such a ureter, although six ureters continued to reflux. On the other hand 72% of ureters with grade III or IV reflux have been successfully treated and, overall, 80% (54)

patients and 85% (96) ureters had reflux ceased or reduced to low grade, asymptomatic disease, with only one or two injections.

The advantages of this technique include the short anaesthetic time, the ability to treat patients on a day-case basis and the low complication rate. If reflux is resolved the patient is advantaged by having had a simple procedure; if reflux persists open ureteric reimplantation can be performed without difficulty, or the grade of reflux may be sufficiently low to be trouble free.

Polytef has been used for the treatment of several hundred children with success rates as high 97%, after completed treatment of up to three injections [72,74,75]. However, the worst results are as low as 62% [142]. In some series, a significant proportion of the ureters treated had less reflux than would generally be treated by open operation [71,140] (Table 25 + 26). Also, the re-injection rate and late recurrence rate vary from author to author, both of which have a significant influence on treatment success [141]. Table 25 and 26 show the results from a number of treatment centres and indicate that the variation in result is independent of the percentage of low grade reflux in the patient populations, which suggests differences in precision of diagnosis at the time of the injection, or variations in the technique used. The variations are despite the general belief that grade II disease is virtually always successfully managed, grade III and IV usually so and grade V reflux in only 50% of cases.

The single-system patients from Adelaide again demonstrate that good results can be obtained with the injection of Polytef paste. Unlike other series, not more than two injections [66,78] were used. Also, grade V reflux, and ureters where the ureteric orifice was sufficiently dilated to allow the 'STINGer' cystoscope to be passed into the ureter, were treated. Other authors have suggested that high grade reflux and/or a very dilated ureteric orifice cannot be successfully treated endoscopically [67]; however, eight of nine (89%) of these ureters had their VUR resolved in this study.

The overall results for the single-system patients, in Adelaide (an 82% cure by one injection and 90% if two injections are used), compare favourably with other larger series, with a similar small proportion of low grade refluxing ureters [69-72,76,78,142].

The reported International success of the STING is shown in Table 26. Most studies quote a rate of improvement to a lower grade of VUR, giving these figures as part of the justification of the technique, rather than using cure rates only. Success after one injection and after completed treatment have therefore been given separately in Table 29.

The remainder of the discussion will be on the use of the STING in infants, the sustainability of a favourable initial treatment outcome, the use in spina bifida and duplex systems, the interpretation of the endoscopic appearance of the Teflon mound, the other urological uses of Teflon, and the role of collagen injections and other injectable substances.

Author/ [Ref. No.]	No. of Ureters Treated per Reflux Grade				Total	Percent corrected
	I/II	III	IV	V		
Rashid [88]	3	7	5	3	18	40%
Burns *et al.* [142]	Distribution Unstated				53	62%
Sweeney & Thomas [71]	80	45	21	7	153	65%*
Brown [99]	12	10	14	4	28	70%
Simsek *et al.*	7	18	16	5	46	74%
Merckx *et al.* [101]	65	26	--	9	100	82%
Dodat & Takvorian [67]	94	93	29	1	217	83%
Puri *et al.* [89]	2517	2499	970	230	6216	86%
Kaplan *et al.* [68]	"Most > Grade III"				20	87%
O'Donnell & Puri [70]	20	78	45	7	150	88%
Puri [69]	7	85	41	10	143	89%
Schulman *et al.* [140]	102	80	24	8	241	92%
Farkas *et al.* [141]	5	57	22	4	88	94%
King & Gollow [72]	"All Grade IV/V"				36	97%

Table 25: Results of completed treatment for **Primary** Vesicoureteric Reflux using *Polytef paste* injection. * = After one injection.

Author [Ref No.]	Percentage corrected with one injection	Percentage corrected after completed treatment
Rashid [88]	40%	40%
Burns *et al.* [142]	Unstated	62%
Sweeney & Thomas [71]	65%	Unstated
Brown [99]	Unstated	70%
Simsek *et al.* [81]	65%	74%
Merckx *et al.* [101]	75%	82%
Dodat & Takvorian [67]	79%	83%
Puri *et al.* [89]	76%	86%
Kaplan *et al.* [68]	Unstated	87%
O'Donnell & Puri [70]	81%	88%
Puri [69]	79%	89%
Schulman *et al.* [140]	87%	92%
Farkas *et al.* [141]	90%	94%
King & Gollow [72]	86%	97%

Table 26: Success rate for one injection in the treatment of **Primary** Vesicoureteric Reflux using *Polytef paste* injection.

Endoscopic treatment of Reflux in Infants

Many infants with grade III-V reflux will not have urinary infections and, in some, the reflux will resolve spontaneously. There are many infants who will not undergo spontaneous cessation of their reflux and those that do, in the meantime, are at risk of developing breakthrough infections as recorded by Dunn and Smith who found significant infections in 34% of children treated medically [143].

The aim of this part of the study was to review the use of the STING in infants under two years of age, a group in whom the procedure had not been studied in detail previously. Compared with older children with VUR, infants are more commonly male [144,145], have a higher average grade of reflux [144] and more frequently cease refluxing spontaneously [146]. Therefore, infants are often managed non-operatively, particularly because of the difficulty of ureteric reimplantation in a small bladder. In this group of patients the STING was successful in resolving reflux in 55 of 60 single-system ureters, including patients with high grade reflux and/or duplex systems. With a short anaesthetic and an endoscopic procedure, seven of 12 duplex and five of seven grade V refluxing systems were rendered reflux free (Tables 14 & 15). Despite including these difficult-to-treat ureters, 31 of 41 patients had their reflux resolved and, although 20 required a second or third endoscopy, 14 of those with repeat injections were successfully treated (Table 16). Therefore, a repeat injection maybe indicated if the reflux is not initially controlled.

The success of the STING in the infant single-system ureters was similar to the results for reimplantation older children [147,148], and the STING results for the infants less than two years are paralleled by those in older patients [71,72].

Long-Term Outcome for Single-systems

When the STING procedure was first used, an intra-operative cystogram was thought to indicate permanent resolution of VUR, however, the rate of recurrence was up to 15%, due to oedema and glycerin adding to the antireflux effect of the PTFE [70,149]. Many Urologists now perform a cystogram after two to three months; Puri *et al.* recorded a recurrence rate of 7% in a series of 75 ureters with at least grade III VUR [85]. Others have had success rates of up to 90% [69,83]. Assessment of published results is marred because persistence and recurrence of VUR are not usually differentiated, although the distinction is obviously important. It seems appropriate to determine whether the treatment failure is due to incorrect paste positioning or volume, or, alternatively, due to a post-operative problem, such as extrusion of the paste.

Few reports have looked at later follow-up [74,75,85], although the possible failure of the STING has been an often expressed concern. The high rate of reflux following treatment with collagen has highlighted the concerns about recurrence [150] and, as indirect radionuclide studies were used to follow patients, the reliability of which has also been questioned, it prompted review of the long-term outcome.

Of the 61 ureters free of reflux after three months, only two (3%) refluxed after 12 months. In one of the two cases with possible recurrence of VUR the IRC may have been a false negative test or bladder instability could have reversed the antireflux effect, as can happen with the open procedure. In the second case, more careful interpretation of the original IRC would have identified the poor bladder emptying, prompting a catheter cystogram at the three month follow-up. Thus, in the majority of patients, and possibly in all patients, the injection of PTFE provides sustained resolution of VUR.

Many centres accept IRC as a appropriate investigation for the detection of VUR, with a sensitivity of 42-82% [151]. Merrick *et al.* [152], in a long-term follow-up study of a large group of children with VUR, reported good sensitivity and accuracy of the IRC technique: it has been suggested that the better kidney-to-background ratio with MAG3 will further improve the sensitivity of this technique. However, this has not been the experience of at least one group [153].

It would appear justified to follow VUR treatment with an IRC after a STING, open operative management or medical management, provided the child can void satisfactorily on command (usually not until after they are at least four and a half years of age). If there is any doubt about the adequacy of any IRC study, the patient should undergo an alternative study (DIC or MCU) to ensure resolution of the reflux.

Spina Bifida

Vesicoureteric reflux is difficult to manage in patients with a neuropathic bladder particularly as the reflux rarely resolves spontaneously and may worsen [137]. Also, ureteric reimplantation is more difficult in these thick walled, trabeculated bladders and antibiotic prophylaxis is hampered by a high incidence of resistant organisms. Polytef injection is a simple procedure in the management of primary VUR, where success rates of up to 92% can be expected (Table 27) [68,72,76]. Good results have also been reported in multiple aetiology, neuropathic bladders [94], including patients with spina bifida [68]. However, occasionally the STING is not able to be performed in the neurogenic bladder because the ureteric orifice is not able to be found within the trabeculations of the bladder; a feature described by the Belfast group [91] and experienced in two of the spina bifida patients in this group. In view of the high incidence of urinary contamination, spina bifida patients are always protected with prophylactic antibiotics during the STING and, where infection is present or suspected injection is not performed.

The overall STING success rate was lower for the Dublin spina bifida series (16 of 29 - Table 6) than reported by Quinn *et al.* [91]; a difference which may be explained by the low re-injection rate in the Dublin group. Half those who had a second STING had reflux resolved, which would suggest that a number of the 10 with persistent reflux after one STING, may have been rendered reflux-free by a further injection. Until now there has been a relatively conservative approach to reinjection in this group, which was to be reviewed following this study. Nevertheless, on the evidence available, the injection of a ureter more than twice; increase the risk of obstruction with little improvement in the success rate.

Reflux ceased in five of the seven Polytef injected grade V refluxing ureters in the Dublin spina bifida study, indicating that this simple procedure can be used successfully in this group, with the subsequent decreased risk of upper tract infection. The three grade IV ureters, which progressed to grade V, reflect the natural history of reflux in spina bifida patients [137] as no upper tract obstruction was evident to explain the change. It is possible that the Polytef paste was not adequately placed in these cases.

The Polytef paste being 50% glycerin that is absorbed, probably explains the recurrence of reflux in patients who did not have VUR on day one after a STING procedure: an MCU is no longer performed intra-operatively or in the first two weeks after the STING as it was for some of the patients in this group; the first investigation is now three months after the procedure.

The current recommendation is to use the STING as the initial management of VUR in spina bifida patients, provided the bladder dysfunction has been adequately managed with anticholinergic medication and intermittent catheterisation. Where a single Teflon injection fails, a further one or two injections should be undertaken.

Author Year - Ref.	**No. of Ureters Treated per Reflux Grade**					
	I/II	**III**	**IV**	**V**	**Total**	**Percent corrected**
Geiss *et al.* 1990 - [154]	Distribution Unknown				27	70%
Kaplan *et al.* 1987 - [68]	"Most > Grade III"				17	76%
Puri & Guiney 1986 - [94]	1	2	8	4	15	87%
Quinn *et al.* 1988 - [91]	0	11	15	15	41	90%
Misra *et al.* [93]	6	9	39	15	69	82%

Table 27: Results of completed treatment for Vesicoureteric Reflux from **Neuropathic** bladders using *Polytef paste* injection.

Duplex System Vesicoureteric Reflux

Ureteral duplication of any degree is the most common anomaly found in the urinary tract [155-157]. Complete duplication is found in 0.2% of autopsy specimens [155] and is two to three times as common in females as in males [156,157]. When the two ureters extend from the kidney to the bladder, the lower pole ureter is usually lateral to its partner, through a short intravesical tunnel [158,159]. Consequently, most complete duplex systems that reflux, do so into the lower pole [160], and 17-25% reflux into both poles [156,157]. Isolated upper pole reflux does occur, but is uncommon [156].

Vesicoureteric reflux into a complete duplex system is unlikely to abate with growth [87,157], and is more difficult to treat than single-system reflux. Therefore, a number of operations have been advocated for management of duplex reflux, including:- common sheath reimplantation [156,157], ureteroureterostomy [157,161], pyeloureterostomy [161], single ureter reimplantation [156], heminephrectomy and the STING.

The reimplantation of a double ureter is frequently successful, but less so than for single ureters, and involves a long hospital stay, plus the risks of a bladder operation [162]. Ureteroureterostomy and pyeloureterostomy [157,163] also require a prolonged post-operative convalescence, and are only suitable where one of the pair of ureters has neither VUR nor obstruction. As well, the ureteric stump can become infected and cause pain [163-165], particularly if the ureter is grossly dilated [166]. Combining a ureteroureterostomy with a ureteric reimplantation is an alternative [167], but this adds significantly to the morbidity.

When the function of the renal parenchyma, into which VUR occurs, is poor, the solution is to remove that segment. However, heminephrectomy carries a risk of injury

to, or loss of, the adjacent pole, and complete removal of a ureter may jeopardise the blood supply of the other ureter of the duplex pair.

This study is the first large single centre series where STING was used to treat reflux into complete duplex systems, although a multi-centre and other studies have shown similar results (Table 28) [80,98,154,168]. Notably, Burns *et al.* had poor results for both single and duplex systems [142] and Farkas had good results for both [141].

The group of duplex system VUR patients had the same high incidence of lower pole reflux expected by the Weigert-Meyer-Stephens rule [156-160]. The intravesical tunnel anatomy predicted by this rule was, initially, thought to be unsuitable for treatment by Polytef injection; the achievement of 66% reflux resolution and 87% complication free improvement, indicates the STING can resolve reflux in a significant proportion of this group. The use of a ureteric catheter to guide the injection, and the Fogarty balloon catheter to pull the distal ureter into the bladder during the injection, have contributed to gaining satisfactory results.

A safer alternative may be to use the STING to prevent upper renal tract sepsis while the function of the lower pole is being further evaluated. If the function of the refluxing renal unit remains acceptable, the reflux has been solved with a day-case procedure. If the subsequent function is poor, the problem of reflux into a residual stump is avoided, without the need for a major operation. Therefore, it is recommended that Polytef paste be injected as first line treatment for reflux into complete duplex systems.

The current policy of the two institutions in which the studies were conducted is to use the Sting for single system and duplex system VUR after appropriate discussion with the parents on the risks of the injection of plastic particles, and the risks of open surgery.

Author (Year)	No. of Ureters Treated per Reflux Grade				Total	Percent corrected
	I/II	III	IV	V		
Burns *et al.* 1990 - [142]	Distribution Unstated				8	13%
Steinbrecher *et al.* 1995 - [98]	8	20	8	1	37	57%
Valla *et al.* 1989 - [168]	Distribution Unstated				26	65%
Sauvage *et al.* 1990 - [80]	Distribution Unstated				13	69%
Farkas *et al.* 1990 - [141]	"All Grade III/IV"				16	94%

Table 28: Results of completed treatment for Vesicoureteric Reflux into complete **Duplex** ureters using *Polytef paste* injection.

Endoscopic Appearance vs Clinical Outcome

Vereecken and Proesmans [75] stated that it is the appearance of the orifice rather than the grade of reflux which determines the volume of the paste required and the likelihood of a successful outcome. During the performance of the endoscopic treatment of VUR the ureteric orifice usually takes on the desired crescent shape with 0.3 ml of the Polytef paste (Fig. 3); the back wall of the orifice should commence movement forward early in the injection. In this series of 55 ureteric orifices, the ureters successfully treated by a single STING received a larger average volume of paste than the nine ureters that continued to reflux (Table 24), indicating that more than just a desirable appearance is required: a suggestion supported by the finding of five with good overall video scores, but continued reflux. Four of the nine failures had a poor overall video score; in three cases the needle placement was initially poor, i.e. not in the six o'clock position and, thus, not ideal. The fourth failure was due to the patient having a ureteric orifice large enough to admit the cystoscope, where an inadequate volume of 0.05ml of Polytef paste was used.

Where the needle is inadvertently poorly placed, and the appearance of the mound is unsatisfactory (Fig. 7), VUR is more likely to persist. Paste extravasation probably also relates to poor needle positioning, with the tip of the cannula too superficial, allowing the Polytef to extrude back along the track; the operator should take extreme care with the insertion of the needle to ensure appropriate positioning.

Failure was more common with ureters with an orifice large enough to admit the cystoscope (23%), however all large orifices injected with at least 0.4ml of Polytef paste were cured of reflux.

Large ureteric orifices also accounted for one quarter of the STINGs that received poor video scores but were cured of reflux (Fig. 7). These poor scores were because

the neat crescent-on-mound appearance is usually not possible with large orifices. However, if adequate volumes of paste were injected, the clinical results were still satisfactory.

As a result of this study, the STING procedure has undergone a minor, but significant change: if the ureteric orifice is relatively small and a satisfactory crescent-on-a-mound appearance is achieved with a small amount of paste, a further aliquot of paste is added, to give a total of 0.3ml of Polytef. Those ureters with an orifice large enough to admit the cystoscope are expected to require more than 0.3ml of paste, knowing that the mound will be less well defined than for those ureters with a smaller orifice.

Polytef appears to be the most appropriate, currently available material for the endoscopic management of VUR. When the safety of the material has been confirmed by long-term studies, only a non-absorbable autologous material or a larger particle inert substance is likely to replace Teflon as the material of choice. The problem then becomes one of identifying the role of the technique, the analysis of which is made difficult by published reports not including details of the delay between the pre-treatment cystogram and treatment, information which influences the interpretation of the results. An analysis of the role of the technique is also made difficult by a shift of treatment toward a lower grade of reflux than is usually treated by open surgery, with as many as 48-52% of the ureters managed endoscopically groups having grade I and II VUR [103,104].

As yet a study has not been performed to compare endoscopic management with other techniques in a controlled trial; endoscopic management of VUR has already a definite place and, when the indications for its use are better defined, it will probably have a more extensive role. Animals studies should be used to clarify the indications for endoscopic treatment, particularly for the postnatal treatment of reflux created surgically in utero. Such a study would address the ureteric bud versus reflux

dysplasia dilemma and would provide an opportunity for a comparison of non-operative versus endoscopic management of reflux in the neonate. These issues are extensively debated in the final section of the manuscript.

Other Uses of Teflon in Urology

Following on the wide-spread use of Teflon for the treatment of vesicoureteric reflux, a number of other uses have been suggested and tried. The authors have usually presented the new technique as a case report with little follow-up; the true value of each of these approaches is, therefore, uncertain: Mizrahi obliterated the urethral diverticulum of a 37 year old woman [138]; Bollock *et al.* injected the bladder wall in a patient with a refluxing, post nephrectomy ureteric stump [169]; Saslawsky *et al.* reported the obliteration of a ureterocutaneous fistula in an 86 year old man, also after a nephrectomy [170]; Daniel Yachia prevented vesicoureteric reflux from an incised single-system ureterocele by the concurrent injection of Teflon during endoscopic incision of the lesion [95] and Viville had success with two similar cases [90]. Teflon has also been used for the treatment of VUR after renal transplantation [82,97] and for the treatment of urinary incontinence, the first indication for Teflon injection in Urology [38,61,135,136,171-178].

Glutaraldehyde Cross-linked Bovine Collagen

Concerns about the migration of particles and malignancy risk of Teflon have led to the development of injectable collagen [76,102-105,110,111,179,180]. For the subureteral injection therapy a paste of a highly purified sterile bovine corium collagen is used [103]. The bovine collagen is treated enzymatically with pepsin and dissolved in acetic acid. As a result of the enzymatic treatment the terminal telopeptides (extrahelical peptides of the collagen molecule), being predominantly responsible for the antigenicity, are removed from the collagen molecule without interfering with its triple-helix structure [181]. Following ultrafiltration and ion exchange chromatography, glutaraldehyde is added in a final concentration of 0.0075% for intermolecular cross-linking of the collagen fibres [182]. This procedure reduces the antigenicity of the collagen, stabilises the molecule and reduces breakdown of the implanted collagen deposits by collageneases [183]. To the suspension of pure collagen which is in phosphate-buffered physiological normal saline solution, 0.3% lidocaine is added. Glutaraldehyde cross-linked collagen is commercially available in 1ml syringes containing 0.75ml of paste in a concentration of 0.35mg collagen per ml. To guarantee its physio-chemical properties the paste must be stored at a temperature of 2 to 8°C and should not be frozen. Following injection of collagen paste, the intracorporeal temperature changes the fluid into a firm but elastic gel [184].

Despite the attempts at stabilising the paste and reducing the immunogenicity, these problems still remain, although collagen can be administered through a finer needle than Polytef, allowing the use of a smaller endoscope. The significant published reports of the use of bovine collagen in VUR are shown in Table 29; both the initial success and the late results are lower than the best results with Polytef injection. For their 78% success rate Capozza *et al.* used an average of 0.90ml of collagen paste with a range of 0.7-2.2ml [185] and Leonard *et al.* used 0.62±0.39ml with an initial 78% success rate [111]. In both studies the paste volume was higher than the volumes in

published series for the use of Polytef. Although the costs of the collagen have been favourable compared to open reimplantation [180], those costs do not allow for the expense of late relapses and lack of success of follow-up injections [110,111,185]. Leonard *et al.* recorded VUR resolution in 47 of 60 at one month (78%), but only 29 of 47 at one year (62%) [111] and 21 of the patients treated by Frey *et al.* developed a late recurrence. The problem with absorption is also seen in the management of incontinence [186], a phenomenon which does not occur with the use of Polytef.

Author Year - Ref.	**No. of Ureters Treated per Reflux Grade**					
	I/II	**III**	**IV**	**V**	**Total**	**Percent corrected**
Lipsky 1990 - [104]	45	31	4	0	80	86%
*Leonard 1991 - [111]	-	-	-	-	-	75%
#Frey 1992 - [103]	33	38	22	4	97	59%
Lipsky 1993 - [179]	68	38	9	-	115	60%
**Frey 1995 - [110]	69	97	32	6	204	63%
Capozza 1995 - [185]	-	-	-	-	-	78%
Leonard 1996 - [180]	20	15	10	-	45	82%

Table 29: Results of completed treatment for Vesicoureteric Reflux using *Glutaraldehyde Cross-linked Bovine Collagen* injection. * After one year follow-up. # There were also six late recurrences. ** After only one injection.

The granulomatous response to Polytef is an often stated difference to the collagen alternative. However, the literature does not support this contention, and raises the concern about the immunogenicity of bovine collagen. Barr and Stegman studied 10 cases who had had subcutaneous injections of collagen, with a persistent positive test site; specimens taken were found to have a granulomatous reaction on histological assessment of the resected area [187]. On-the-other-hand, Frey *et al.* did not find granuloma at the site of the collagen injection at the time of subsequent reimplantation [103], whereas, Leonard *et al.* found glutaraldehyde cross-linked collagen engendered a minimal localised inflammatory reaction without granuloma formation in seven patients who under went reimplant three to 19 months after a collagen injection, although they did find infiltration of eosinophils and late calcification at the injection site [102].

The presence of the eosinophils is consistent with the concern about immunogenicity, which has been dealt with in different ways by different authors. Frey *et al.* have not routinely performed a skin test before treating the VUR, as do Leonard *et al.* in all their patients [111]. This latter approach would seem reasonable given the findings of Leonard *et al.*; bovine collagen antibodies were identified in three of 10 patients, 14 to 41 months after one to four collagen injections [188]. These worrying results have also been recorded by others: Elson, in a study of 118 patients with skin augmentation found 3% of patients demonstrated hypersensitivity to the test implant and six patients had a hypersensitivity reaction after a negative skin test [189]; Shortliffe treated a group of 17 patients with incontinence, finding a rise in titres of antiglutaraldehyde cross-linked antibody in the majority of patients: although in patients with detectable antiglutaraldehyde cross-linked collagen antibodies no apparent hypersensitivity reactions were noted [190]; Cooperman and Michaeli, in a prospective study, found a localised, self limiting inflammatory responses to the implant material in two of the 61 subjects (3%): only in these two subjects could elevated levels of circulating antibodies be measured by radioimmunoassay [191]; in a retrospective study of 72 patients

treated with injection for dermal defects a reaction was seen at the implant site alone in 31, generalised symptoms without implant site involvement occurred in 35 and six had both. The systemic complaints could not be correlated with either skin reactions or antibody titres, but the antibody titres correlated with the local reactions [192]. The concerns about allergy were highlighted in a letter to the editor of the Journal of Urology, from Francesco Aragona, pointing out that studies had shown a humoral response to Zyderm collagen implant was usually seen, when studied with the more sensitive enzyme linked immunosorbent assay [193]. Lipsky reported the important case of a child who developed an anaphylactic reaction to the collagen injection for VUR, despite negative skin testing prior to the procedure [179]!

Obviously, the safety and efficacy of glutaraldehyde cross-linked collagen for the treatment of vesicoureteric reflux in children has not been confirmed; in making this statement the author would not be in a position to suggest it is unsafe.

Other substances

Silicone injectable material has been used by surgeons for breast augmentation [115-121] and the treatment of skin creases [122,123] for many years. More recently the material has been used for the treatment of VUR. The silicone used (Bioplastique) has a larger average particle size (100-150μm) than Polytef (90% < 40μm) and when injected into mice could not be found to migrate to the regional nodes as Polytef does [129]. Also the success rate for the treatment of VUR appears to be similar to that of Polytef, the development of Bioplastique was a significant stimulus to the conduct of the studies in Part II [124-127,194].

Another polymer, polyvinyl alcohol (150-250μm), has been used for experiments in rabbits [108] and monkeys [114], with good VUR resolution results; similar

encouraging results have been obtained with Bioglas, a microparticulate form of glass [130], none of these three substances has been used for the treatment of patients with VUR. Atala *et al.* in Boston have also developed a detachable balloon, which is injected into the bladder and urethral wall for the treatment of VUR and urinary incontinence. This device has not yet been reported in clinical use [106,195].

Suitable autologous substances have been sought for the endoscopic management of VUR - fat [131], blood [112], chondrocytes [109,196] and human collagen have all been suggested, but none have yet proven satisfactory. More recently, a product which consists of beads of starch (Deflux^{R}) has been used in both a rat model and in human for the treatment of VUR, with encouraging results. Unfortunately, an extensive study of all the potentially worrying factors has not been conducted on each of these new substances, before they have been used in patients [107].

PART II

Risks of Plastics - Clinical and Laboratory Studies

Introduction

Overview

The recent publicity of the risks of breast implants has heightened public awareness about the use of plastics in medicine. Much of the argument in the Law Courts has used expert witnesses to explain the phenomena under consideration, rather than a review of the available scientific data. The way in which recommendations on the injectable material to be used for the treatment of VUR are reached, has similarly lacked scientific input. To better understand the phenomena of concern, studies of the malignancy and migration risks of the available PTFE and silicone were designed, case material reviewed, evidence for plastic migration in intravenous therapy and migration from vascular access devices assessed. The latter work was performed to place the results from the laboratory and literature in the context of other uses of plastic in the care of children. The literature was studied to answer two questions, namely, Do plastics cause malignancy? and Do plastics migrate? The review served as a template on which to design subsequent studies.

Do Plastics Cause Malignancy?

Plastic materials have a number of applications in medicine, including both solid and injected implantable devices [115,197,198], as syringes and intravenous fluid conduits [199-201]. Many studies have investigated the malignancy risk associated with the implantation of foreign material in rodents [20-22,24,25,50,202], identifying that tumours develop in the capsule around the implants. It has been suggested that the risk of malignancy is reduced when the implanted foreign body is perforated [20], or in a powder form [22]. The currently available injectable forms of plastic are PTFE, with small particles, and silicone with a larger average particle size; these two products had

not previously been studied concurrently in a rat model. The carcinogenic potential of plastic implants is suggested, but not proven, by recorded cases of tumour adjacent two *injectable* PTFE [42,43], and by the development of lymphoma in patients with silicone arthroplasties, where particles had migrated to axillary lymph nodes [203,204].

Part II of this manuscript examines the malignancy risk of two injectable plastics which are currently used for the management of urinary incontinence and VUR. There may be a different risk of particle migration, due to the larger average particle size of injectable silicones; however, the larger particle may conceivably have a greater malignancy risk, judging from previous rat studies.

Do Plastics Migrate?

Polytetrafluoroethylene has been used for the management of VUR since 1981 [62], and has been popularised by the work of Puri and O'Donnell [64,65]. Malizia *et al.* were the first to research the migration of particulate plastics in 1984 [17], although the migration of Polytef particles had been identified as early as 1967, by Boedts [26]. Malizia *et al.* used paste volumes similar to those used for the management of urinary incontinence; volumes which have resulted in episodes of adverse effects secondary to migration of plastic particles [32], whereas migration from small volume implants has not yet been associated with complications.

Polytetrafluoroethylene has been the most used injectable plastic substance for the management of VUR [62,64], urinary incontinence [176] and vocal cord paralysis [6]. It is currently available as a 50/50 mixture with glycerin and marketed as Polytef. Alternatively, injectable silicone has been used for tissue augmentation by plastic surgeons [115] and more recently for the treatment of VUR [124-127,205]. The injectable form is suspended in a hydrogel carrier and marketed as Bioplastique^{R}. Recent reports have suggested that the larger particle size of Bioplastique (100-

150μm) avoids the migration that occurs with Polytef (90% <40μm) [124]. However, a number of studies have documented, not only the migration of injectable PTFE and silicone [115,117,125,206-209], but migration from solid plastic implants [49,198,203,210,211] and intravenous and haemodialysis tubing as used in the treatment of adult patients [60,199-201,212-215].

It has been previously accepted, but is not generally recorded, nor extensively documented, that migration to the lung often occurs when plastics are used in the treatment of human disease, even when a patient is simply given an infusion of intravenous fluid [200]. Reports have suggested that particles can also migrate to the brain following the injection of particulate plastics [17,58,209], including one unsubstantiated report which suggested that migration of plastic to the brain led to a stroke in a six year old girl who had been treated with Teflon paste injection for VUR [216]. The histological reaction to these plastics in the brain has been investigated in the monkey and dog [17,57,209,217,218], and adjacent to rat peripheral neural tissue [219]; however the results are not consistent.

In the dog, PTFE has been suggested to cause demyelination in one of two studies [56,218], whereas in the monkey and rat there was no continued significant inflammatory reaction nor demyelination [17,219]. Because of this variation in outcome, the histological response in the brain to plastic particle injected via the carotid artery, was included as part of this study. The sheep was chosen to include a further species, and the study was designed to compare the tissue reaction of PTFE and silicone.

Experimental Framework

The "risks of plastics" investigations were divided into seven studies. The first four were designed to assess the short and long-term histological response to PTFE and silicone in the subcutaneous space and lung of the rat, and the sheep brain. The intravenous and carotid injections were used to produce a migration model, to circumvent the lack of migration in the subcutaneous injection model.

Evidence had been previously presented for the migration from various tubing used in clinical practice, but no study had been conducted on the use of roller pumps at rates appropriate to the administration of intravenous fluids for Paediatric patients. Thus, an *in vitro* study was performed and followed by an assessment of the fibrous sheath associated with a solid, implanted vascular access device; energy dispersive x-ray analysis of a replica enabled analysis of the elemental content of the Infuse-a-port as used in a number of patients. Further evidence of the occurrence of migration of plastics in patients was supplied from a case treated for incontinence.

LABORATORY STUDY 1

Short-Term Response to Subcutaneously and Intravenously Injected Polytef and Bioplastique in a Rat Model

Materials and Methods

Fifty Sprague-Dawley rats were included in this study. Twenty-three were injected with Polytef, 25 were injected with Bioplastique and two with normal saline as controls. Eight of the animals in the Polytef group had 0.01ml injected into the internal jugular vein and 15 had 0.1ml of the plastic injected into the subcutaneous tissues of the back, whereas eight animals had Bioplastique injected intravenously and 15 had a subcutaneous Bioplastique injection. The intravenously injected animals were sacrificed either at 10 minutes or 12 weeks, and the subcutaneously injected animals were sacrificed either at 30 minutes, 12 weeks or six months (Table 30).

The Polytef used consists of 50% PTFE particles of which 90% are less than 40μm; most of the remainder of the injection volume was glycerin carrier, with a small amount of polysorbate. Bioplastique is 38% silicone, with particles as small as 5μm, but more than 80% between 100-150μm. The bulk of the injection is a hydrogel carrier which is excreted in the urine. The injectable plastics were identical for Laboratory Studies One to Four and the Polytef used clinically.

Subcutaneous Injection Technique

The rats were held in a secure grip by an assistant; an injection of 0.1ml of either Polytef or Bioplastique was injected into the subcutaneous space of middle of the back. Sighting of the bleb during injection ensured placement of the plastic in the subcutaneous space.

Intravenous Injection Technique

The animals were anaesthetised with intraperitoneal barbiturate for the procedure and 0.01ml of paste was injected into the internal jugular vein under direct vision through a lateral neck incision.

Technique of Rat Sacrifice

Rats were euthanised with lethal intraperitoneal injections of sodium pentothal. As soon as death occurred, the subcutaneous injection site (where applicable) was excised and placed in 10% formalin, along with the brain, lungs, heart and great vessels, liver, kidneys and spleen from each animal.

Technique of Handling the Tissue Specimens

A representative section of each organ was then taken, and the subcutaneous injection site was submitted *in toto* for histological examination. Sections were stained routinely with haematoxylin and eosin. As well, injection site slides were stained with Masson trichrome stain to assist in assessing the degree of reactive fibrosis, and lung slides were stained with both Movat pentachrome and elastin stains to delineate blood vessels. Polarised-light and phase-contrast microscopy was used to assist with identification of aggregates of Polytef and Bioplastique respectively.

Results

The following groups of animals were included in this phase of the study.

	Polytef	**Bioplastique**	**Control**
SC 30 min	5	5	-
SC 3 mths	5	5	-
SC 6 mths	5	5	-
IV 10 min	3	5	2
IV 3 mths	5	5	-
Total	23	25	2

Table 30: Numbers of rats sacrificed in the early follow-up plastics-in-rat study. SC = subcutaneous. IV = intravenous

Controls

Sections from the rats who received intravenous injections of normal saline were unremarkable. In particular, no plastic particles were found in any of the organs examined.

Subcutaneous Injections

Polytef at thirty minutes: Organ sections from the rats who had received subcutaneous injections of Polytef 30 minutes prior to death were unremarkable, demonstrating no evidence of foreign material. The injection sites showed more cohesive aggregates of polarizable synthetic material composed of smaller particles than the Bioplastique (Fig. 8A). The lack of adjacent tissue response was similar to those seen for Bioplastique.

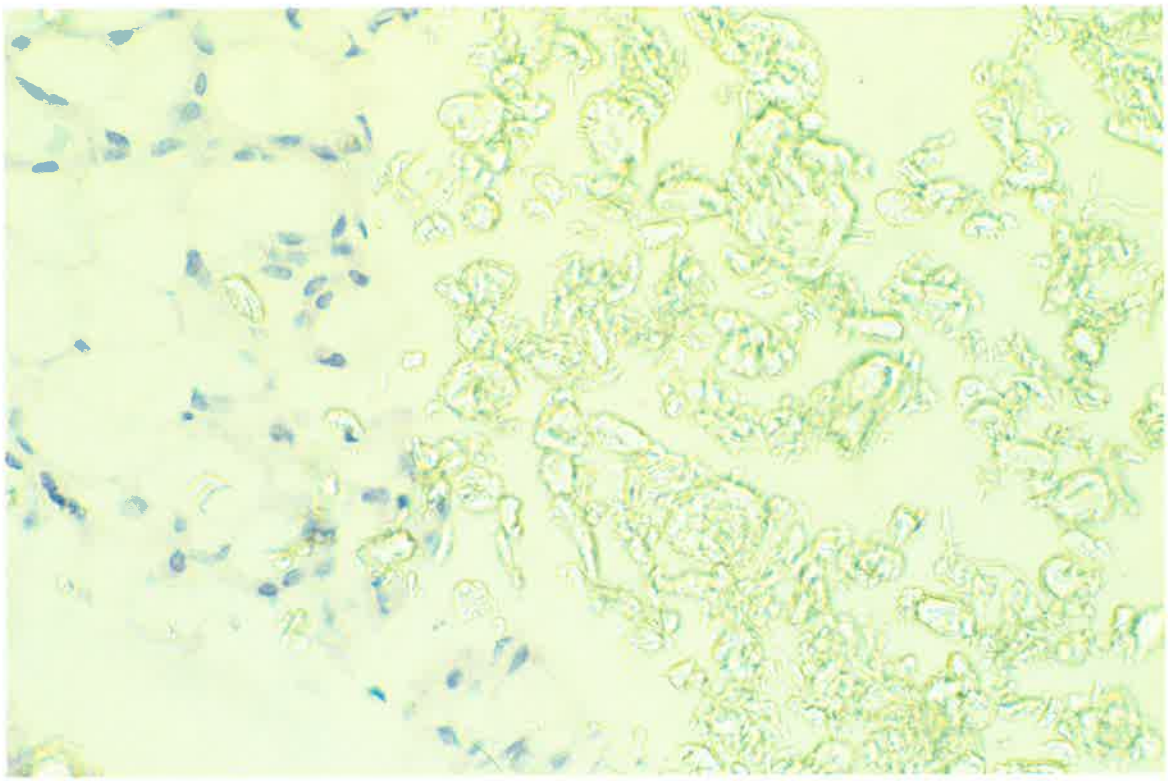


Figure 8A: Biopsy demonstrating small size particles of *Polytef* abutting subcutaneous adipose tissue. (Hematoxylin and Eosin (H & E), original magnification x250).

Bioplastique at thirty minutes: Organ sections from the rats who had received subcutaneous injections of Bioplastique 30 minutes prior to death were unremarkable, demonstrating no evidence of foreign material. The injection sites showed loosely aggregated, poorly-cohesive masses of Bioplastique, which did not polarize, but which was more easily seen using phase-contrast microscopy. The synthetic material usually formed one large collection bordered by fibro-connective and adipose tissue (Fig. 8B). Occasional smaller satellite aggregates of Bioplastique were often present within the subcutis, adjacent to the main mass. No inflammatory reaction was present in any of the sections.

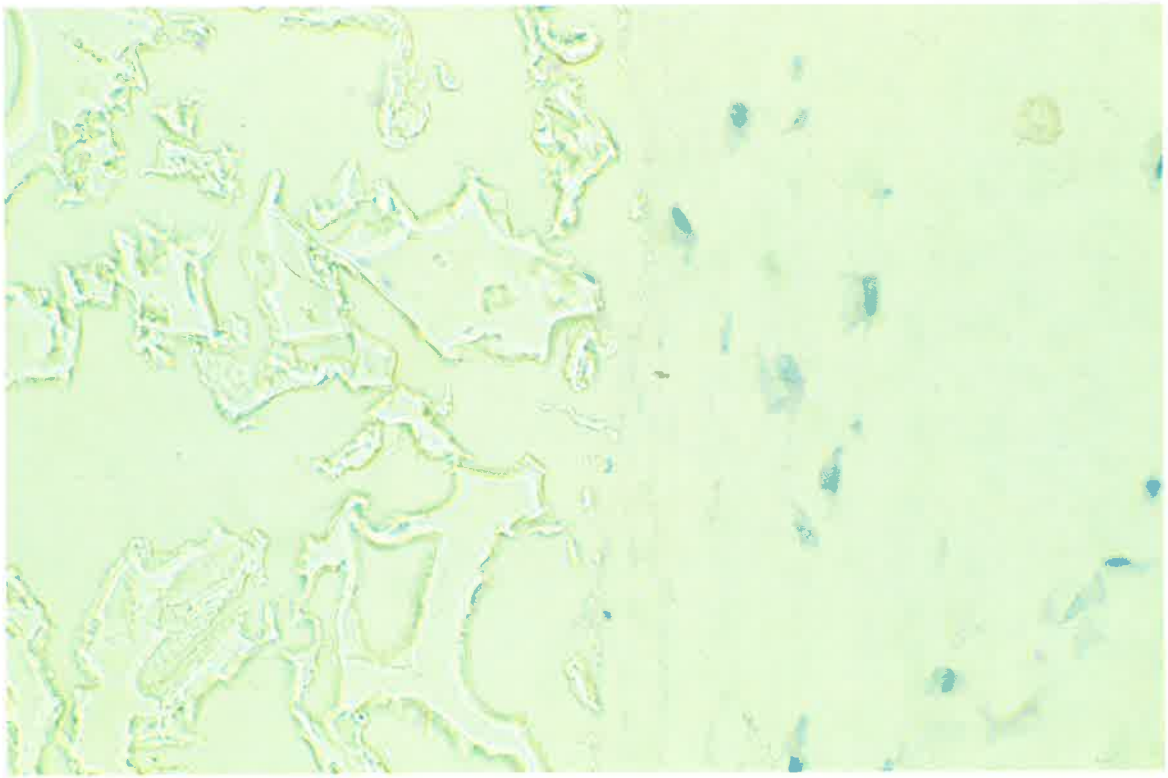


Figure 8B: Section from subcutaneous injection site of *Bioplastique* at 30 minutes showing large particles of *Bioplastique* abutting loose subcutaneous connective tissue. (H & E, original magnification x250).

Polytef at three and six months: Organ sections from the rats who had received subcutaneous injections of Polytef three and six months prior to death demonstrated no evidence of foreign material or granulomatous inflammation on histological examination, such changes were only found at the site of Polytef injection.

The injection sites at both three and six months showed a similar picture, with a localized foreign-body giant cell reaction around small aggregates of Polytef surrounded by fibrous stroma containing both multinucleated giant cells and other chronic inflammatory cells. The degree of fibrosis was less than with Bioplastique, with closer approximation of the smaller particles. The giant cells were also smaller in size (Fig. 9A). There was again no inflammatory infiltrate in the tissue immediately adjacent to the Polytef, which consisted of variable amounts of fibrous pseudocapsule and unremarkable adipose tissue.

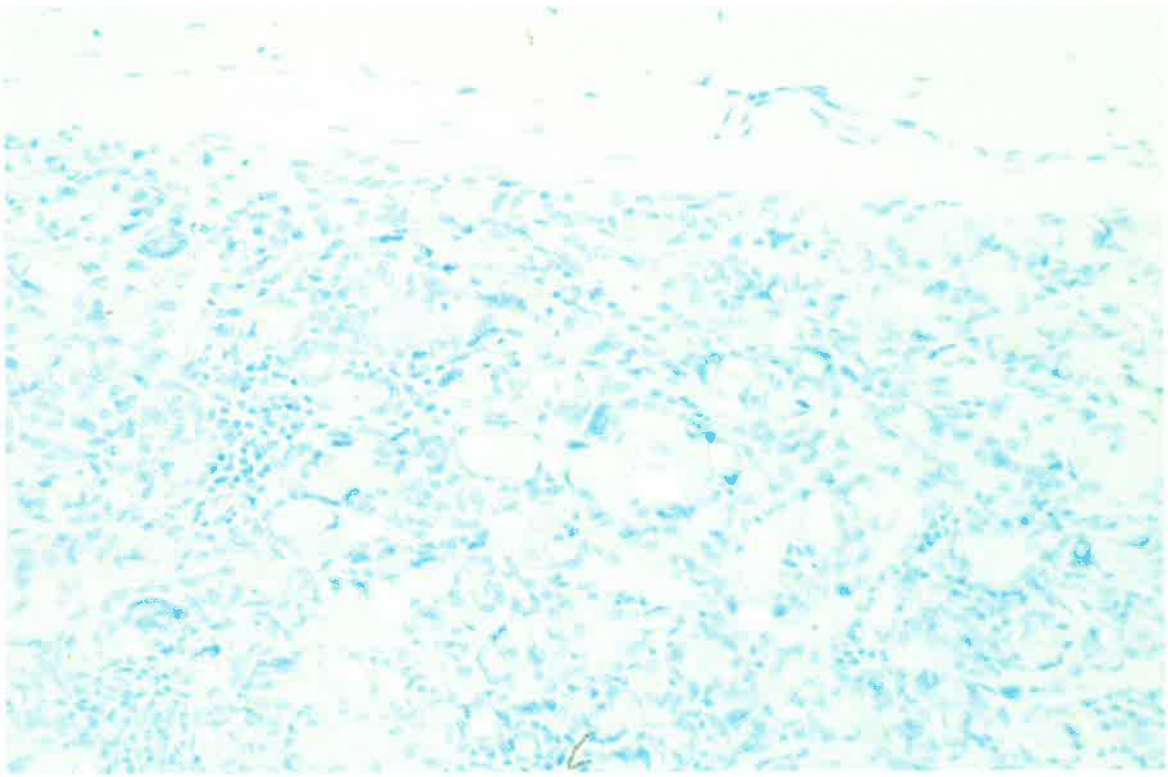


Figure 9A: Subcutaneous injection site demonstrating smaller *Polytef* particles six months after injection with a surrounding chronic granulomatous inflammatory infiltrate. The multinucleated giant cells are smaller than are found with *Bioplastique* injections. There is minimal reaction in the subcutaneous tissue immediately adjacent to the deposit. (H & E, original magnification x250).

Bioplastique at three and six months: Organ sections from the rats who had received subcutaneous injections of Bioplastique three and six months prior to death demonstrated no evidence of foreign material or granulomatous inflammation on histological examination.

The injection sites at three and six months were similar, with a marked localized foreign-body giant cell reaction around small aggregates of Bioplastique surrounded by dense fibrous stroma containing both large multinucleated giant cells and other chronic inflammatory cells (Fig. 9B). There was no inflammatory infiltrate in the tissue immediately adjacent to the Bioplastique; the tissue consisted of variable amounts of fibrous pseudocapsule and unremarkable adipose tissue.

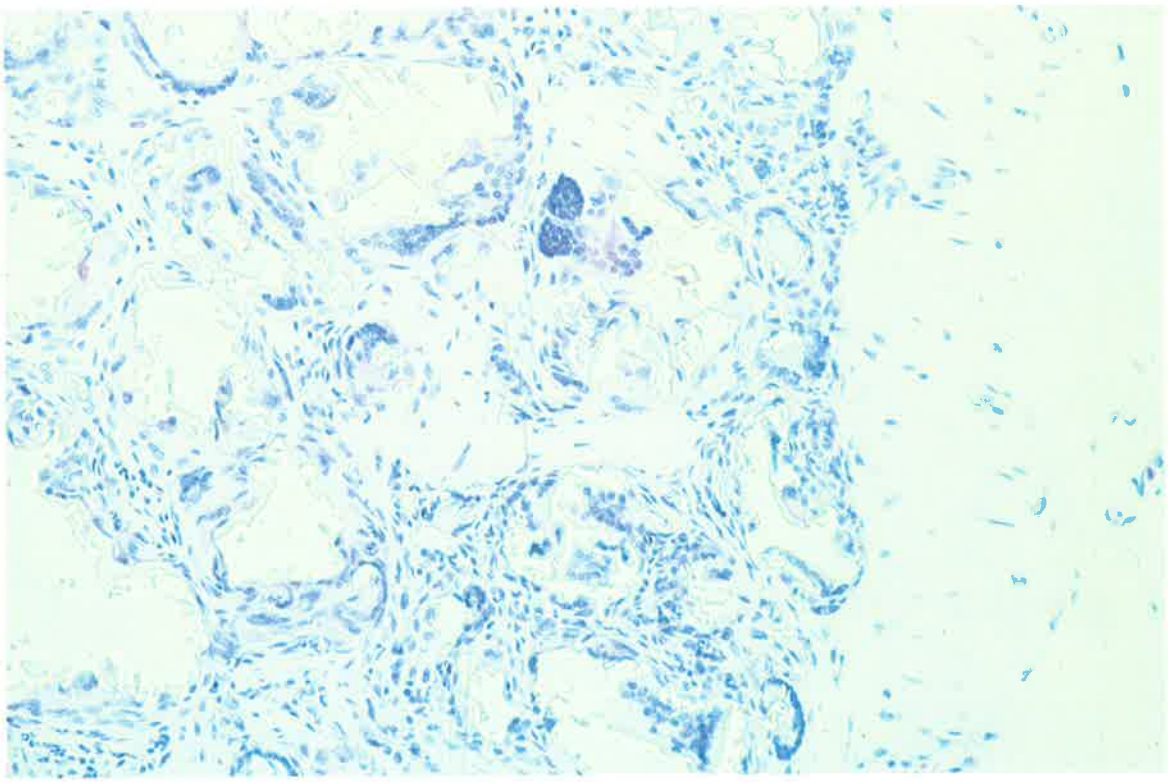


Figure 9B: Subcutaneous injection site six months after injection with *Bioplastique* demonstrating marked fibrosis and chronic granulomatous inflammation with large multinucleated giant cells surrounding aggregates of *Bioplastique*. There is minimal inflammation in the subcutaneous adipose tissue immediately adjacent to the deposit. (H & E, original magnification x250).

Intravenous Injections

Polytef at ten minutes: Sections from the lungs of rats 10 minutes after receiving intravenous injections of Polytef showed a similar appearance to those from rats who had received injections of Bioplastique, except that greater amounts of synthetic material were found within the smaller arterioles and pulmonary capillaries (Fig. 10A). There was, however, no evidence of Polytef in any of the other organs examined. Again there was no tissue response to the intravascular material.

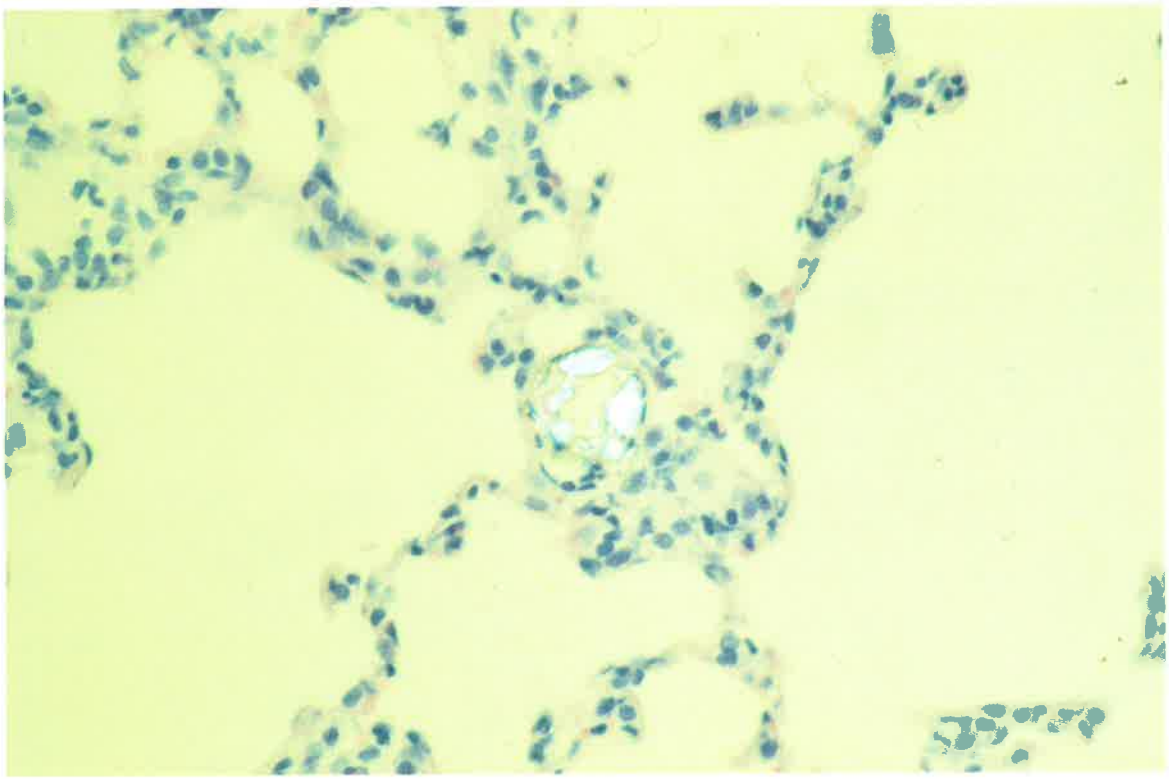


Figure 10A: Section from the pulmonary interstitium 10 minutes after an intravenous injection with *Polytef*, demonstrating filling of smaller pulmonary arterioles with particles of *Polytef*. (H & E, original magnification x250).

Bioplastique at ten minutes: Sections from the lungs of rats who died 10 minutes after receiving intravenous injections of Bioplastique showed aggregates of synthetic material filling small pulmonary arterioles (Fig. 10B). There was no inflammatory reaction, thrombosis or endothelial alteration within the affected vessels, nor was there any evidence of pulmonary infarction. Bioplastique was not demonstrable in any other organs.

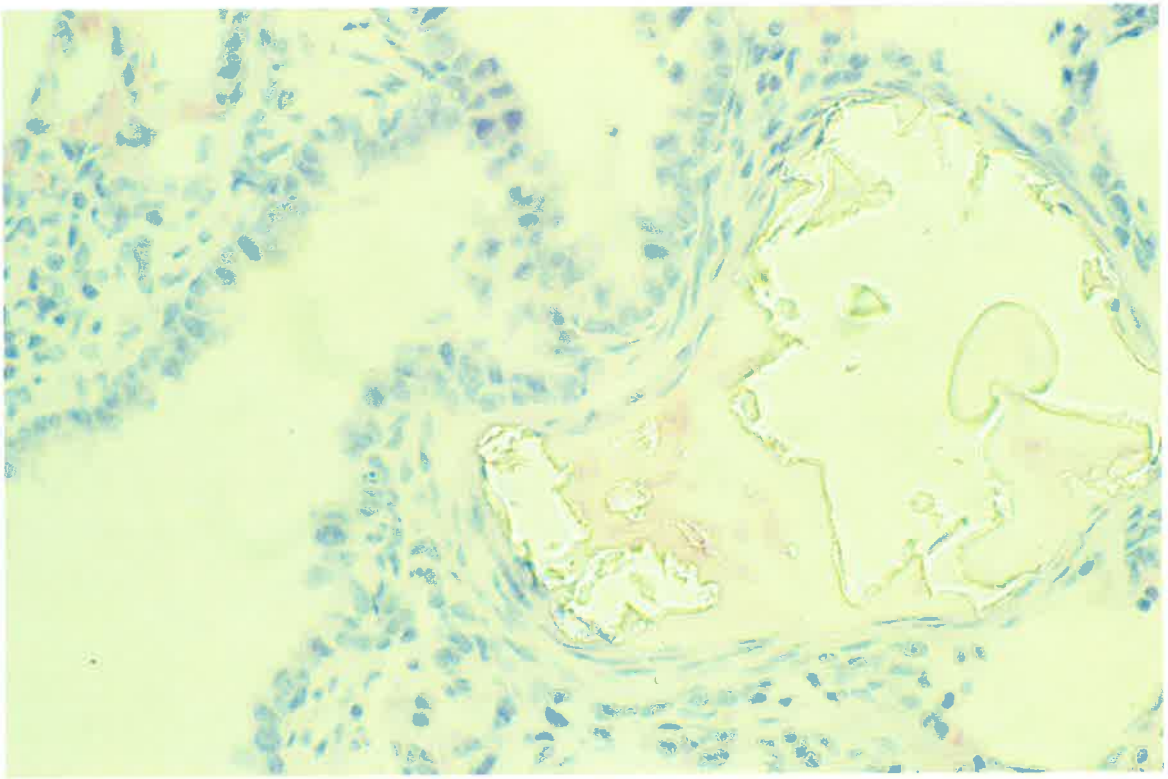


Figure 10B: Section of pulmonary parenchyma 10 minutes after an intravenous injection of *Bioplastique*, demonstrating a bronchus with surrounding alveoli and a pulmonary arteriole filled with large particles of *Bioplastique* (H & E, original magnification x250).

Polytef at three months: Sections from the lungs of rats who were sacrificed three months after receiving intravenous injections of Polytef demonstrated scattered aggregates of periarterial, interstitial chronic granulomatous inflammation surrounding fragments of Polytef, as well as smaller granulomas that were present within the interstitium some distance away from bronchioles (Fig. 11A). There was neither evidence of pulmonary hypertension, nor of pulmonary infarction. Polytef was again not demonstrable in any of the other organs, and there was no evidence of granulomatous inflammation beyond the lung.

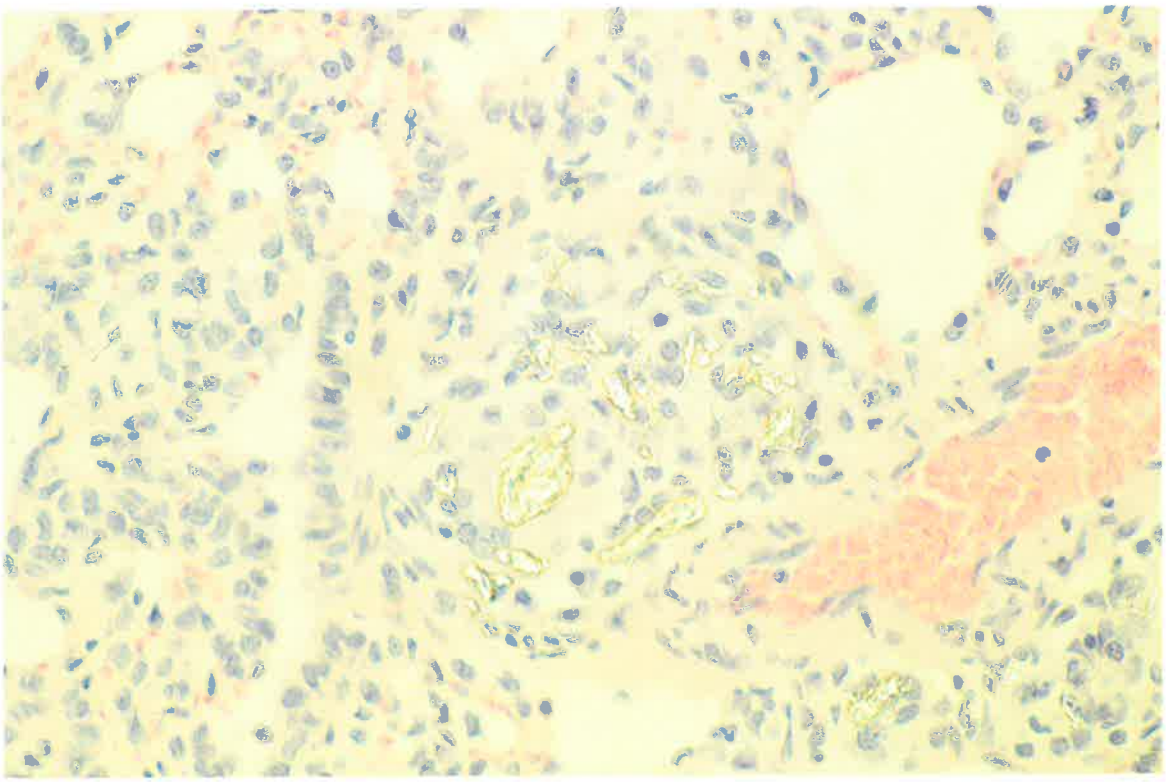


Figure 11A: High power photomicrograph demonstrating chronic granulomatous inflammation surrounding smaller particles of *Polytef* within the interstitium of the lung, adjacent to a pulmonary arteriole, three months after an intravenous injection. (H & E, original magnification x560).

Bioplastique at three months: Sections from the lungs of rats who were sacrificed three months after receiving intravenous injections of Bioplastique demonstrated scattered aggregates of periarterial, interstitial chronic granulomatous inflammation surrounding fragments of Bioplastique (Fig. 11B). There was neither pulmonary vascular hyperplasia, nor evidence of pulmonary infarction. Bioplastique was again not demonstrable in any of the other organs, nor was there any evidence of granulomatous inflammation.

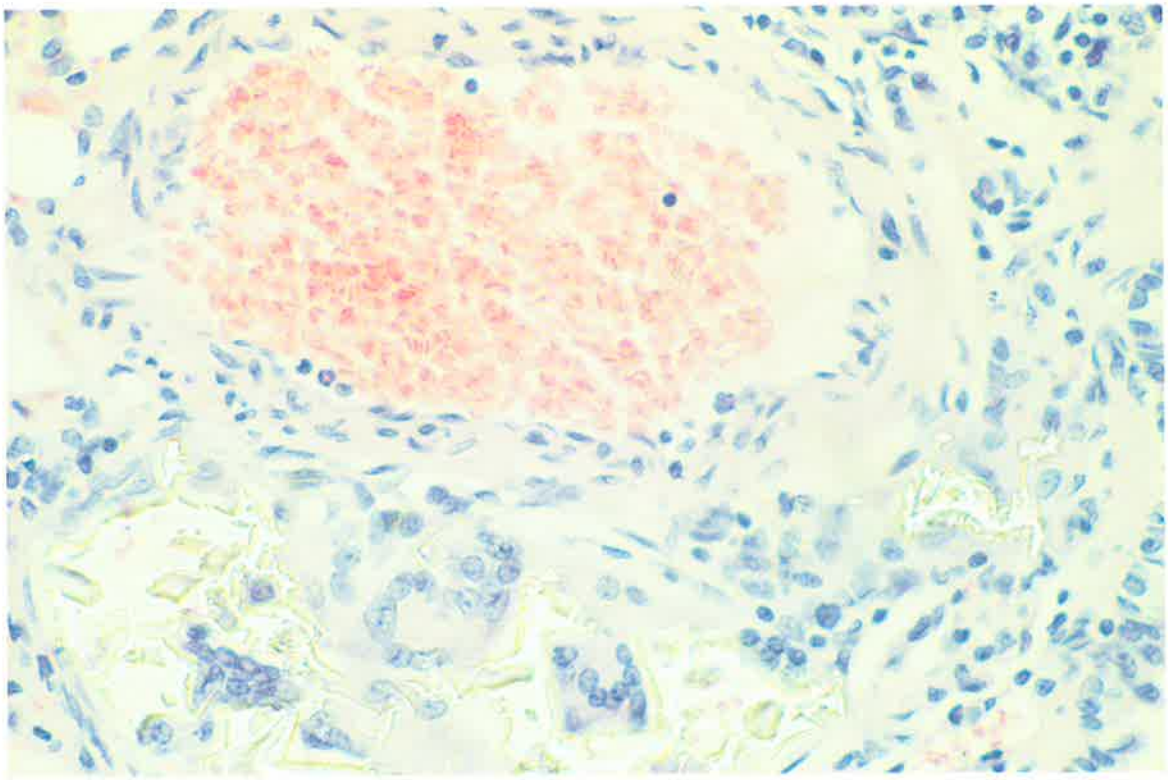


Figure 11B: Section of lung three months after an intravenous injection of *Bioplastique*, demonstrating aggregated giant cells surrounding particles of *Bioplastique* adjacent to a pulmonary arteriole. (H & E, original magnification x250).

LABORATORY STUDY 2

Long-Term Histological Response to *Subcutaneously* Injected Polytef and Bioplastique in a Rat Model

Materials and Methods

One hundred and fifty infant Sprague-Dawley rats were divided into three groups; 50 had 0.1ml of Polytef injected under the skin of the back, 50 had 0.1ml of Bioplastique injected and 50 animals acted as controls.

The animals were sacrificed at two years, or when there was evidence of ill-health or tumour development. Rats were euthanised with lethal injections of intraperitoneal sodium pentothal. Upon death, the subcutaneous injection site was excised and placed in 10% formalin, along with the brain, lungs, heart and great vessels, liver, kidneys and spleen. Representative sections of each organ and tumour were taken, and the subcutaneous injection site was submitted *in toto* for histological examination. Sections were stained routinely with haematoxylin and eosin. Also, injection sites were stained with Masson trichrome, to help assess the degree of reactive fibrosis, and tumours were stained immunohistochemically. Polarised light and phase-contrast microscopy was used to delineate aggregates of Polytef and Bioplastique respectively.

The number of tumours in each group and the relative numbers of breast and pituitary tumours were compared using the Chi^2 test.

Results

Six rats were not available for follow-up assessment due to technical problems; two from each group. The distribution of total tumour numbers is given in Table 31, and the numbers of breast and pituitary tumours are give in Table 32.

No. of Tumours	Polytef	Bioplastique	Control
0	18	14	12
1	16	18	15
2	5	12	13
3	6	3	5
4	2	1	3
5	1	-	-
Total	48	48	48

Table 31: The total number of tumours per animal for each of the study groups.

	Numbers of tumours in each group		
Tumours Identified	**Polytef**	**Bioplastique**	**Control**
Breast Adenoma	15	22	27
Pituitary Adenoma	18	18	20

Table 32: The numbers of the two most common tumours identified in the rats.

Using the limited sampling technique, taking only a representative section of each organ, no PTFE or silicone was found beyond the injection site in the implanted animals, nor were plastic particles found in the controls.

Polytef

Of the 48 Polytef injected animals studied, 30 (63%) had one or more tumours (Table 31 + 32). Breast adenomas and fibroadenomas were common and often multiple, being present in 15 (31%) animals. Pituitary adenomas were seen in 18 (38%) rats. The other tumours seen in this group included breast adenocarcinomas (4), lymphoma (1), ovarian tumours (2), uterine carcinoma (1) and a hepatocellular carcinoma (1). No sarcomas were seen, and no tumours were found in the region of the injection site.

The injection sites at two years showed a similar reaction to that previously recorded at six months, but with some reduction in the cellularity of the localized foreign-body giant cell reaction around small aggregates of Polytef. The particles were surrounded by fibrous stroma containing both multinucleated giant cells and minimal numbers of other chronic inflammatory cells (Fig.12). There was no inflammatory infiltrate in the tissue immediately adjacent to the injection site, which consisted of variable amounts of fibrous pseudocapsule and unremarkable adipose tissue.

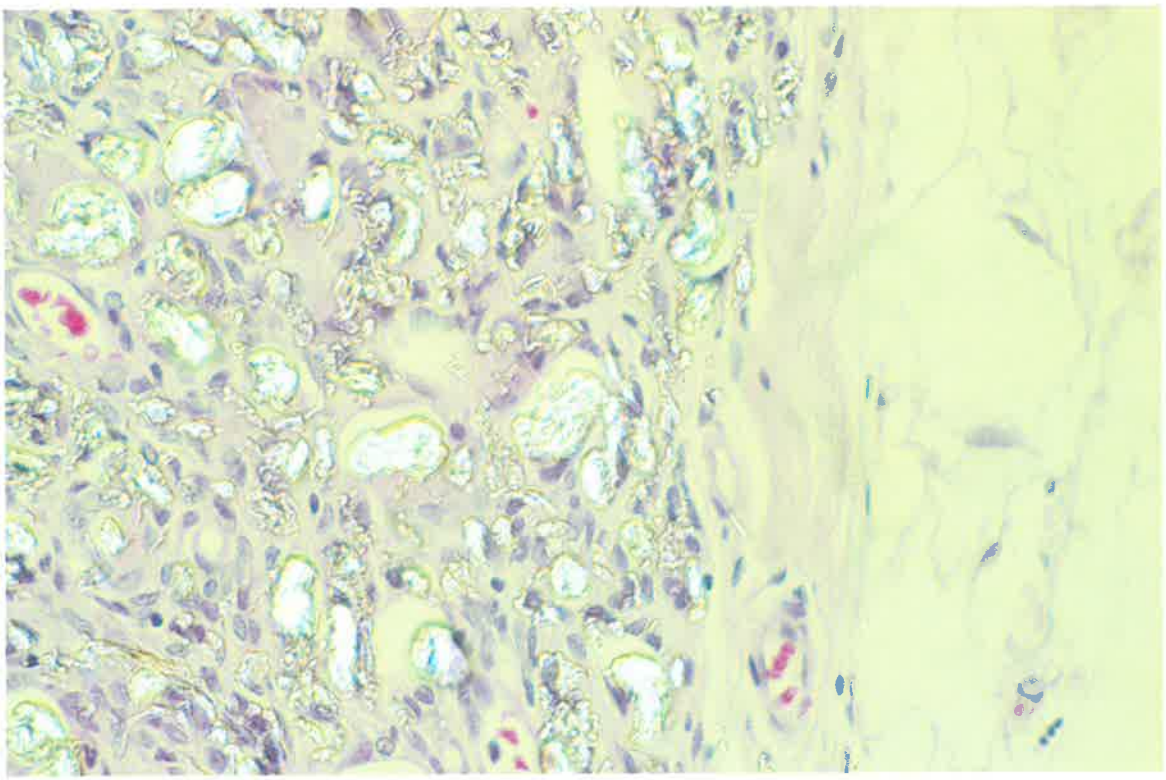


Figure 12: Subcutaneous Polytef particles within foreign body giant cell with a quiescent adjacent inflammatory reaction after two years.

Bioplastique

Thirty-four (71%) of the 48 Bioplastique injected animals studied developed one or more tumours. Twenty-two (46%) had a breast adenoma, many with multiple tumours, and pituitary tumours were seen in 18 (38%). The other tumours in this group included a mediastinal hibernoma (2), retroperitoneal liposarcoma (1), squamous cell carcinoma of the foot (1), breast adenocarcinoma (1). The three tumours associated with the injection site were in this group; these three tumours were all poorly differentiated sarcomas which were immunohistochemically positive for vimentin and focally positive for muscle specific actin and smooth muscle desmin, consistent with lieomyoscarcoma: Bioplastique particles were seen within the tumours (Fig. 13). One other rat had a sarcoma located on it's back which differed from the other three in that it was separate from the injection site and was of neural origin (Fig. 14).

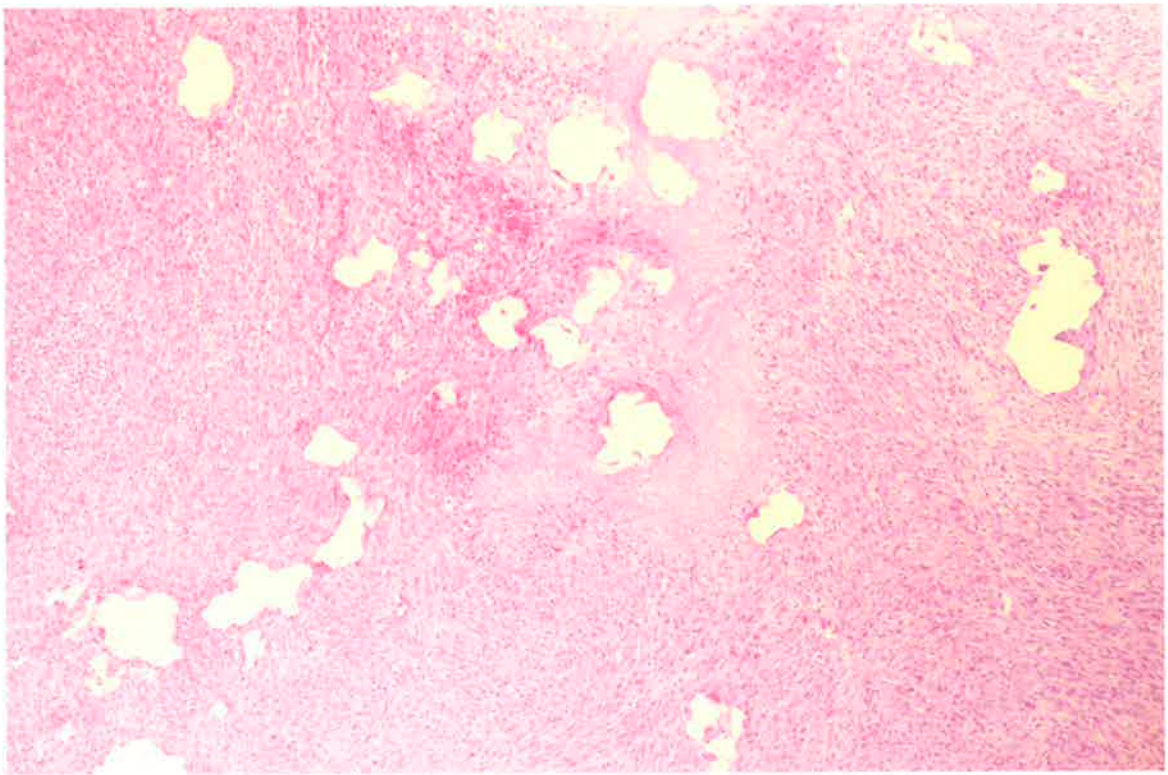


Figure 13: Bioplastique within a poorly differentiated, subcutaneous sarcoma from one of the three animals with sarcoma and Bioplastique co-located.

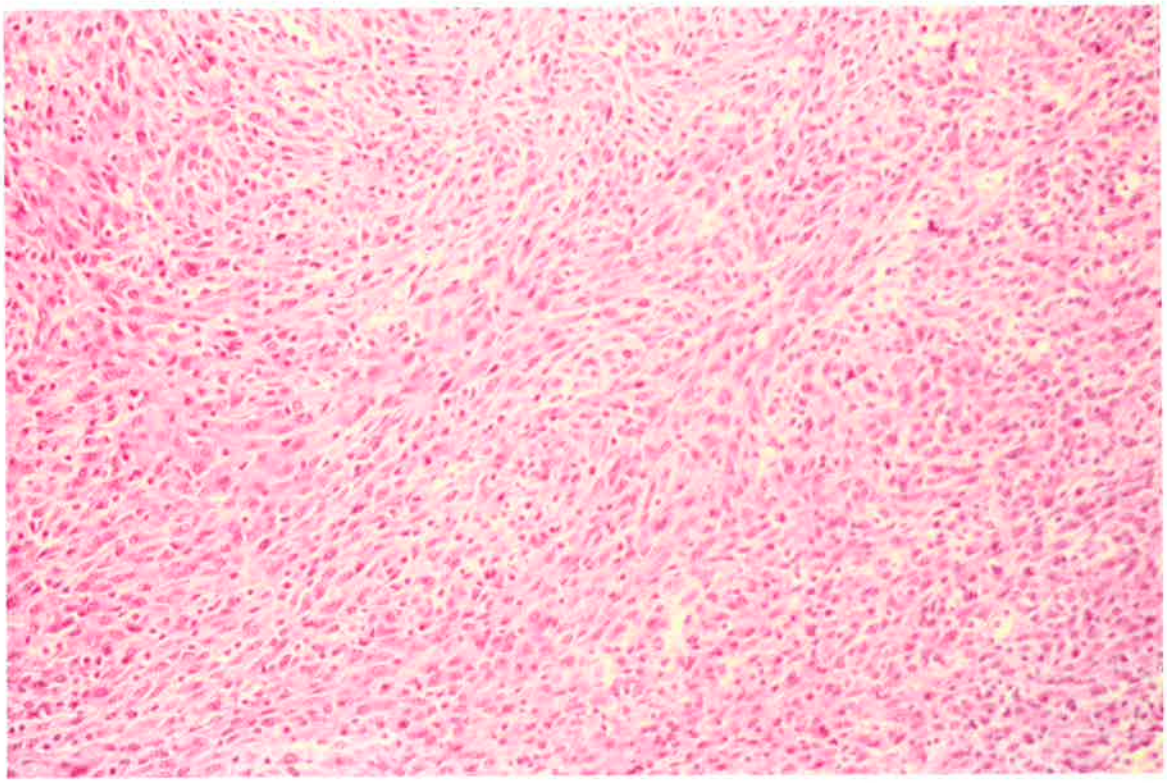


Figure 14: A sarcoma with neural features, from the back of a rat, but not associated with the Bioplastique injection site.

In the remaining 44 rats, the reaction to the implant was similar to that seen at six months, with some reduction in the degree of cellular infiltrate within the fibrous pseudocapsule, around which were particles phagocytosed by giant cells (Fig. 15).

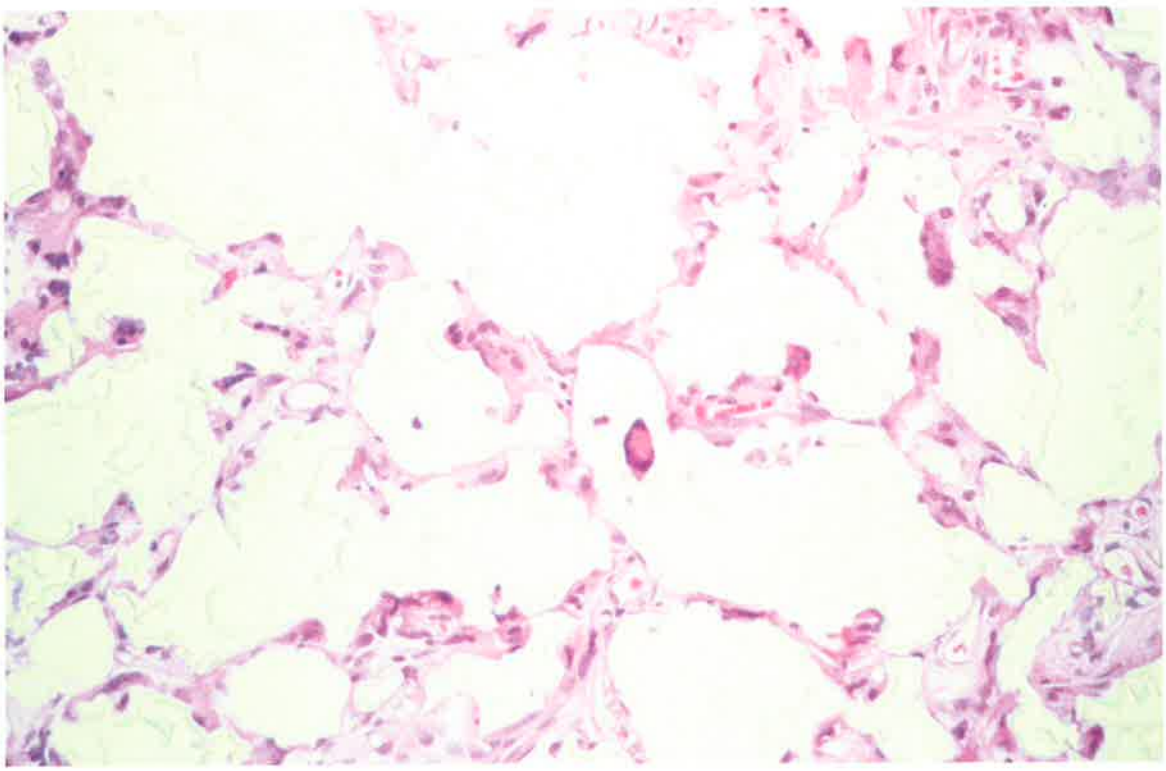


Figure 15: Bioplastique particles from a tumour free subcutaneous site.

Control

Forty-eight of the 50 control animals were available for assessment of which thirty-six (75%) had developed at least one tumour. The tumours included 20 (42%) pituitary adenomas of various sizes, a variable number of breast adenomas in 27 rats (56%), adenocarcinomas (three), renal carcinoma (one), metastatic carcinoma of unknown origin (one) and a liposarcoma. No fibrosarcomas were identified in the control group nor were there any tumours evident on the back of any of this group of rats.

Statistical Analysis

Chi^2 tests were used to compare the tumour incidence between the groups. There was no significant difference between the three groups for the total number of tumours [$X^2=7.5$; $(0.4<p<0.5)$ - Table 31], and there was no significant difference in the number of breast and pituitary tumours [$X^2=1.19$; $(0.25<p<0.5)$].

LABORATORY STUDY 3

Long-Term Histological Response to *Intravenously* Injected Polytef and Bioplastique in a Rat Model

Materials and Methods

Twenty-one Spraque-Dawley rats were included in this part of the study. *Eleven* were sacrificed at 12 months, *nine* at two years and *one* died of a pituitary tumour at 18 months: *10* were injected with Polytef and *11* with Bioplastique.

All sections were stained routinely with haematoxylin and eosin, and lung slides additionally with Movat pentachrome and elastin to more clearly delineate the vessels. Polarised and phase-contrast microscopy were used to help identify aggregates of PTFE and silicone respectively.

The control group for this part of the study was the same animals as described in Laboratory Study Two and consisted of 50 animals, 48 of which were available for analysis.

Results

Incidental tumours were found in a number of rats, but no particles of plastic were found in the tumours. The rate of tumour formation was the same as for the control rats. In all but one animal the lungs were macroscopically normally. The abnormal lungs were found in a Polytef injected animal with a sizeable breast adenocarcinoma, which had metastasised to the lung and largely filled the thoracic cavity. In the small areas of normal lung that remained the reaction to the plastic material was the same as in the other animals, as indicated below. A pituitary adenoma was also found in the same animal and in three other Polytef injected animals. A breast adenoma was

discovered at autopsy at two years in one, both a breast and a pituitary adenoma in another, and the third appeared to have died from a large pituitary adenoma at the age of 18 months. Tumours were also found in three Bioplastique injected animals. One had a pituitary adenoma, one a breast adenoma; the third had both a breast and pituitary tumour.

Lung Changes

The appearance of the histologic reaction was virtually the same two years after the injection of either plastic as it had been at 12 months.

Polytef

Sections of lung demonstrated focal periarterial, chronic granulomatous inflammation adjacent to larger airways with aggregated giant cells containing birefringent fragments of PTFE. The number of inflammatory cells, in addition to the giant cells, was minimal (Fig. 16). Similar deposits of the plastic were present within the lung parenchyma beside small arterioles, some distance from the large airways (Fig. 17). There were no pulmonary hypertensive changes, nor evidence of lung infarction. Polytetrafluoro-ethylene was not seen in any other organ and there was no foreign body granulomatous inflammation outside the lung.

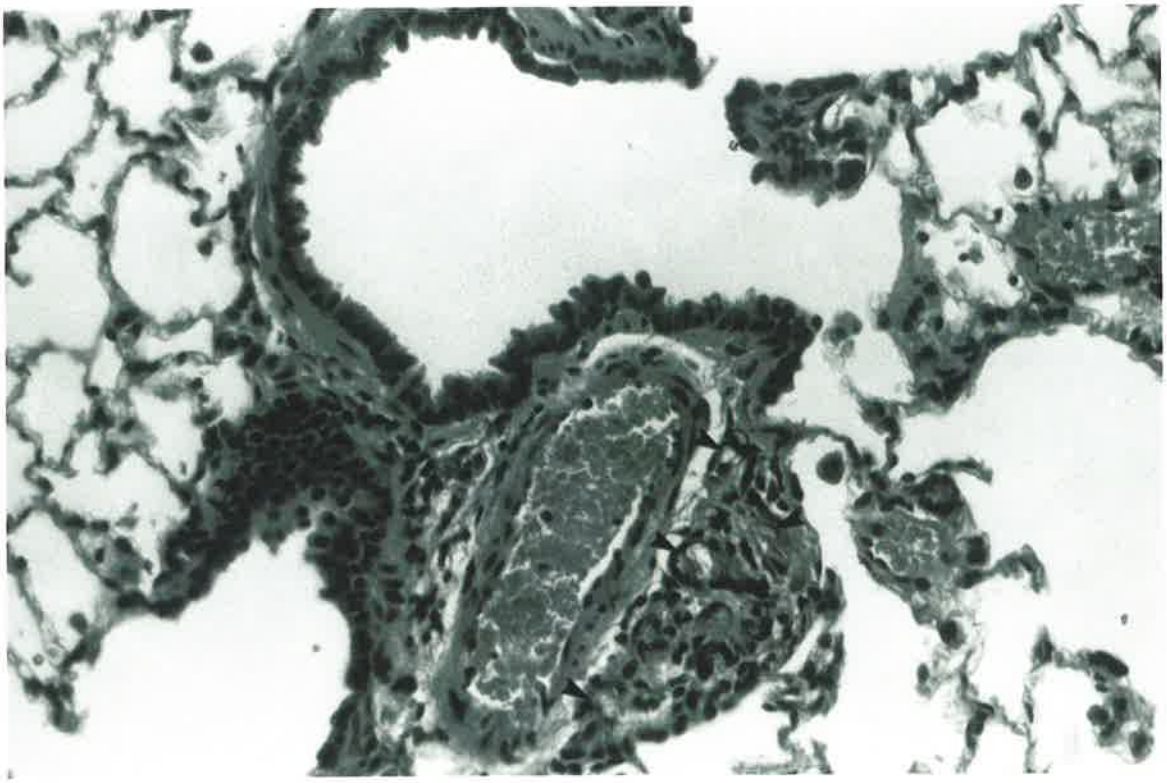


Figure 16: PTFE particles within aggregated periarterial giant cells within the lungs.

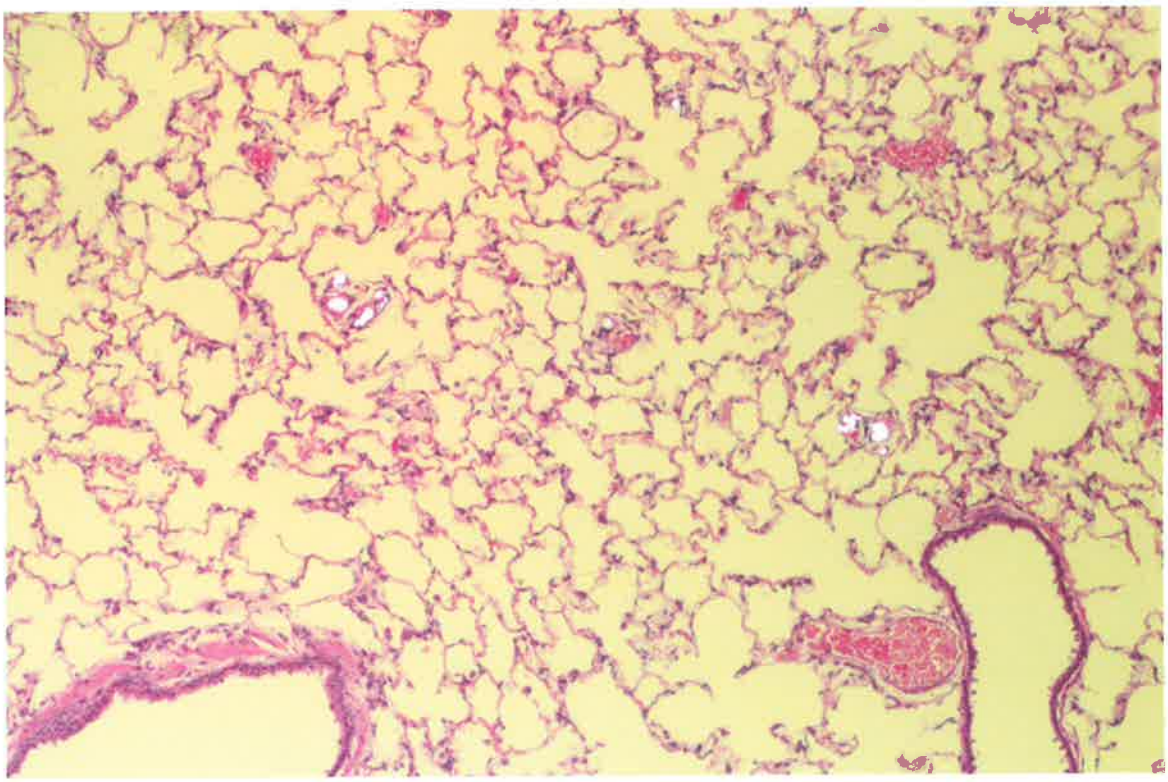


Figure 17: PTFE particles within the lung parenchyma, adjacent to small arterioles, some distance from larger airways.

Bioplastique

Lung sections demonstrated focal periarterial, chronic granulomatous inflammation, again adjacent to larger airways, with aggregated giant cells which were larger than those seen with PTFE (Fig. 18). Phase-contrast microscopy highlighted the silicone particles within the giant cells. Other inflammatory cells adjacent to the giant cells were few in number. As with Polytef injected animals, there were no pulmonary hypertensive changes, no evidence of lung infarction, and no plastic or granulomata in any of the other organs.

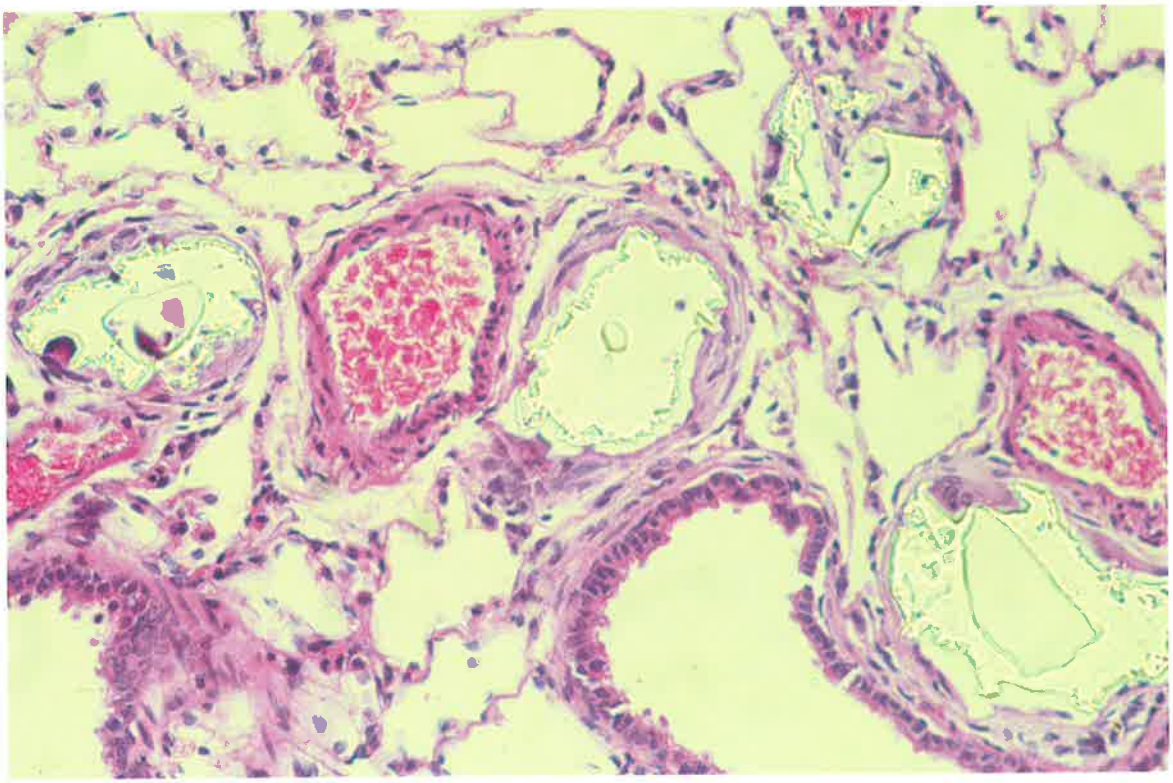


Figure 18: Silicone particles within aggregated giant cells, larger than those seen with PTFE (Figs.16+17). The granulomatous inflammation is associated with only a minimal number of other chronic inflammatory cells.

LABORATORY STUDY 4

Histological Response to Injected Polytef and Bioplastique in the Sheep Brain

Materials and Methods

Four sheep had a direct vision, carotid artery injection of 0.1ml of either PTFE-containing Polytef, or silicone-containing Bioplastique. The sheep were anaesthetised with nitrous oxide, halothane and oxygen and a small longitudinal neck incision was made. The carotid artery was isolated and the paste injected using an intravenous cannula. The paste was injected over a 10 minute period. One PTFE and one silicone injected animal were then sacrificed and the entire brain submitted for sectioning. One animal injected with PTFE and one with silicone were sacrificed at six months. Again the entire brain was sectioned. The animals sacrificed early were to confirm the ability to identify particles after a 0.1ml injection, and those sacrificed at six months were included to assess the histological response.

Polarised and phase-contrast microscopy were used to identify the PTFE and silicone respectively. Luxol fast blue stain was used to assess demyelination. Confirmation of the nature of the particulate matter, using electron microscopy and energy dispersive X-ray analysis, was not felt necessary because the plastics had been introduced via a direct carotid artery injection. Also the PTFE group acted as a control for silicone particles, as did the silicone group for PTFE.

Results

No particles of silicone were seen in the Polytef injected animals; likewise, no PTFE particles were found in the Bioplastique injected sheep.

Ten minutes

Polytef

Readily identifiable intra-arterial Polytef fragments were seen within meningeal vessels and within the grey and white matter of the cerebrum and particularly the cerebellum. PTFE was also present within the choroid plexus of the fourth ventricle. No inflammation was seen.

Bioplastique

Occasional intra-arterial fragments of silicone were identified, but were more difficult to find than was the PTFE with polarised light, as the Bioplastique consisted of 38% silicone compared to the 50% plastic in Polytef. No inflammatory reaction was seen in the region of the silicone.

Six months

Polytef

Sections revealed plastic material within vessels at all levels of the brain sampled. Generally there was no reaction to the particles which remained within the vessels. In one vessel in the cerebellum there was an "onion skinning" appearance resulting from proliferation of the vessel wall. In several vessels within the cerebral cortex there was a giant cell reaction around extravasated material. Luxol fast blue staining failed to reveal any demyelination around vessels containing the particles (Fig. 19). Polytef particles found within the brain substance were in some instances associated with chronic granulomatous inflammation (Fig. 20).

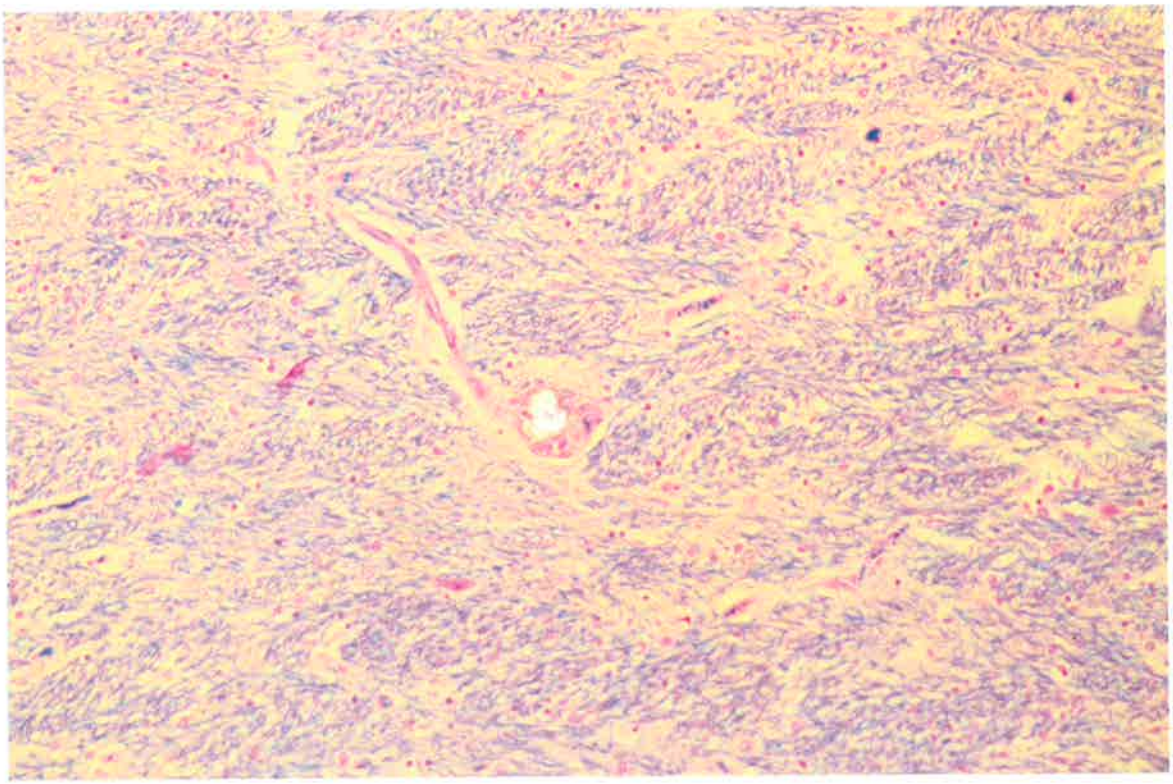


Figure 19: Luxol Blue staining showing no evidence of demyelination adjacent to PTFE.

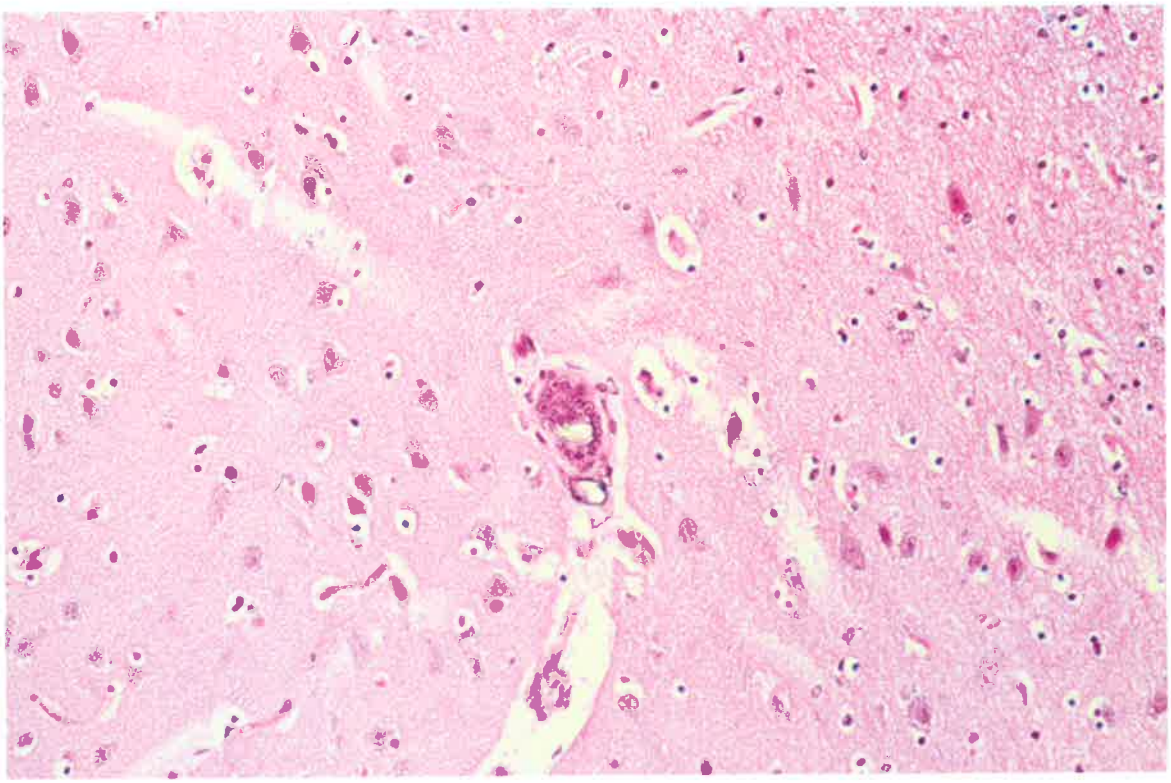
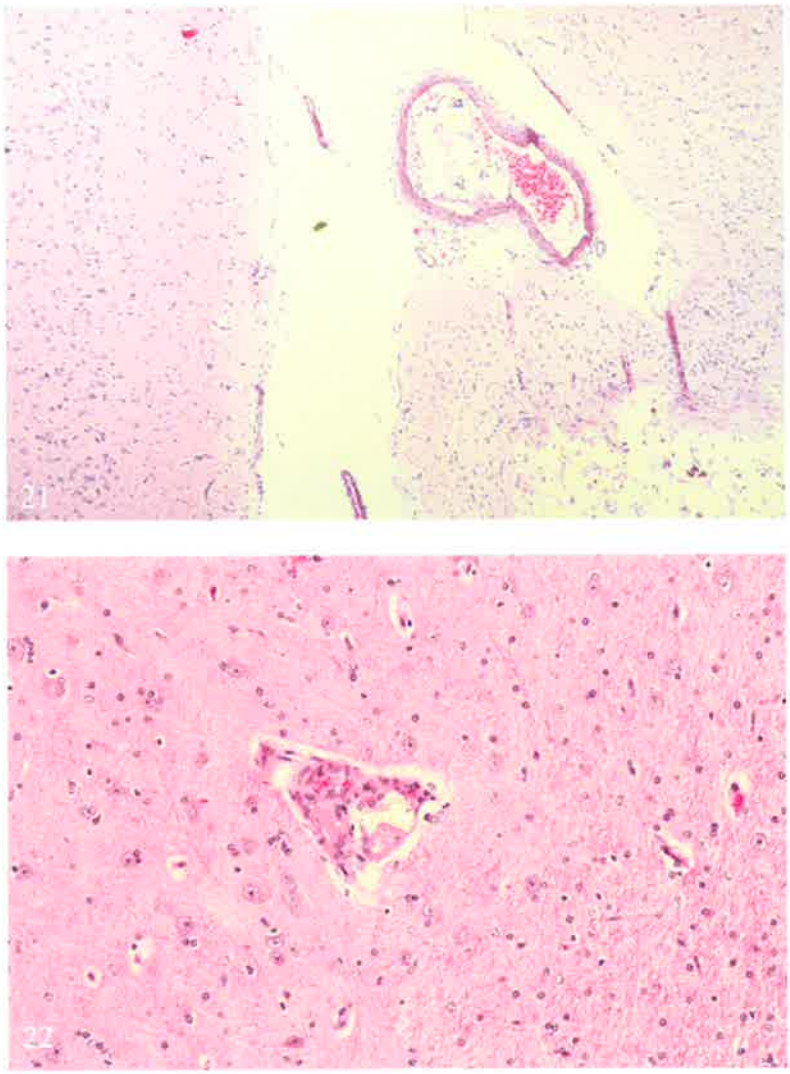


Figure 20: PTFE particle in the brain six months after injection showing a minimal granulomatous reaction.

Bioplastique

Particles of silicone were located within the meningeal vessels (Fig. 21) and also in a juxtavascular position where they were often surrounded by giant cells (Fig. 22). Very occasional intravascular aggregates of silicone were identified within vessels in the brain substance. There did not appear to be any associated demyelination. Bioplastique particles tended to be found more readily in meningeal vessels, although they were also present within the deep vessels of the brain.



Figures 21+22: Silicone particles in the brain vessels (upper) and parenchyma (lower) six months after injection, showing minimal chronic inflammatory reaction.

LABORATORY STUDY 5

An *In Vitro* Study of Silicone Migration from Intravenous Fluid Tubing as used in Paediatric Practice

Materials and Methods

Microfilter Usage

In total 11 cellulose acetate microfilters were examined. Four filters had fluid delivered via a pump over three hours and one over 72 hours, three had fluid but no pump action on the tubing and three filters were unused.

Scanning electron microscopy (SEM) was performed on seven and Energy Dispersive X-ray Analysis (EDXA) on four separate filters; two that had received fluid pumped for either three or 72 hours, one exposed to non-pumped fluid and one unused filter.

Silicone Tubing Usage

Eight sets of silicone tubing were examined. Four were housed in the finger pump running at 60ml per hour for three hours, and one for 72 hours. Three were unused sets. One of the portions of silicone tubing was also examined by EDXA.

Specimen Preparation

In Vitro Perfusion

For the four infusion sets used for three hours, a 100ml bag of 0.9% Sodium Chloride intravenous solution was pumped through a 560 IVAC finger pump via an IVAC Universal infusion set, which has a silicone segment to sit within the pump chamber. The pump was run at 60mls per hour for a total of three hours. The solution was

recirculated through the pump, such that a total of 180mls passed through the pump and back into the bag. The fluid was then vacuum filtered through a 2µm cellulose acetate filter. When the fluid was not passed through an IVAC pump, the same type of tubing was used and 180ml was vacuum extracted directly from a 500ml bag, through a similar filter.

When the pump was run for 72 hours, a one litre bag of saline was recirculated through a finger pump at 60ml per hour, and compared to a vacuum pumped one litre of 0.9% saline.

Scanning Electron Microscopy (SEM)

The filters and segments of the tubing were mounted on aluminium stubs, glow discharged and shadowed with gold during rotation through 360° in a vacuum evaporator. The filter and tubing samples were examined in a JEOL JSMT20 SEM at 12.5 kV and 30° tilt. The control samples were prepared in the same manner.

Energy Dispersive X-ray Analysis (EDXA)

The cellulose acetate filter samples were prepared by cutting sections of the filter approximately 10mm square from the 50mm diameter filters. These sections were glued to 12.5mm diameter aluminium pin stubs using double-sided adhesive tabs which were then coated with 20nm of carbon in a vacuum evaporator.

All samples were examined in a Philips XL20 SEM with an integrated EDAX-DX4i EDXA Analyser. The X-ray detector was of the ultra-thin-window type and can efficiently collect x-rays over the elemental range Boron (Z = 5) to Uranium (Z = 92), but note that Hydrogen was not within the detectable range of the analysis. The accelerating potential used was 20 kV.

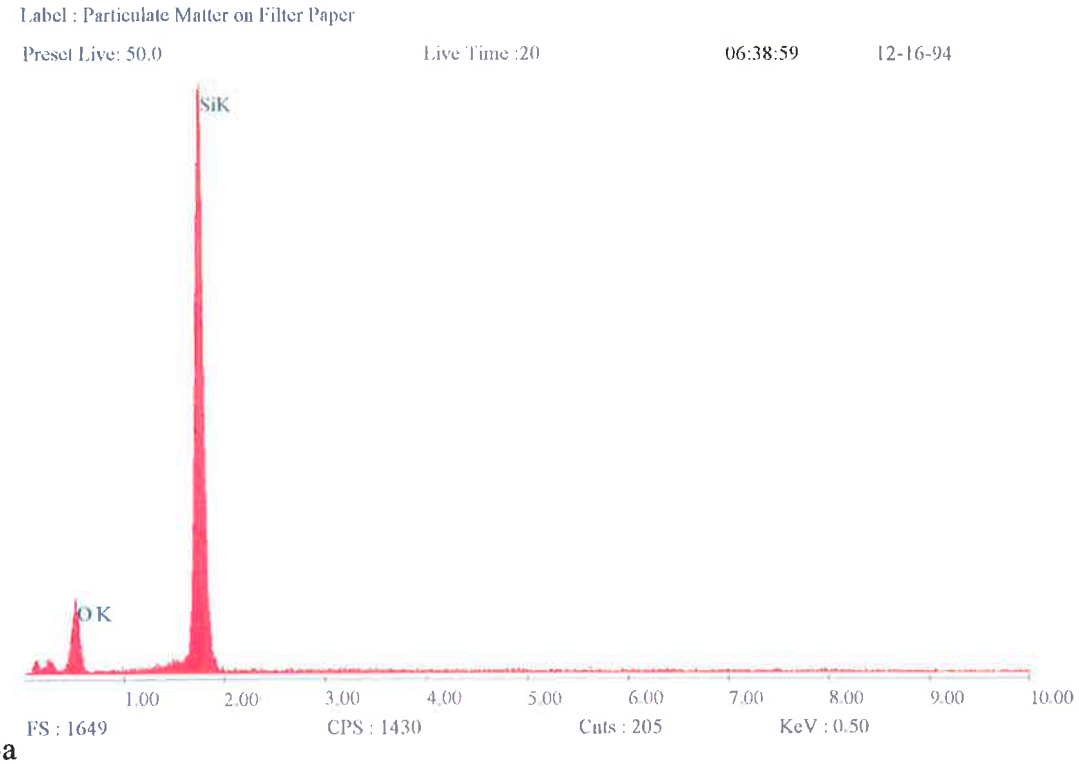
Spectra were recorded from an area with no apparent particulate matter and from several of the particles on the surface.

The author was involved in the setting up of the infusion system and the filtering of the fluid and the SEM and EDXA was performed as technical assistance by those with expertise in using the equipment. The interpretation of the data was through discussion with the technicians and the author.

Results

Cellulose Acetate Filters

The filters exposed to pumped fluid had particulate matter randomly spread over the surface. Particle analysis showed elemental Silicon (Si) in some, and Sodium and Chloride in others, whether the pump had been used for three or 72 hours (Fig. 23). All filters through which pumped fluid had passed contained particles which contained elemental silicone. Particles were seen on the two filters after 180ml, and one after one litre had been suctioned through them without finger pump action on the IV line; EDXA identified Sodium Chloride on the filter through which 0.9% saline had passed (Fig. 24), but no Si was found.



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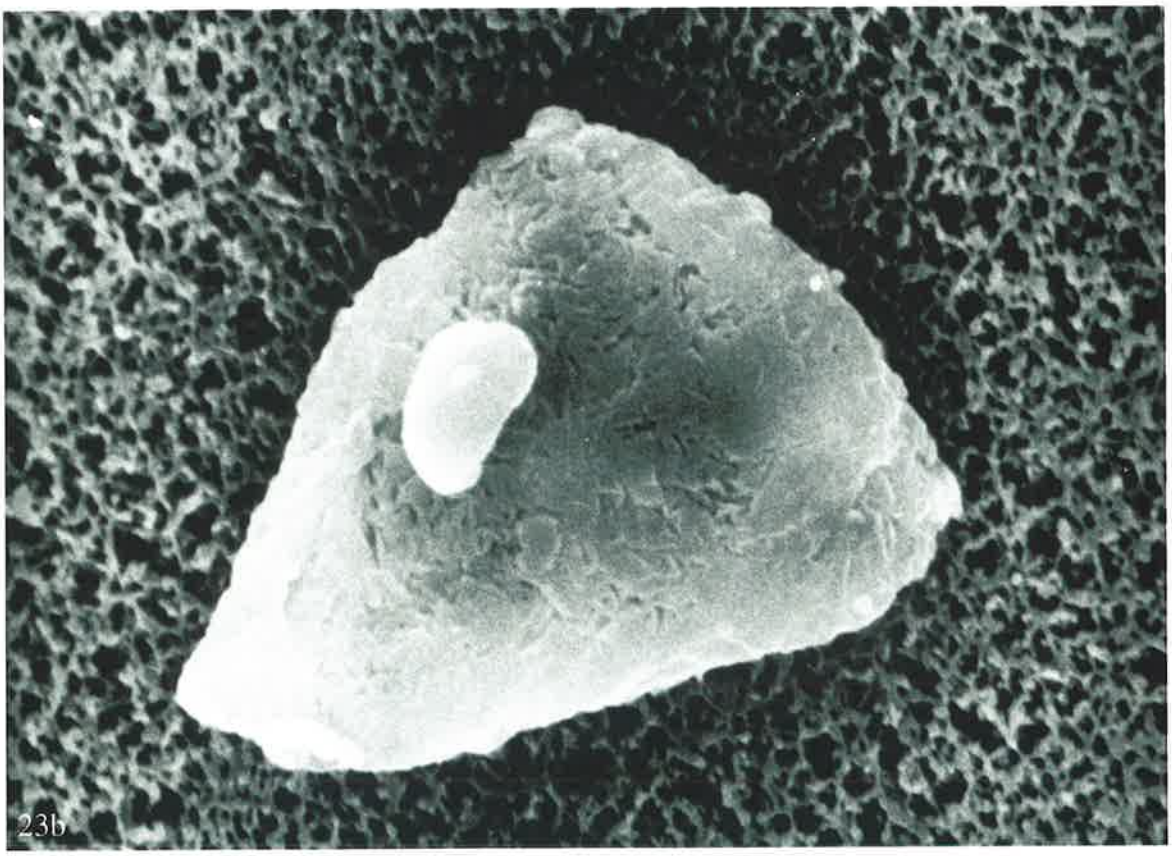
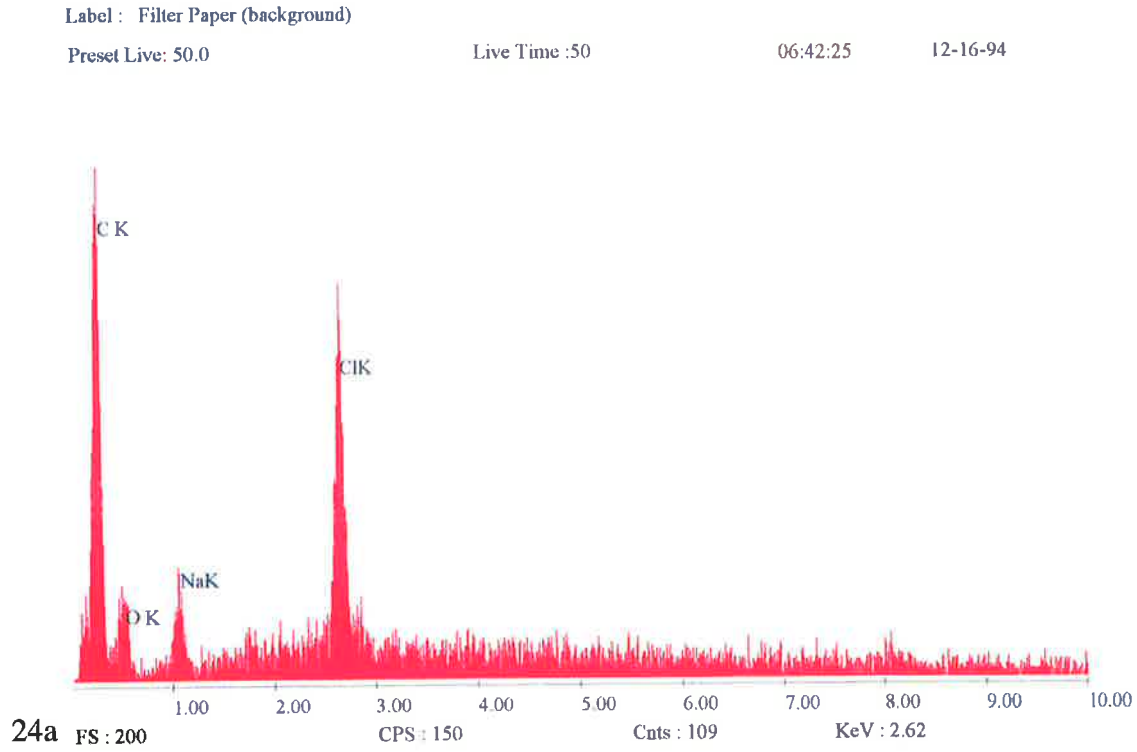


Figure 23: (A) EDXA of a particle on a filter exposed to fluid pumped through the finger pump. (B) A Scanning Micrograph of the silicone containing tube through which non-pumped fluid had been passed.



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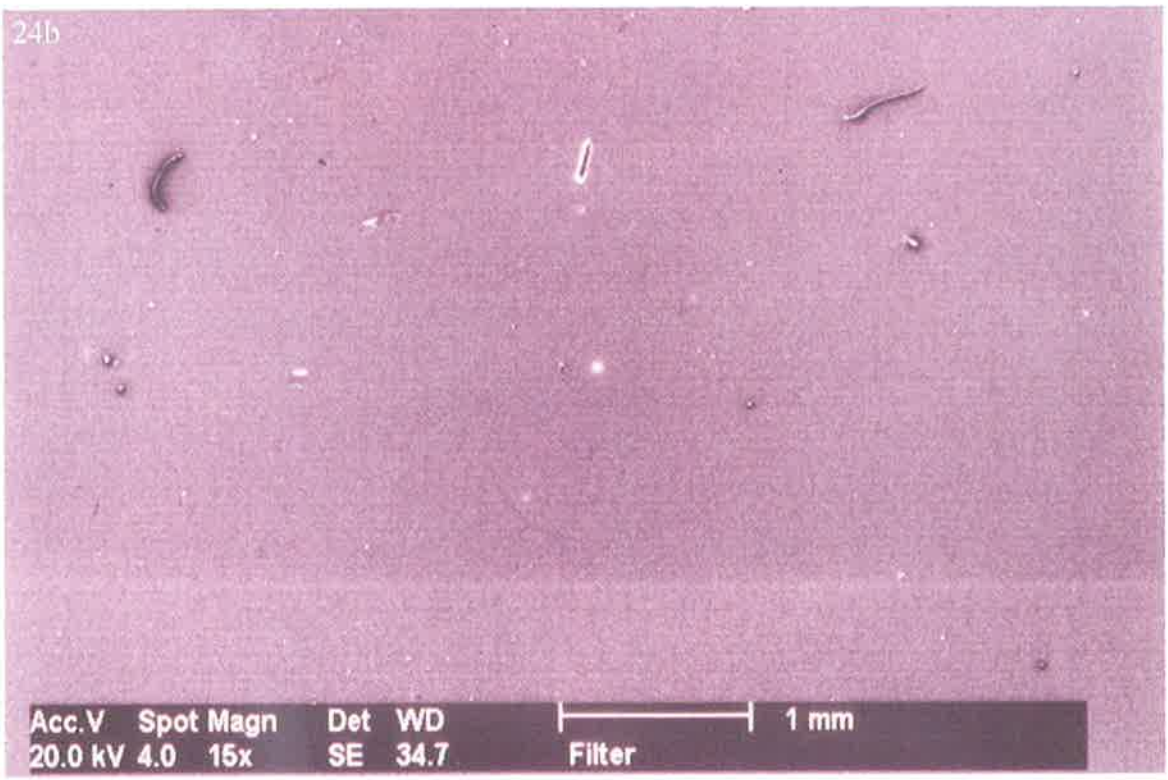
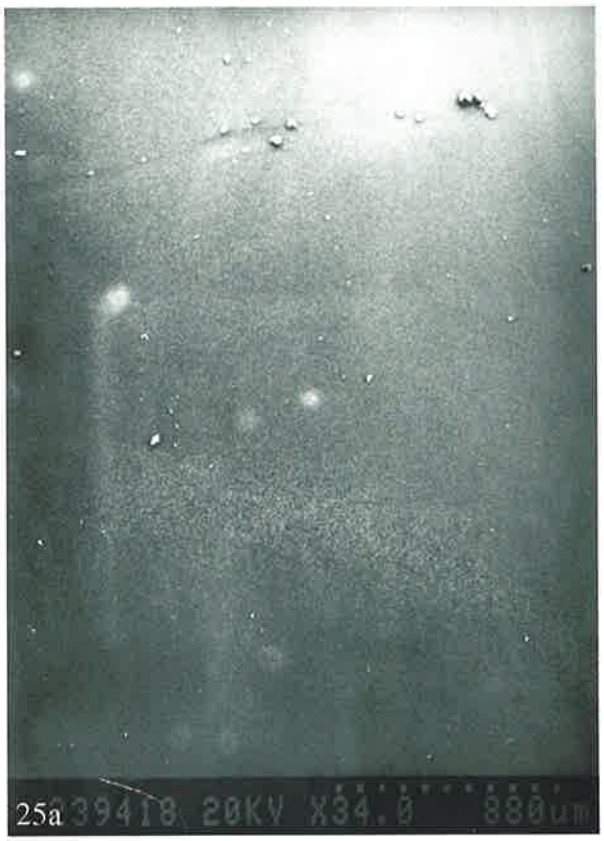


Figure 24: (A) Spectral analysis of a filter which received non-pumped fluid; NaCl was found, but not Silicon. (B) A number of particles are, however, evident on the filter surface.

The three unused filters had microparticles on their surface none of which had the appearance of silicone particles. Also, the EDXA spectra of one of the filters showed only the carbon and oxygen present in the cellulose acetate filter and no evidence of Si (Fig. 24).



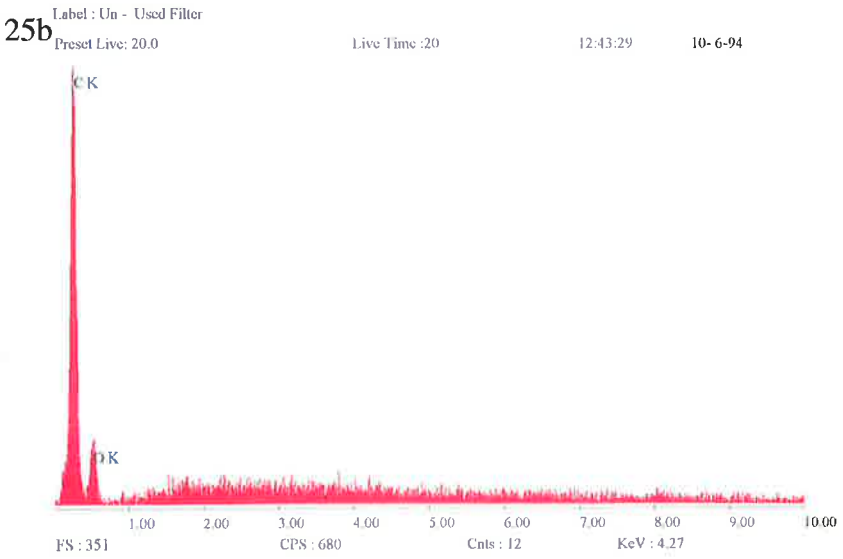


Figure 25: (A) Scanning electron micrograph of an unused filter and (B) the associated spectral analysis.

Silicone Tubing

All of the four pieces of tubing used in the pump for three hours were free of the surface particles noted on the unused tubing (Fig. 26), and the tubing used for 72 hours had a roughened inner surface (Fig. 27). Two of three unused sets of pump chamber tubing had a number of particles on the inner surface of the tubing (Fig. 28) and the third was free of particles. Spectral analysis of one of the pieces of infusion pump tubing confirmed its Si content (Fig. 29).

Figure 26: Scanning electron micrograph of pump chamber silicone tubing after three hours at 60ml per hour showing the smooth inner wall.

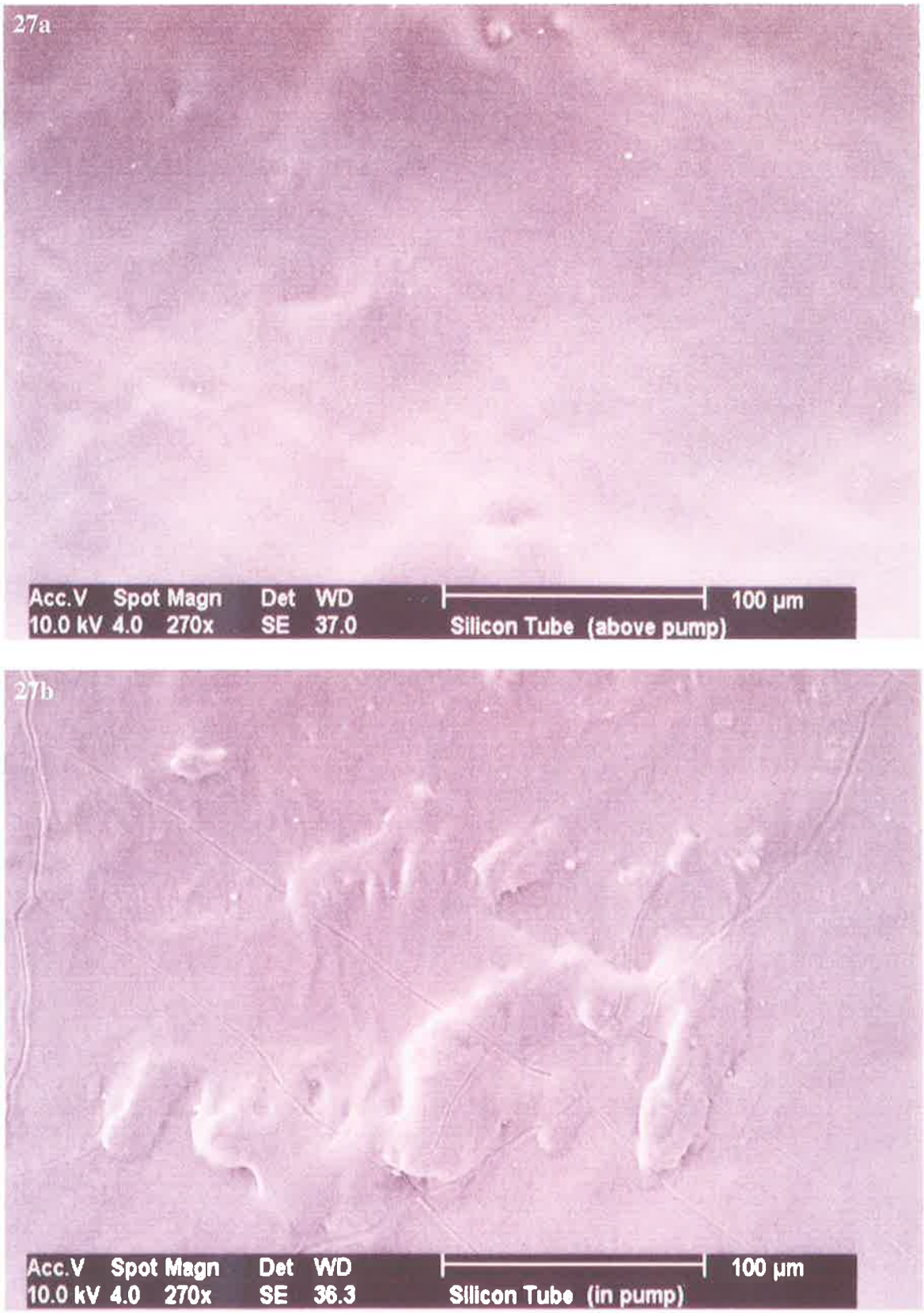


Figure 27: Scanning electron micrograph of the inner layer of the pump chamber tubing above (A) and at the level of (B) the finger pump impact. Note the irregular surface created by the pump over 72 hrs.

Figure 28: Particles seen on the inner layer of unused silicone tubing.

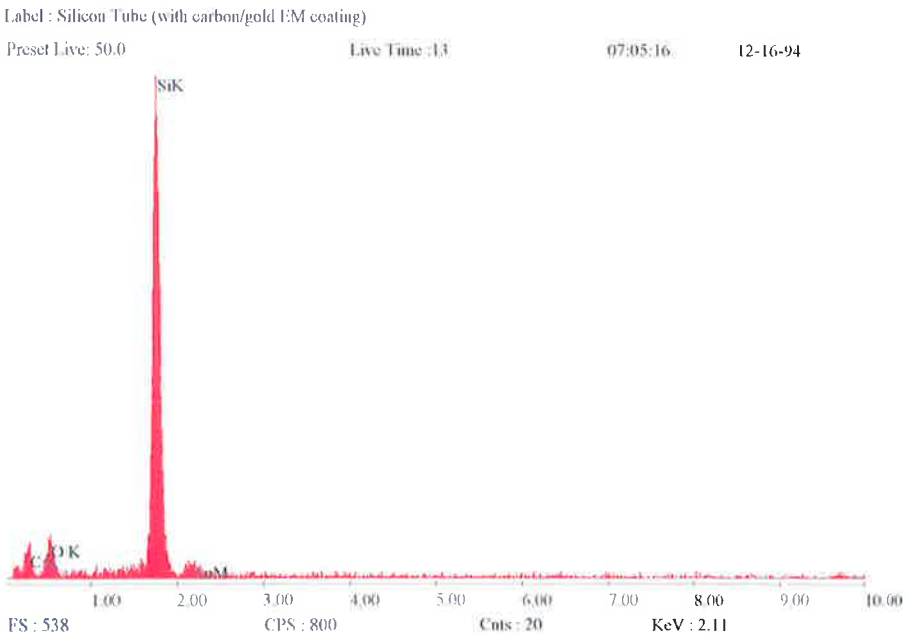


Figure 29: One section of tubing was examined with EDXA, confirming it to contain elemental silicon, as reported by the manufacturers.

STUDY 6 - Case Report of Migration

Skin Migration Following Periurethral PTFE Injection for Urinary Incontinence

The inclusion of this case, a woman treated for incontinence, stemmed from the presentation of the early laboratory results and subsequent access to the clinical and histologic information of this woman's unfortunate complication. The case adds significantly to the debate on the risks of particulate plastic and therefore was included in this work.

Case Report

A 49 year old women was referred to the Urology Department at the Tauranga Hospital, New Zealand with a periurethral mass and incontinence in July 1986. She initially presented elsewhere with incontinence in July 1983 and was treated with over 10ml of Polytef paste injected into the region of her bladder neck. Incontinence recurred after a few weeks, leading to a nylon-sling bladder neck suspension procedure, which also failed to produce lasting continence.

In July 1986 post menopausal bleeding lead to an examination under anaesthetic at which a fleshy, vascular lesion in the right para-urethral region was noted. A biopsy was taken of the vaginal mucosa which revealed refractile foreign material within multinucleated giant cells of the lamina propria. A cystoscopy was subsequently performed which confirmed the presence of an area of granulation tissue and induration at the level of the bladder neck. An oval, mobile mass approximately 10cm in diameter was attached to, but not invading, the anterior bladder wall.

In November 1986, a laparotomy was carried out at which the retropubic mass was removed. The mass had an irregular thick wall with a central cavity lined by a serosa-

like surface, containing foreign material. A Marshall Marchetti Kranz bladder neck suspension operation was then carried out to facilitate subsequent continence.

Histology of the resected material revealed refractile material consistent with PTFE particles, surrounded by numerous foreign body giant cells. Approximately 10 days later the woman developed malaise, a skin rash and migratory polyarthropathy; she was noted to have swelling of numerous small joints of the hands, her wrists, right knee and cervical spine. She had a macular red rash involving the hypothenar eminence and anterior aspect of both thighs, her Erythrocyte Sedimentation Rate (ESR) was raised to 59 and her platelet and neutrophil counts were elevated. Antibody studies exhibited negative antinuclear factor, mitochondrial and smooth muscle antibodies; a knee joint aspirate was sterile; and a skin biopsy demonstrated small vessel vasculitis and an inflammatory reaction containing foreign body giant cells containing refractile material consistent with PTFE.

A full recovery followed bed rest and analgesia. One year post surgery she was continent and otherwise fit with no detectable long-term sequelae.

STUDY 7 - Vascular Access Device

A Study of Particulate Migration *In Vivo* from Implanted Vascular Access Devices used in Paediatric Practice

Introduction

This report is of a study of 11 children who had an indwelling intravenous access device for between 27 and 1854 days, as part of treatment for malignancy or gastrointestinal disorders. When each device was removed, the surrounding reactive fibrous capsule was examined by SEM and EDXA, to identify particulate matter, and by phase-contrast, light microscopy to determine the corresponding histological response. A detailed account of the device used and the findings of one of the patients is also given.

Materials and Methods

Series Analysis

Eleven patients having removal of a vascular access device were selected for the study, including three boys and eight girls. Two separate segments of the capsule of two of the patients were dealt with as two separate samples. The patients all had intravenous access devices *in situ* for between 27 and 1854 days (median 346 days), for treatment of malignancy in eight cases and gastrointestinal disorders in three (Table 33). The devices that had been used for intravenous access were Infuse-a-ports in five of the cases, Therex low profile port in four, a Portocath and a Microport.

The venous access device and the surrounding soft tissue capsule were removed during surgery.

Specimen Analysis

For all cases, samples of the capsule were examined by a SEM and EDXA to identify the elemental content of any particulate matter present. These samples had been mounted in paraffin wax blocks for histological sectioning, so needed to be extracted from the wax before SEM analysis. Sections of the wax mounted tissues were taken from the samples and repeatedly washed in xylene, rinsed in alcohol and then in acetone, prior to being dried in a critical point drying apparatus. The samples were then mounted on aluminium stubs and coated with a thin (20nm) carbon layer in a vacuum evaporator to reduce the effects of electron build up in the microscope. Sections of five catheter samples and one of the devices were also mounted in a similar way to the tissue and coated with a carbon layer as above. They were then analysed in a Philips XL20 SEM with an integrated EDAX - DX4i Energy Dispersive X-ray Analyser. The detector collects X-rays over the elemental range boron (Z=5) to uranium (Z=92) and was of the ultra-thin window type. A 20 kV accelerating potential was used.

Infuse-a-port

The constituents parts of an Infuse-a-port were examined by EDXA and SEM (fig. 30).

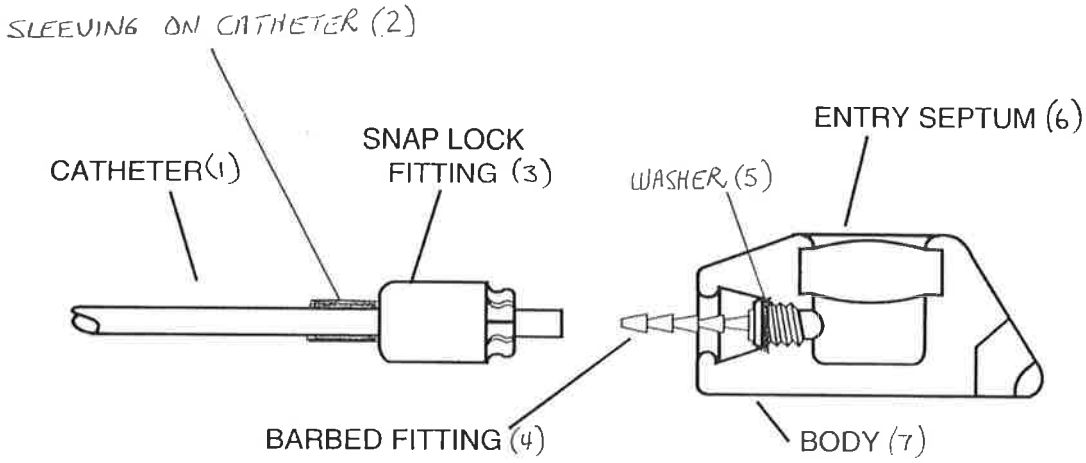


Figure 1
Cross-Section View of **Infuse-a-Port®** Assembly

ENERGY DISPERSIVE X-RAY ANALYSIS CARRIED OUT ON NUMBERED COMPONENTS OF ASSEMBLY. (1-7)

Figure 30: A diagram of an Infuse-a-port indicating the sites samples were obtained from for the results shown below. The numbers are referred to in subsequent figures.

Sex	Diagnosis	Duration	Tissue Sample
F	Short gut	1854	1
M	Gastrointestinal bleeding	997	2
F	Hodgkin's disease	202	3
F	Ewing's sarcoma	334	4
M	Hepatoblastoma	346	5
F	Germinoma	310	6
F	Osteosarcoma	624	7
F	Sacrococcygeal teratoma	338	8
M	Short gut	27	9, 10
F	Rhabdomyosarcoma	613	11, 12
F	Wilms' tumour	470	13

Table 33 : Patient sex, diagnosis, duration of intravenous access implantation, and capsule tissue sample number.

Results

Series Analysis

Foreign body inclusions were found in the excised capsule of six of the eleven patients who had an intravenous access device removed (Table 34). The six capsules which contained silicon were from around devices that had been implanted for 202 to 1854 days (median 470 days). The samples which did not yield a positive result on the EDXA were from capsules surrounding devices that had been implanted from between 27 and 997 days with a median of 346 days. Foreign body inclusions which were positive for Si also contained calcium, aluminium, sodium and chloride; and one of the samples was positive for copper and zinc, consistent with the presence of brass.

A foreign body giant cell reaction was seen in five of the samples (five patients). Elemental silicon was identified in three of these capsules and foreign material, resembling suture thread, but no Si was seen in the remaining two on EDXA. However, the two tissue samples were small and may not be totally representative of the whole sample. In patients in whom a giant cell reaction was seen the device had been implanted for the treatment of short gut, Hodgkin's disease, sacrococcygeal teratoma, Wilms' tumour, and osteosarcoma.

Tissue Sample	Si	Ca	Al	FB Giant cells	Chronic inflam.	Device
1	yes	yes	yes	-	yes	Infuse-a-port
2	-	-	-	-	-	Portacath
3	yes	yes	yes	yes	-	Therex port
4	-	yes	-	-	-	Infuse-a-port
5	-	-	-	-	-	Therex port
6	yes	yes	yes	-	yes	Therex port
7	-	-	-	yes	-	Infuse-a-port
8	yes	-	-	yes	yes	Therex port
9	-	-	-	yes	yes	Infuse-a-port
10	-	-	-	-	yes	Infuse-a-port
11	yes	yes	yes	-	-	Microport
12	-	-	-	-	-	Microport
13	yes	yes	yes	yes	-	Infuse-a-port

Table 34: Capsule Histology, EDXA findings, and device type. Si - silicon: Ca - calcium: Al - aluminium

A fibroconnective tissue capsule was seen in tissue samples from all of the patients. This was associated with an acute inflammatory infiltrate in two of the cases, and with a hyalinised connective tissue appearance in ten of the samples (nine patients). Six of the samples (five patients) in which fibroconnective tissue was seen had negative EDXA findings. Of the ten samples with hyalinised connective tissue, there were four with negative EDXA findings.

Focal chronic inflammation was seen in five of the tissue samples (four patients). Silicon, calcium, aluminium, sodium and chlorine were identified by EDXA in two of these samples; no inclusions were identified in the remaining three. The intravenous access device in these cases had been used for the treatment of short gut (two), germinoma and sacrococcygeal teratoma.

Energy dispersive X-ray analysis was performed on five of the catheters that had been used (Therex low profile - two, Infuse-a-port - two and Microport - one). They were all found to be a Si based material embedded with titanium oxide, similar to the elemental make-up obtained from the tissue samples. The Infuse-a-port device examined had elemental composition true to the label, namely the silicone rubber and the Sulfinated Epoxy (PolySulfone). The presence of a small amount of carbon in all spectra is due to the thin layer of carbon (<10nm) that has been evaporated over the samples to reduce the effects of electron charge build up in the microscope.

Detailed Patient Specimen

The two views of sample 13 showed particles distributed over the inner surface of the capsule (Fig. 31 & 32). The bright spots in the image are areas of higher atomic number than the darker background. Analysis of the particulate material (in the size range of sub-micron through to 10 micron) showed that the bulk of the material was high in silicon, aluminium, calcium and oxygen, with traces of magnesium, sodium and chlorine.

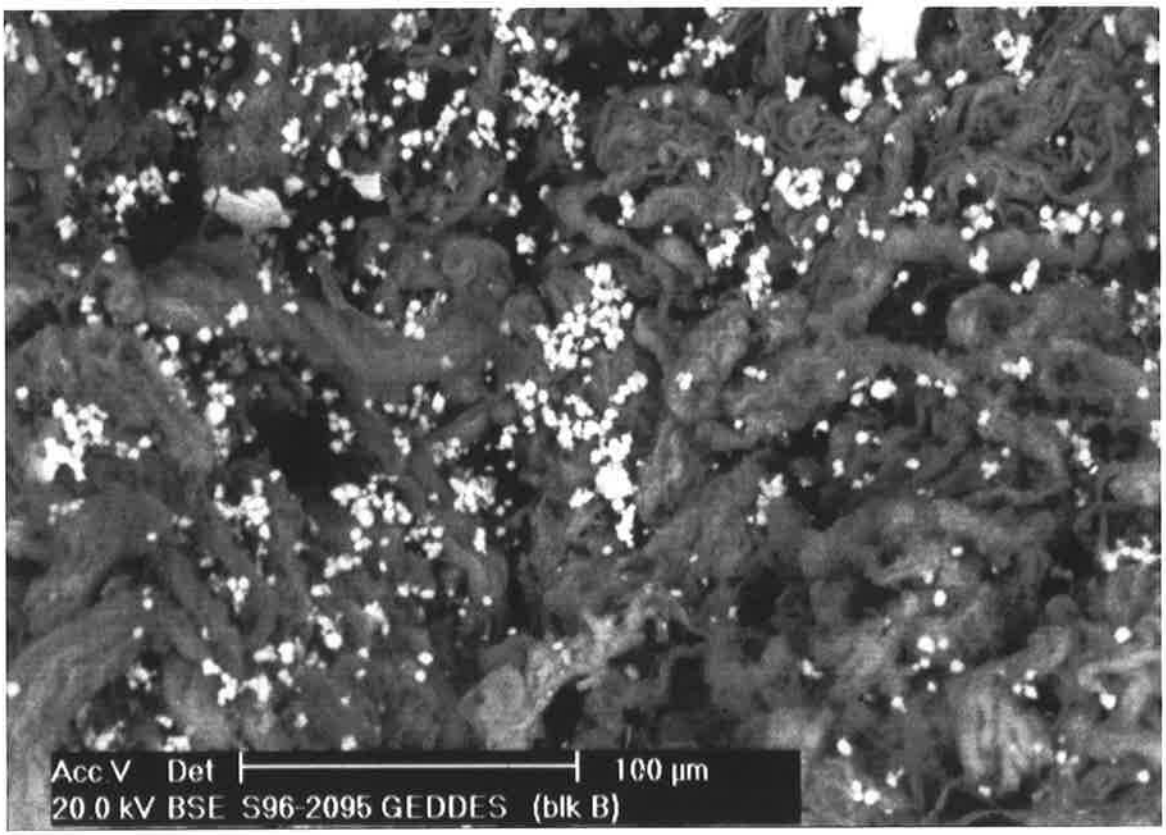


Figure 31: A large number of particles can be seen on the inner surface of the Infuse-a-port sheath in the samples tested.

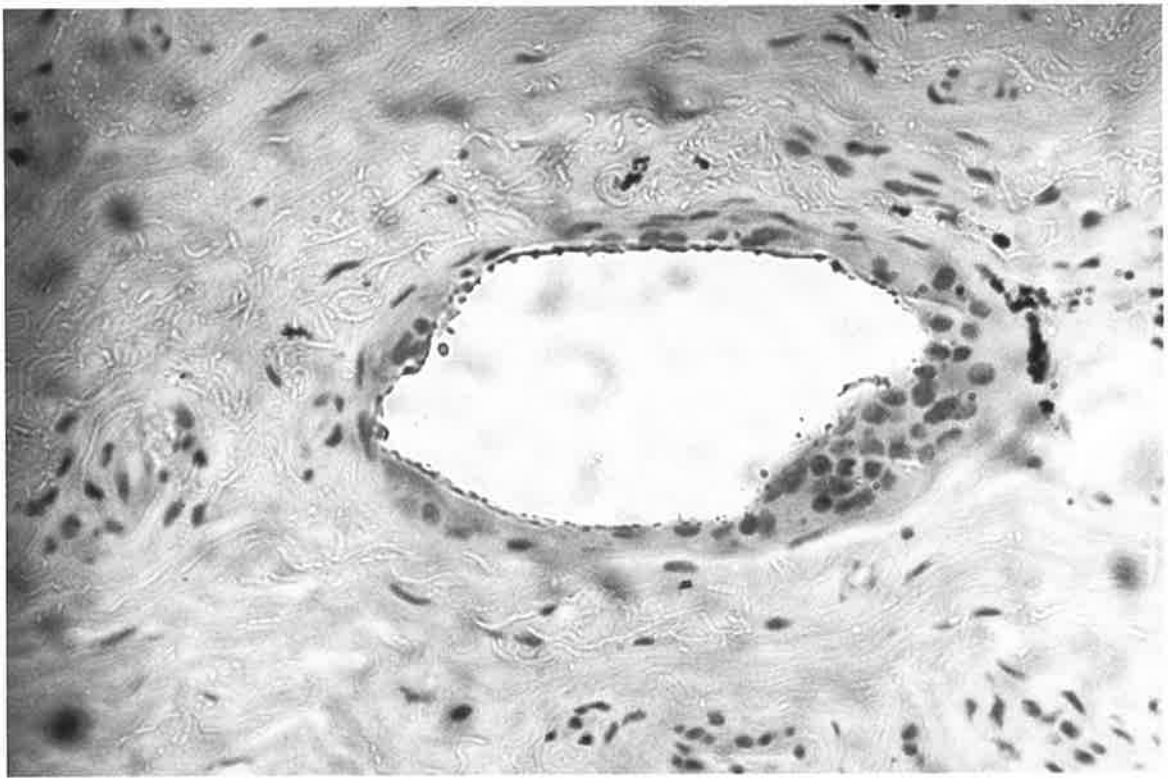


Figure 32: A foreign body giant cell containing material consistent with silicone from an Infuse-a-port.

A few particles of much higher mean atomic number, which were indicated by the presence of brighter spots, were found to contain copper and zinc (Fig. 33).

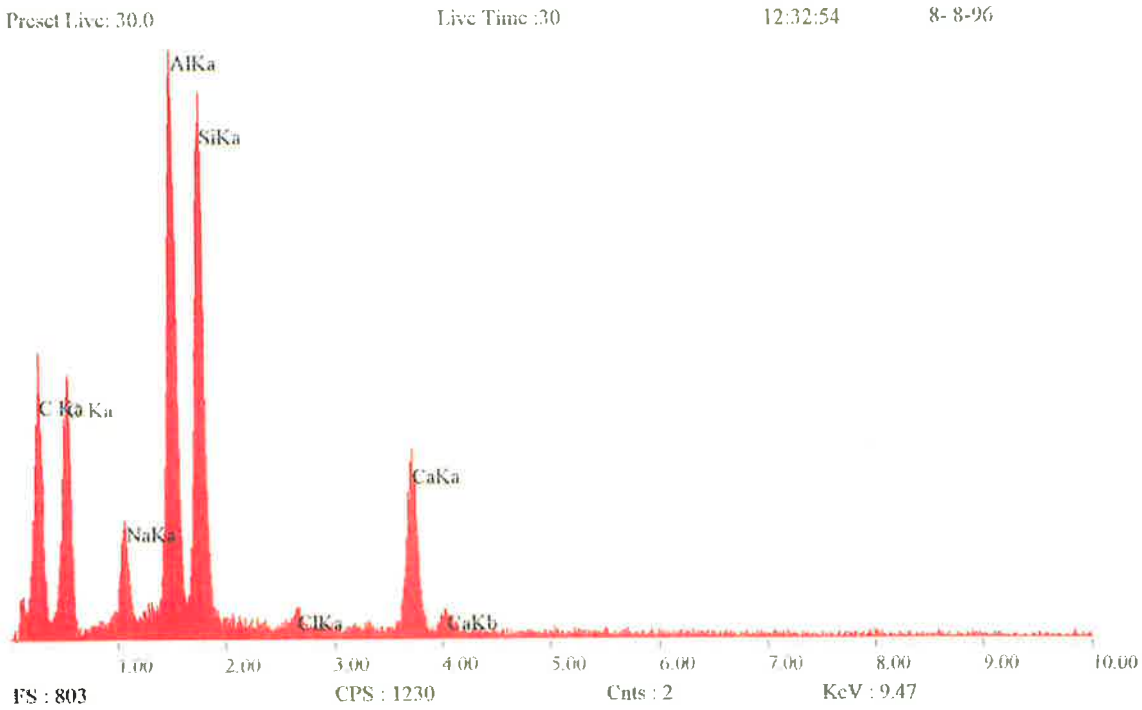


Figure 33: An EDXA image showing a number of different substances were on the capsule that had developed around the Infuse-a-port, including elemental silicon.

Infuse-a-port Results

The SEM and EDXA for the seven studied components of the Infuse-a-port are seen in Figs. 34-40. Elaboration of the results follows each of the pictures.

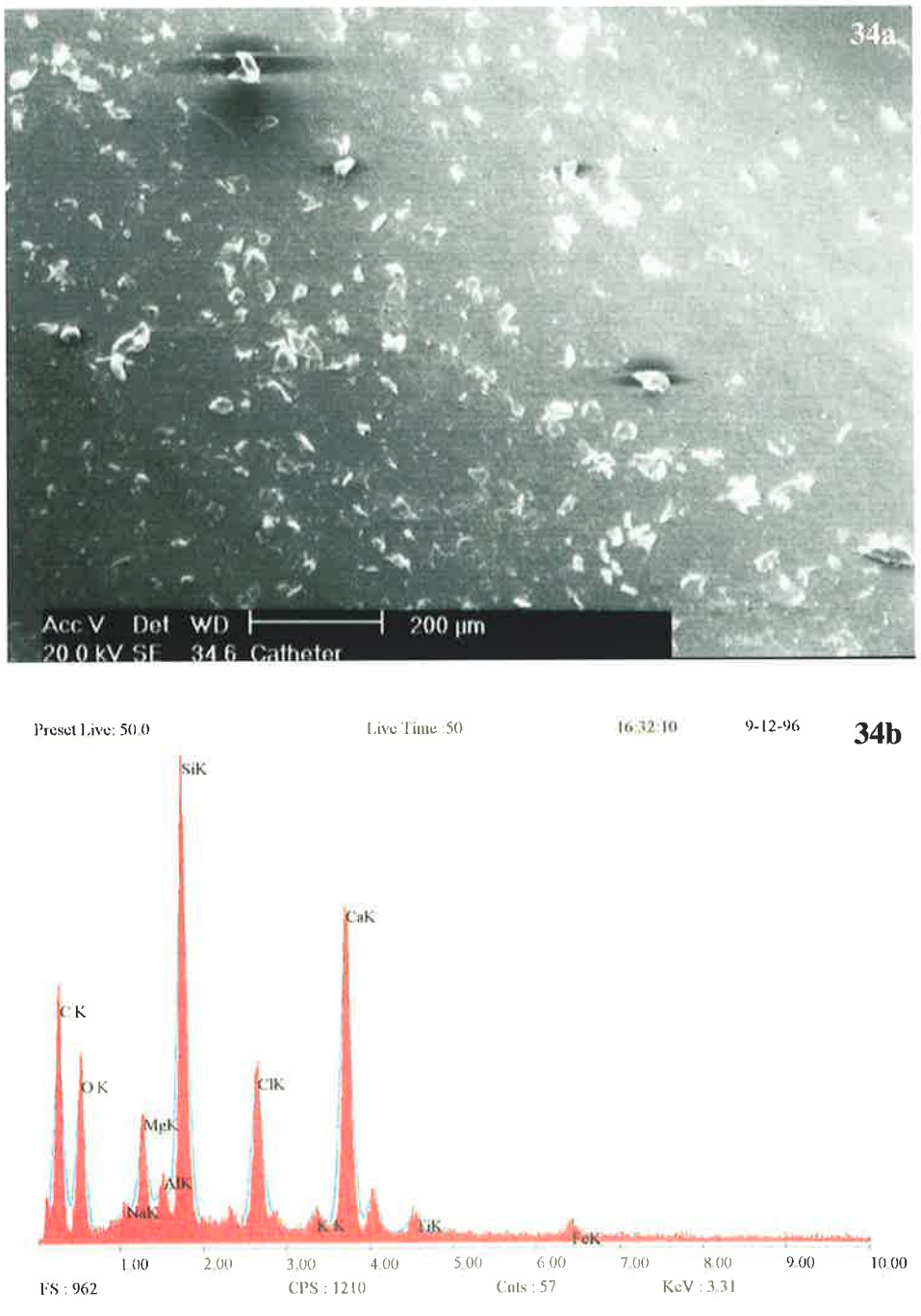


Figure 34: Infuse-a-port specimen 1 = silicone catheter. The SEM image shows a number of particles over the surface of the catheter (A). The range of substances within the catheter are shown in this EDXA image (B).

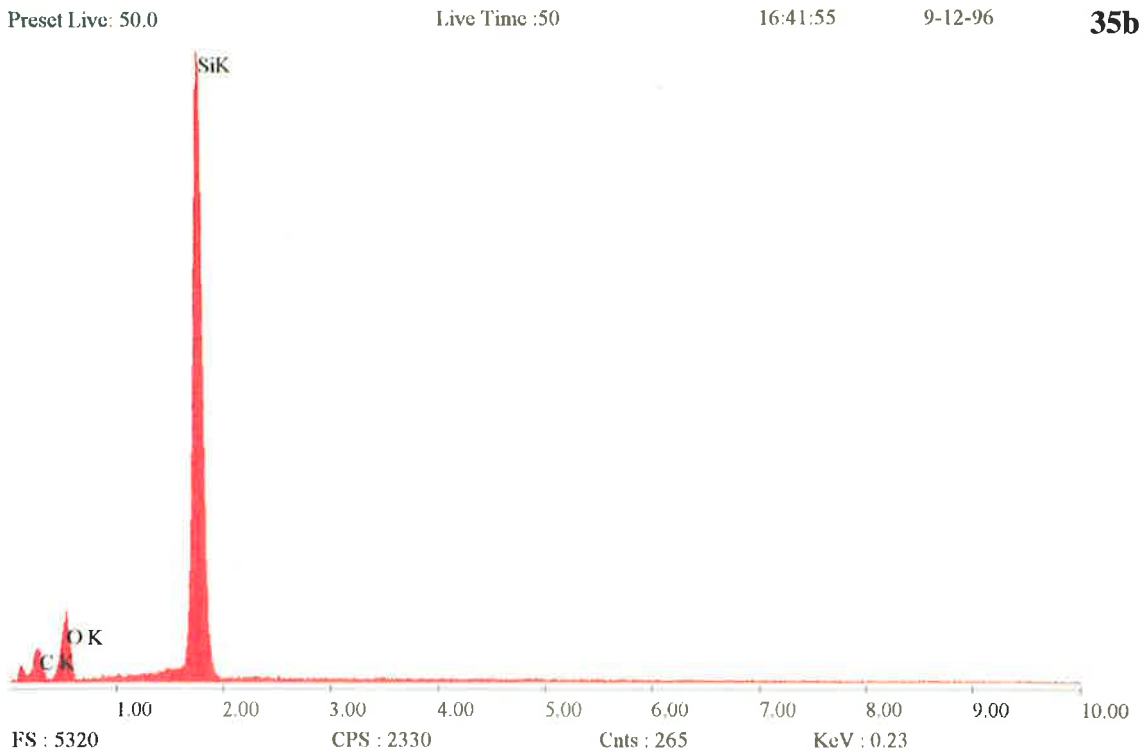


Figure 35: Infuse-a-port specimen 2 = sleeve on the housing end of the silicone catheter, at its junction with the snap-lock fitting. An SEM image of a relatively smooth surface (A) shown to be mostly silicon on EDXA print-out (B).

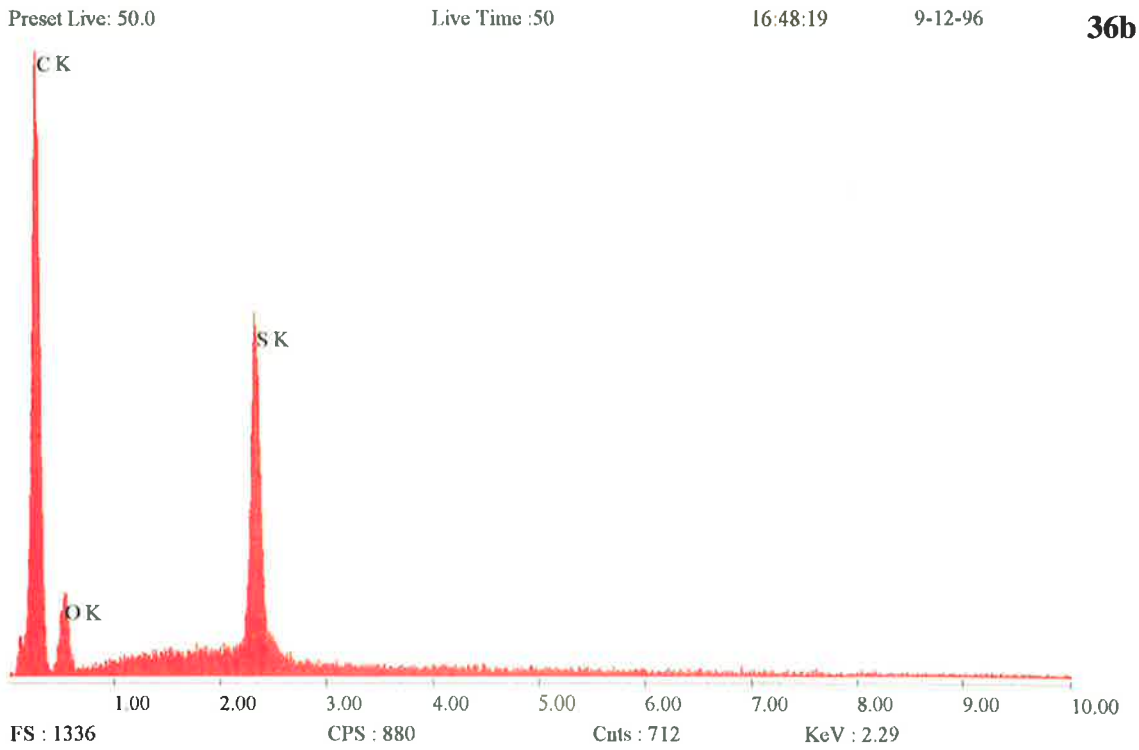
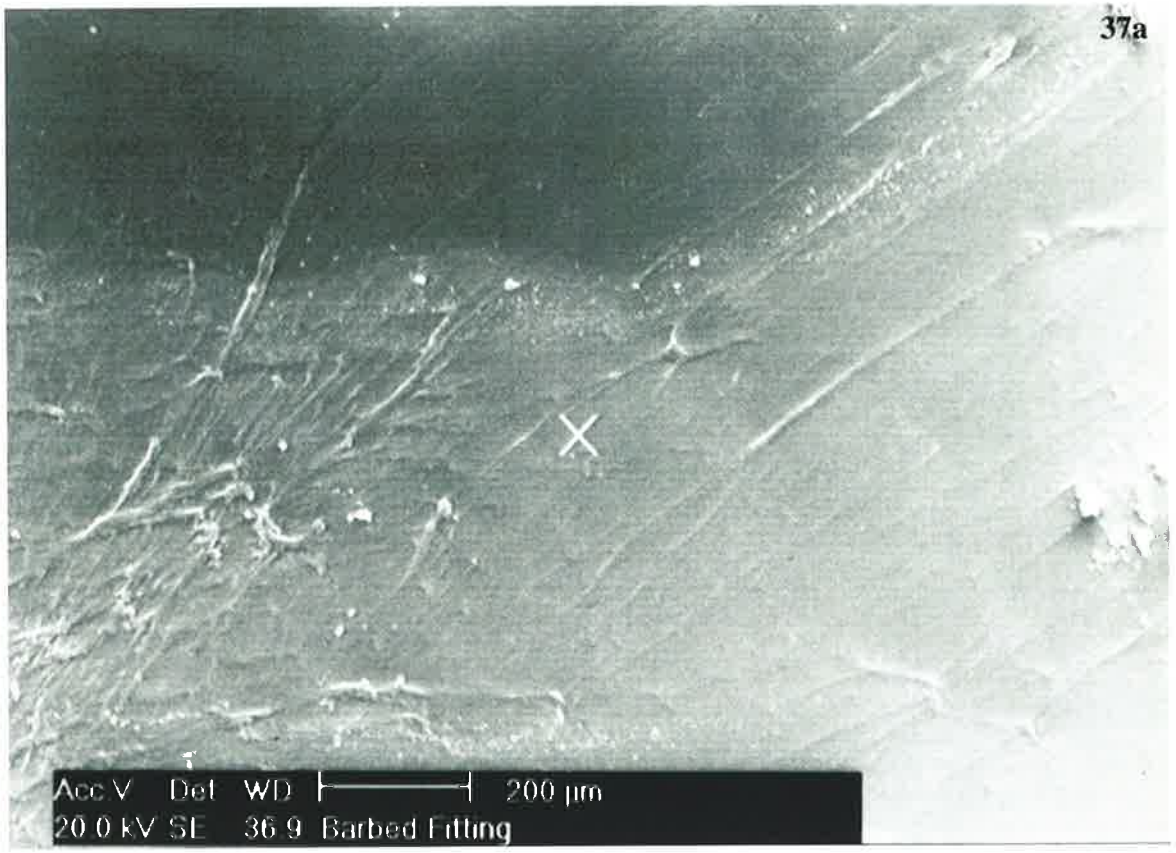


Figure 36: Infuse-a-port specimen 3 = snap-lock fitting. A slightly grooved surface (A) made of carbon and sulphur (B).



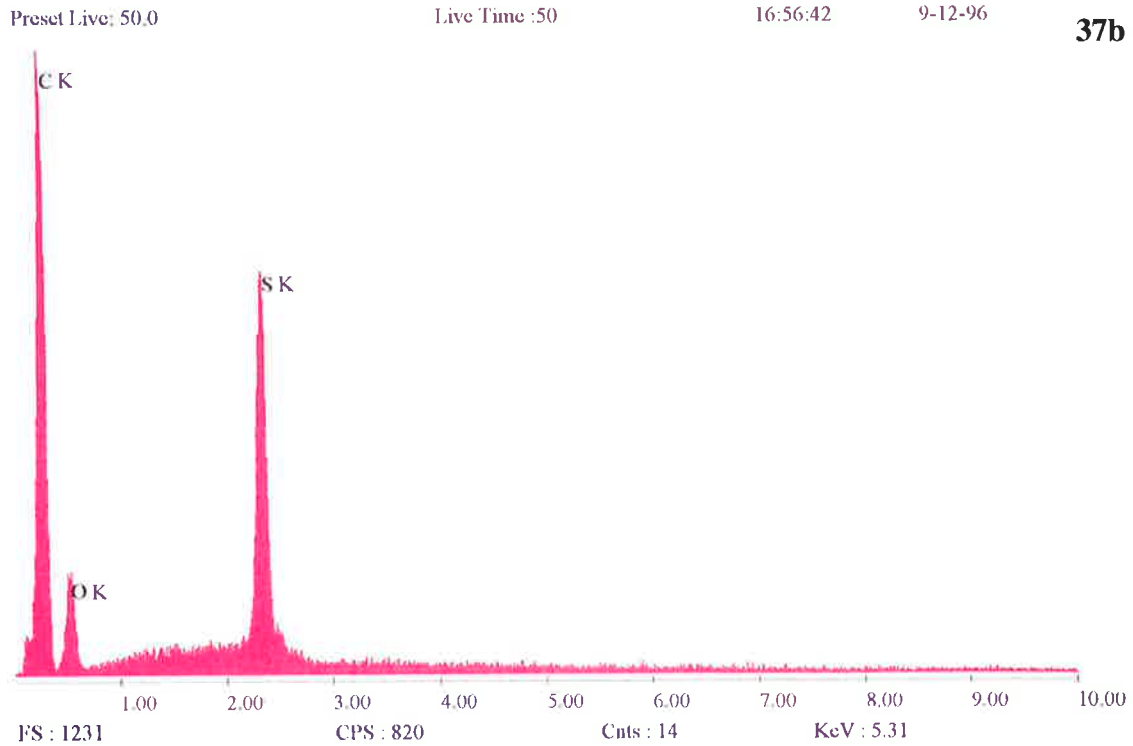
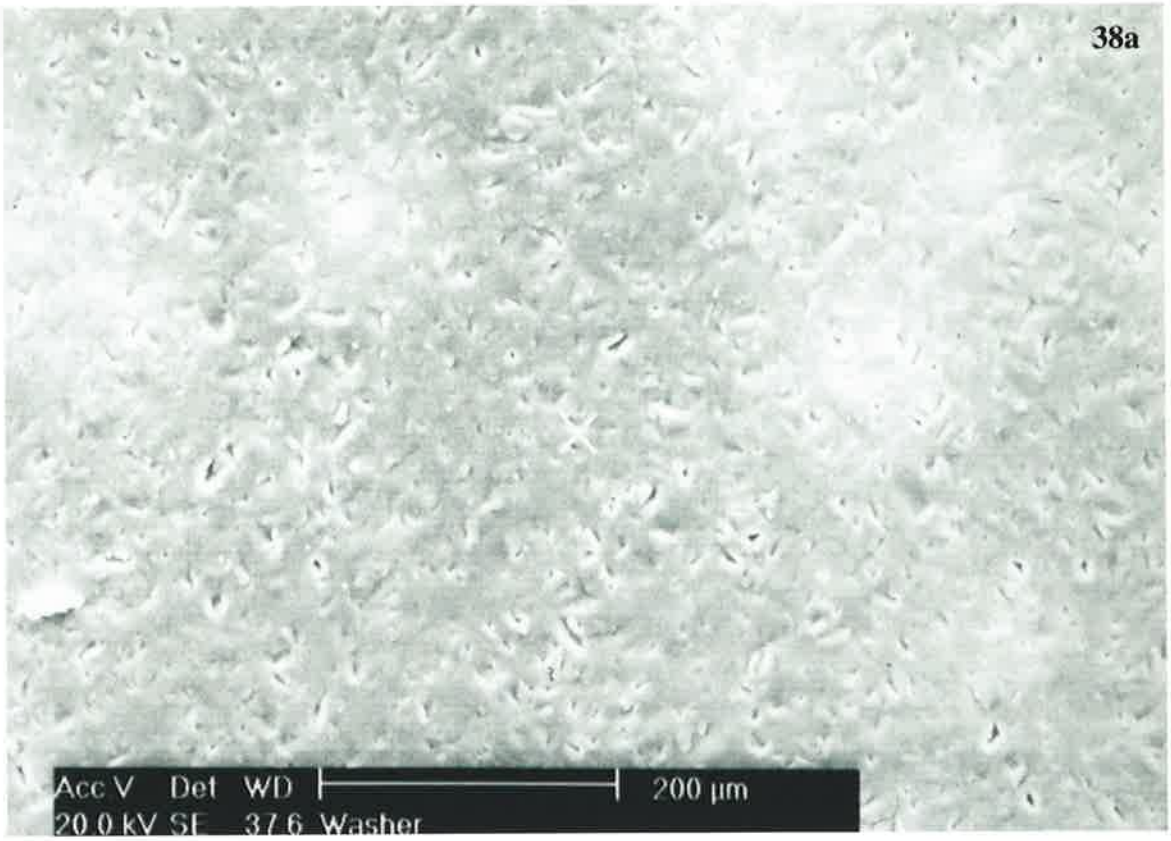


Figure 37: Infuse-a-port specimen 4 = barbed fitting for the catheter to the housing. A smooth surface (A) of sulphur and carbon - Polysulphone (B).



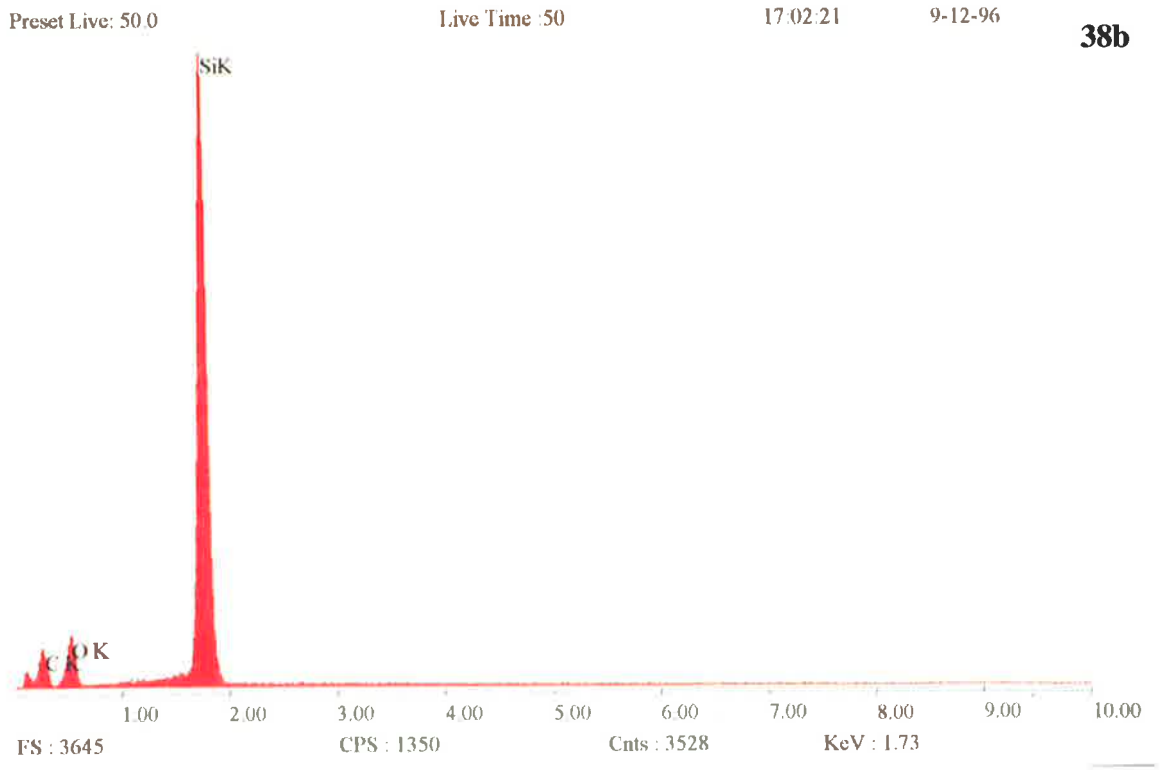


Figure 38: Infuse-a-port specimen 5 = washer between barbed fitting and catheter housing connection. A slightly irregular surface structure made of Silicon.

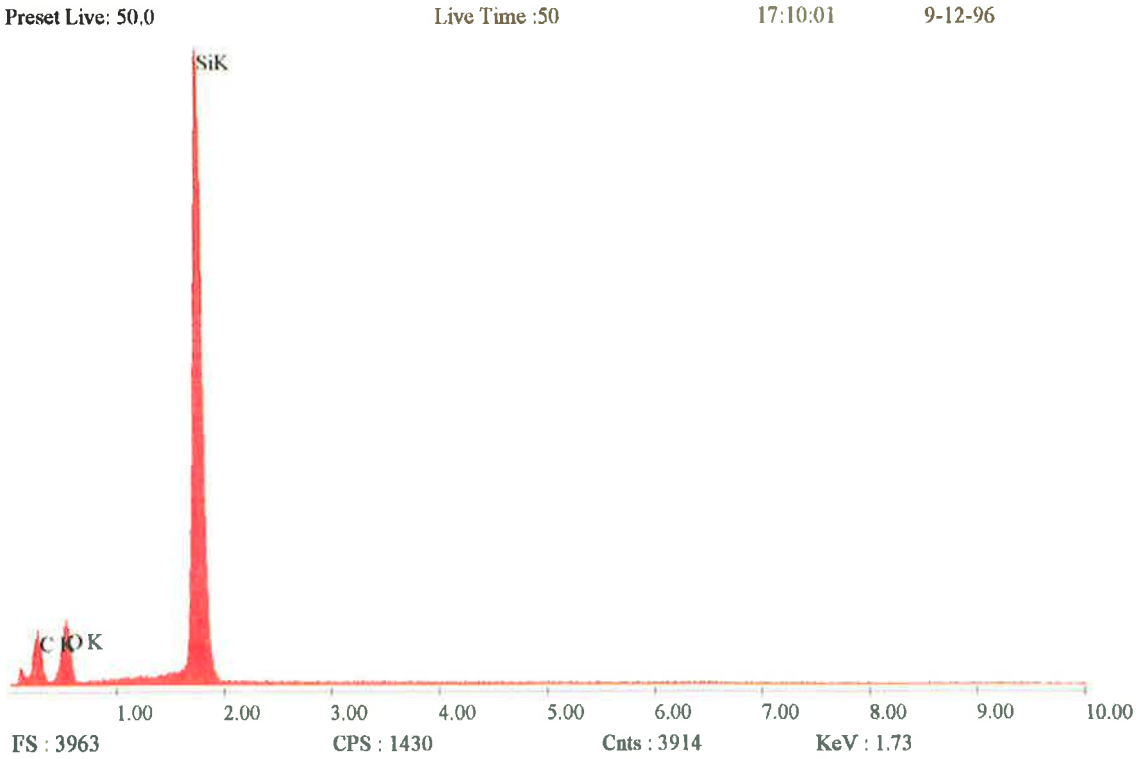
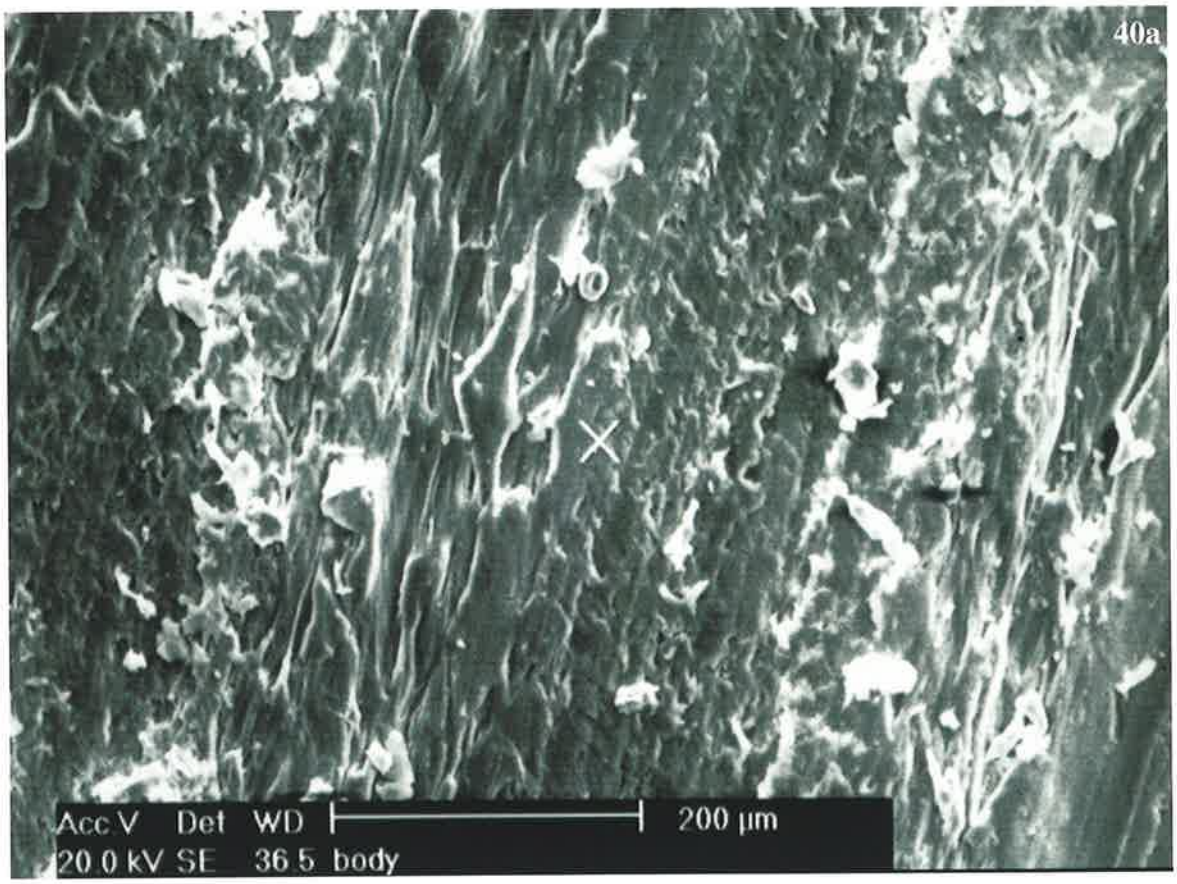


Figure 39: Infuse-a-port specimen 6 = the silicone septum from within the body of the device. The dissected particles were pure silicon.



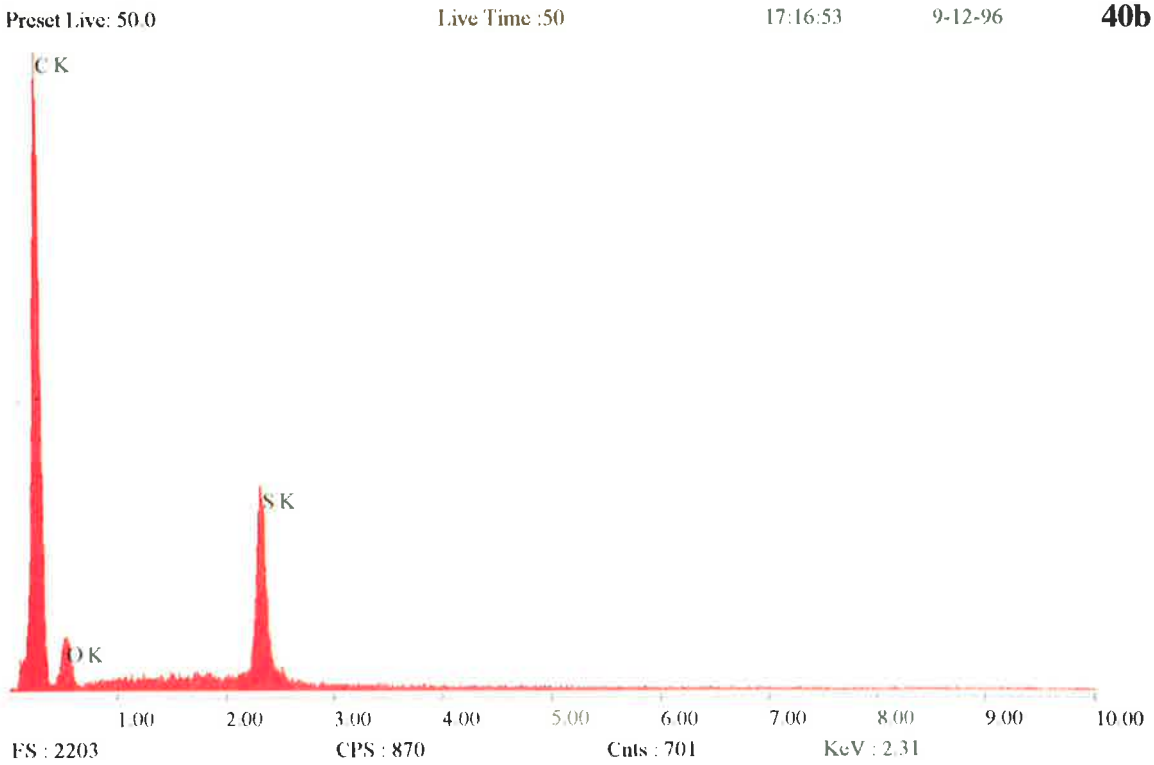


Figure 40: Infuse-a-port specimen 7 = body of the device housing. A roughened surface (A) of carbon and sulphur (B).

Infuse-a-port and Sample 13 - Summary

Specimens from the catheter tubing (specimen 1 - Fig. 34), the sleeving on the catheter immediately adjacent to the body of the device (specimen 2 - Fig. 35), the washer between the barbed fitting and the Infuse-a-port housing (specimen 5 - Fig. 38) and the silicone septum within body of Infuse-a-port (specimen 6 - Fig. 39) were all composed of elemental silicon. In addition, a number of particles were seen over the surface of the catheter and sleeve, which consisted of a variety of substances, including sodium, magnesium, aluminium, potassium, calcium, titanium and iron (specimen 1 - Fig. 34).

In contrast, the snap-lock fitting (specimen 3 - Fig. 36), used to hold the catheter to the barbed fitting, the barbed fitting which connects the catheter to the body of the Infuse-a-port (specimen 4 - Fig. 37) and the body of the housing itself (specimen 7 - Fig. 40) consisted of Sulfinated Epoxy (PolySulfone).

The presence of a small amount of carbon in all spectra is due to the <10nm of carbon evaporated over all the samples to reduce the effects of electron charge build-up in the microscope. For silicon, aluminium, calcium, magnesium, sodium and chloride, the elemental content of the catheter and the device was consistent with particles found on the inner surface of the capsule which had formed around the device. There were ***five exceptions***: **zinc** and **copper** were found and could have arisen from the needles used to access the Infuse-a-port for the administration of the chemotherapy; **Iron** and **Titanium** found in the Infuse-a-port were only in small amounts on the catheter, therefore may not reach sufficient tissue levels to be detected. The **sulphur** from the Infuse-a-port was in large quantities in the device, but may have been absorbed after breakdown in the tissues. These findings would appear to confirm that the foreign body elemental load in the capsule reaches its inner surface by fragmentation from the device during the duration of its placement.

Discussion

General Comments

Silicone (polydimethylsiloxane) is widely used as an implantable or injectable plastic in many areas of medicine, including reconstructive and plastic surgery. Silicone has been shown previously to migrate from the inner surface of tubing commonly used for haemodialysis and cardiac by-pass, resulting in silicone embolisation but, thus far, virtually no adverse affects have been noted. However, detailed, long-term studies and studies of low volume, intravenous infusions have not been previously conducted. Furthermore, silicone has been generally accepted as biologically inert, but in recent years, immunological abnormalities have been reported in patients with silicone devices such as ventriculoperitoneal shunts, catheters for long-term venous access and breast implants [51-55].

Other plastics are also widely used in medicine, to the extent that it would be impossible to practise modern medicine without the temporary or permanent implantation of plastics. This high exposure to plastics, combined with the occasional case report of adverse reactions to implanted foreign material, necessitate a rational approach to data collection on the risks of plastics. The desire to have a rational approach was at the forefront of planning when collating the data and considering the literature.

The discussion of Part II has been divided into sections, fitting with the various clinical and laboratory investigations conducted. The results of the experiments, and related literature on the subcutaneous reaction to particular plastics will first be reviewed, followed by discussion of the reactions seen when the plastics are found in the lung, brain and other central tissues. The discussion of particles reaching essential organs will consider migration from both solid and particular implants and, more importantly, as a result of the use of infusion pumps. Next, the reaction of the immunological

system will be debated from the extensive literature and investigations additional to those included in this study. Finally, the new substances, which have been suggested for the injection treatment of VUR, will be discussed and the relative merits considered.

Reaction to Particulate Subcutaneous Plastics

Adverse reactions at the site of a local injection of plastic material relate to the chronic inflammatory response. These have been seen to produce urethral obstruction [37] and a vaginal granuloma in patients treated for urinary incontinence [38], and a neck granuloma in patients treated for vocal cord paralysis [8,28,36]. However, more Polytef paste was used in these cases than for the management of VUR in children. The present study has demonstrated that the inflammation around a small volume of plastic particles becomes quiescent, a result which has also been demonstrated up to 18 months after injection in the dog [9,10] and up to 22 years in humans [7,220].

Failure to identify systemic migration in the acute and follow-up phases may be a feature of the rat model utilised. It is possible that the method of examination of the histological specimens may be less sensitive than other methods [210], although the lack of any granulomatous inflammatory lesions in the liver, spleen, brain, pulmonary lymph nodes and kidneys does lend support to the apparent absence of systemically embolised particles on routine, phase-contrast and polarised microscopy. But, as migration has previously been well documented in larger animals, documenting the presence or absence of a histological response to migration was of greater interest, rather than confirming migration itself. Therefore, EDXA, as was used by Barrett *et al.* [210], was not included in the study. However, the presence of embolised plastic material without an associated histological reaction, seen in some of Barrett's cases

may indicate that a positive EDXA, in the absence of granulomata is not a clinically significant finding.

The rat is well known to have a high incidence of tumour formation when foreign materials are implanted under the skin of the back, and a number of studies have previously investigated the malignancy risk associated with the implantation of foreign material in rodents [20-22,24,25,50,202]. Oppenheimer, in addition to finding tumours associated with the capsule around solid implants [50], also demonstrated that removal of the solid implant, after a critical period of time, did not prevent the formation of malignancy in the capsule; removal of the capsule was necessary to eliminate the formation of a fibrosarcoma [21]. Their findings have not always been confirmed [25], and the fibrosarcomas identified by Oppenheimer's group differed from the fibrous histiocytomas found by Maekawa *et al.* [24]. Further work by Oppenheimer *et al.* has suggested the malignancy risk is reduced by perforation of the implanted foreign body [20] and virtually eliminated if the implant is a powdered polymer [22]. These findings are consistent with the outcome of this study, in which three sarcomas developed at the injection site in the silicone injected group, with more fibrosis around the individual silicone particles; 90% of the Polytef particles are less than 40μm in diameter and appear to have less fibrosis around the individual particles. This intralesional fibrosis may act as a miniature capsule for the generation of tumour formation as recorded by Oppenheimer [21,50], however, there may be an inherent difference in the malignancy risk of silicone and PTFE; thus a comparison of particles of the same size and substance is needed to test this possibility.

A causal relationship between the presence of implanted plastics and tumour formation has not yet been proven in humans, although there have been a number of cases in which plastic may have stimulated tumour formation [41-43,203,204]. These include tumours found adjacent to a PTFE vascular graft [41], and in silicone-containing regional lymph node lymphomas of a number of patients with silicone arthroplasties

[203,204], and in two patients with a thyroid malignancy after vocal cord injection of PTFE [42,43].

The difference between the tumour numbers at sites remote from the injection in rats is of interest, particularly the tendency for a higher tumour frequency in the control group. This finding, and the possibly important difference in the injection site outcome for the two different plastics, should be further explored both in animals and humans.

Reaction to Particulate Intrapulmonary Plastics

The histological response in the lung appears similar to the subcutaneous site, where there had been no significant change in the inflammatory response beyond three months post injection. The response was similar to material taken from one of the patients who had an open operation after a failed STING procedure, and is similar to that seen by others in both the bladder [35] and vocal cords [26].

The introduction of injectable PTFE, for the management of VUR in children in 1984 [64,65] has stimulated a great deal of discussion about the use of plastic materials in Urology, resulting in a number of alternatives being suggested; these include silicone (Bioplastique) [124,205], Polyvinyl Alcohol [108,114], particulate glass (Bioglass) [130] and implantable, inflatable micro-balloons [106]. The stated advantage of the newer substances is the larger particle size and an assumed reduction in the risk of particle migration. This view is supported by a number of studies which have demonstrated migration of PTFE particles from Polytef paste in which 90% of the particles are less than 40μm in diameter [17,56,57,136,173,208,209]. These studies include the small injection volumes used for the endoscopic treatment of VUR. The suggestion that a larger particle will prevent migration is not supported by studies that have shown silicone spread from the peritoneum to various organs in mice [206,207]

and to the lung in humans [115,117], or by the clinical consequences of migration having been seen with both injectable PTFE [32] and silicone [115,117]. The intravenous injection of Polytef and Bioplastique in rats also demonstrates that particle size was not significant in particle migration to distant organs, as both the PTFE and silicone particles were dealt with, in the long-term, in a similar manner in the rat lung, after intravenous injection. In the short-term, however, the smaller size of the Polytef particles resulted in the involvement of a greater number of small pulmonary arterioles and capillaries than for Bioplastique; therefore a more peripheral spread of the PTFE was evident. Importantly, neither silicone or PTFE granulomas were found in any other organs in the rats.

It would appear that children undergoing a plastic injection procedure for the management of VUR are probably destined to have plastic particles in their lungs, but perhaps it is the load effect of the plastic which is more important: the load of foreign material delivered to the lung during a STING procedure is relatively minute, as highlighted by a histological picture of a rabbit injected with PTFE, produced by Vandenbossche *et al.*; it shows a lung after hypochlorite digestion with *one PTFE particle* in the field, but a *large number of other foreign particles* [208]. Therefore, the contribution of the PTFE to the total foreign body load in the lung was probably not significant. The comparison with the load of systemic plastic from intravenous infusions should be assessed to allow for logical conclusions to be drawn.

Reaction to Particulate Intracerebral Plastics

The dose of plastic injected intra-arterially, and subsequently lodging in the brain, was larger than would be expected from a usual peripheral injection of particulate plastic, although, systemic spread to the liver [115] and lung [32,117,208], after injection of a

large volume of PTFE and silicone particles, has been proven. Nevertheless, neither neurological sequelae nor evidence of brain migration have been confirmed in humans.

The reaction of the brain to plastics has been examined previously; however, this study differs in that it looks at both PTFE and silicone. An additional new finding is the movement of particles into the brain parenchyma, from the lumen of vessels. This transvascular movement was similar to that seen in the rat lung and may well represent a similar process to the separation of particles from solid implants [197,198,203,210,221,222], probably due to macrophages.

In experiments using PTFE, Aaronson *et al.* found demyelination in a dog model [56], which may have been due to a demyelinating disease (personal communication). Malizia *et al.* found no reaction to the intra-cerebral particles [17,57] and Miyakita and Puri noted a mild inflammatory response, but did not record the presence of particles outside the vessels [218]. The cerebral response to particulate silicone has not previously been studied. These sheep showed a minimal response to the plastics with giant cell formation and little inflammatory response after six months.

In view of the documented migration of plastic from a number of sources used to treat patients and the mild inflammatory response in the sheep brain, the long-term response in animals and the response in the human brain should be further studied; particularly as plastics will certainly continue to be widely used in medicine.

Migration of Particulate Plastics

The phenomenon of plastic particle migration to various areas of the body has been confirmed, not only from particulate injection, but also from a number of different implants. Confirmation of migration has been facilitated by the use of EDXA

techniques in a number of studies, which eliminate the inaccuracies of electron and light microscopy described by Miyakita and Puri [218]. These authors made the point that lung and brain emulsification can give confusing results, because of the difficulty in differentiating PTFE and hypochlorite on light and electron microscopy. Despite this, the weight of evidence would suggest that migration to the brain is possible, as both Malizia *et al.* [17] and Aaronson *et al.* [56] used EDXA to confirm the presence of PTFE particles in the brain in their bladder injection studies.

Despite wide use of injectable PTFE for vocal cord augmentation [26], urinary incontinence management [32,33,136] and, more recently, for the treatment of VUR [62], only one case of clinical significance has been previously reported [32], whereas particle migration itself has been confirmed in animal studies [17,58] and in humans [26,32,33,136], with both large [17,32,33,136] and small volume injections [26,58,136]. The migration in humans has been identified in blood vessels [26], lymph nodes [48,136] and the lung [32,33]. The larger particles of injectable silicone have also been recorded to migrate [117,220,223].

Furthermore, reports of distant migration from breast implants [49], heart valves [59], artificial urinary sphincters, penile prostheses [198,210] and finger joint prostheses [211,221,224] have been published. The phenomenon of movement of particles from the blood vessels into the lung and brain parenchyma has not been previously published. To date there has not been a report of clinically apparent adverse effects of plastic particle migration after a small volume injection.

There may well be two different mechanisms of migration of plastics in the body; the particulate materials may embolise or be phagocytosed and carried by the macrophages in which they have been entrapped, as is the likely mechanism for the solid and larger particle plastics. As yet there has not been a study designed to specifically assess the embolic phenomenon in a larger animal model, although the rapid onset of the

specifically assess the embolic phenomenon in a larger animal model, although the rapid onset of the symptoms, and the apparent volume of PTFE particle migration in the case presented by Claes *et al.* [32], may indicate that the injection had been, in part, intravascular. However, it seems certain that phagocytosis plays a role in migration to regional lymph nodes thereafter. It is unknown whether particles trapped by macrophages can subsequently migrate beyond the lymph nodes and the long-term effect of particles lodged in the lung and brain is uncertain; as yet, long-term histological follow-up of plastics in humans is not well documented in the literature.

Migration from Solid Plastic and Tubes

In the Urological literature there has been much discussion on migration resulting from the injection of particulate plastics, but little attention paid to similar problems recorded with solid plastic implants, including shedding of plastic from artificial urinary sphincters [198,210], breast implants [49,223,225], artificial joints [203,204,211,221], and heart valves [59]. Many have expressed concern about the possible consequences of injected particles, but are unconcerned by the possible consequences of the particles produced by the implantation of a solid device [17,32].

Although particle migration has been previously identified in animal studies and in humans with both large [17,32,33] and small volume injections [26,58,136], and migration in humans has been identified to blood vessels [26] and the lung [32,33] with injectables, the particles from solid plastics have only been found to migrate to lymph nodes [48,49,136,225] and the capsule surrounding the implants. This finding was also found with my examination of the Infuse-a-port capsule. The substances identified matched closely the constituents of the implant, particles which would have remained in the patient had the capsule not been removed. The finding of this particulate material on the capsule is an important feature as it may be relevant to the findings of Oppenheimer *et al.* who identified that it is the capsule that predisposes to the development of the malignancy in their rat model. Was it the retention of this foreign material that induces the change, or the capsule itself? Also, there have been a number of patients who have had finger joint replacements who have developed an axillary lymphoma in which silicone particles have been found [203,204,225,226]. However, a cause and effect relationship is unlikely as these patients have had rheumatoid arthritis which has a known increased incidence of lymphoma.

The study of vascular access devices is the first to look at the implantable plastic devices studied. Elemental silicon tissue levels were measured by Evans and

Baldwin, 1996, in capsules surrounding removed Port-a-Catheter devices in 15 patients [227]. Although elemental silicon was found in only six of the 15 patients, the associated histological response was not reported. The level of Si determined in their 15 patient group was greater than those measured in their previous study of cadaver tissue, from patients without medical Si contact [228].

Silicone was originally chosen for medical use because of its chemical inertness and the assumption that it would also be biologically inert [198,204,210]. There have however, been reports of scarring and dystrophy of the arm after silicone migration [229]; and cellulitis, facial nodules and ulceration occurring in the long-term following small volume silicone injections [220]. However, reports on the histological response to silicone have been somewhat variable. Foreign body giant cell granulomas have been described, with silicone inclusions and a surrounding inflammatory infiltrate often seen as a fibrous tissue capsule around the device [17,49,128,210,230].

Not surprisingly, it is thought that the particulate nature of the injectable material, and the technique of administration, are responsible for the plastic migration. However, the results from studies looking at solid implants refute this concept (at least in part), as do studies which show particle shedding during either cardiac bypass or haemodialysis [60,212-215,221,225,230]. Furthermore, migration of silicone to the liver and myocardium from haemodialysis tubing have both been documented [60,212].

In fact, analysis of either the fluid or the tubing used through a finger or roller pump shows fragmentation of Polyvinyl Chloride [231], Polyurethane [232] and silicone [201,215]. The pumps investigated in the published studies are usually used for the administration of intravenous fluids to children post-operatively, but were used at

higher rates in these studies, and larger total volume infusions than the *in vitro* study presented here. The significance of these findings is highlighted by a neonatal death recorded after a prolonged illness, where the infant had died from pulmonary hypertension secondary to silicone-induced, granulomatous, inflammatory-lung disease [200]. No other case of clinically significant migration from intravenous tubing has been recorded and little consequence of plastic migration has been identified, except when large volumes have been used [115,117,118], although one unsubstantiated report suggested that migration of plastic to the brain led to a stroke in a girl who had been treated endoscopically for VUR [216]. Adverse effects of these large volumes have included lung disease [33,115,117,118,200] and myocarditis [212].

The results of the *in vitro* study confirm that intravenous migration of silicone particles is possible when a finger pump is used to administer fluid at rates appropriate to paediatric practice. The embolisation appears to come from particles adherent to the inner surface of the tubing in the short-term, rather than fragmentation, as occurs after longer periods. The wear and tear effect on the tubing was seen over 72 hours, which is the period of use of the tubing in standard ward practice.

The identification of embolisation of silicone particles during routine pump assisted infusions in children adds support to the use of microfilters in the line, although it could be argued that a small systemic load of silicone is not harmful, as large numbers of children have already been exposed to this form of treatment without any recorded adverse events. However, given the possibility of unrecognised adverse affects, further study of intravenously administered plastic is needed, particularly given the possible high incidence of systemic silicone suggested by a recent finding of elemental silicon in nine of 10 cadavers with no history of intracorporal plastic silicone [233]. These results show that silicone does migrate from long-term indwelling devices, and does cause a local inflammatory response. To determine the significance of these

findings, longitudinal studies are necessary, both to confirm these findings and to monitor carcinogenicity. The use of these devices in children for the last several years without any reports of long-term adverse events suggests complications are rare. More research, however, is needed to enhance our understanding of the long-term sequelae of these devices in children. A further adverse effect of intravenous infusions comes from the solubility of the plastic softening agent, Phthalate, from the infusion set. This substance can be toxic, causing mutagenic effects and infertility in rats and respiratory disease in children [234,235]. The presence of this toxin should not be taken out of context of the usual dose received during most intravenous infusions, but should be considered if raising concerns and comparing therapies for the management of vesicoureteric reflux.

In the manuscript study of the capsule around intravenous access devices, from a group of 11 children, the devices had been implanted for between 27 and 1854 days. On removal, Si was identified in six of the capsules, with an associated fibroconnective and hyaline connective tissue capsule in six; two of which showed foreign body giant cell reaction; two others showed a focal chronic inflammatory reaction. All the capsules with Si had been implanted for longer than 202 days with a median of 470 days. This phenomenon is also in need of further study.

Immunological Response to Plastic

The development of a capsule around implanted devices and the inclusion of plastic particles into macrophages suggests some degree of 'recognition' by the immune system. This response to plastics is extensively discussed in the literature and is presented here, in part, as it is an important aspect of the debate on the use of plastics in medicine.

Goldblum *et al.* reported two patients with an alleged severe immune-mediated reaction to a silicone ventriculoperitoneal shunt, resulting in inflammation of the tissue surrounding the shunt and its subsequent blockage [51]. They used an ELISA and reportedly found these patients to have IgG which bound to silicone tubing at a consistently higher rate than for control patients, concluding that antibodies to silicone bind specifically via the IgG Fab fragment. Despite using a number of variations to block the non-specific response experienced when using the assay described by Goldblum *et al.*, it was not possible to reproduce their results [236]. In particular, all the controls were positive and all patients tested produced similar results, whether they had known exposure to silicone or not. The control groups included the use of fetal cord serum and pooled AB serum, a commercial pool from a large number of people, were not expected to give positive results; pooled AB serum consistently gave the highest IgG values.

A review of the literature revealed mixed reports of the development of an ELISA test for silicone antibodies and that other groups have also been unable to reproduce the results of Goldblum *et al.* A study by Wolf *et al.* [237] used a modified ELISA to confirm the findings of Goldblum *et al.* To prepare the antigen, they coated plates with 0.1% bovine serum albumin and then 0.1% silicone and each serum sample was assayed in the presence of albumin only and with albumin complexed with silicone to allow for non-specific antibody binding. They found statistically significant reactivity attributed to IgG to silicone in sera of the high exposure group (patients with confirmed ruptured or leaking breast implants) when compared to that of controls (no implants), diabetics (silicone exposure from syringes) and the low exposure group (intact implants). Rose *et al.* [238] tested positive sera provided by Goldblum, and obtained similar results, but they also found a high positive response in patients with connective tissue disease, yet no history of silicone implants. Also, they obtained high values for normal sera when silastic tubing was used as the antigen, but significantly lower values using elastomer discs adhered to the wells used for performing the test.

They conclude that further work is needed to establish whether these results are due to silicone-specific antibodies and that this is hindered by the high levels of non-specific protein binding on plastics. Rosenbau *et al.* [239] tested a number of different ELISA methods in an attempt to demonstrate IgG antibodies specific for silicone. They varied such parameters as the silicone used, the blocking agent to prevent non-specific protein binding to the antigen, their washing methods and their controls. Similarly, Wolf *et al.* coated wells with 1% bovine serum albumin, as a blocking agent, then either polydimethylsiloxane or its hydroxy-terminated form. Using positive sera from Goldblum *et al.*, they were unable to obtain any positive reactivity with any of their three methods or the method similar to Wolf *et al.* They failed to obtain a positive result with any sera. With only one method were they able to demonstrate a significant result for silicone breast implant patients, compared to normal serum pool. They conclude that it is extremely difficult to set up an ELISA test for silicone antibodies and that these antibodies may not even exist. Kossovsky *et al.*, also concluded that the binding is non-specific [240,241]. In the light of the above studies and that of other groups, we conclude that the method reported by Goldblum *et al.* is not a reliable assay for antibodies to silicone. The silicone tubing used as the antigen may be inappropriate as it may be technically difficult to wash properly and it appears that we were unable to adequately block non-specific protein binding to the plastic surface. It would appear that an immunological response to implanted plastics has not been confirmed, however, there **is** a tissue response, as evidenced by the histological findings of inflammation, which should be further studied.

PART III

Vesicoureteric Reflux - Clinical and Laboratory Studies

Introduction

Vesicoureteric reflux is a common condition for which a general consensus for management is yet to be reached. This is despite it affecting from one to ten percent of the population [242], the recognition of which most commonly follows investigation of a UTI or a pre-natal diagnosis of hydronephrosis. Interestingly, boys are more commonly affected by VUR after a pre-natal diagnosis of hydronephrosis and following a UTI in infancy [145,243], and are also more likely to have renal anomalies associated with the VUR than girls [2,243-248]. Two recent studies are the work from Craig Peters in Boston, whose group has induced high grade VUR associated renal parenchymal anomalies, with ablation of the ureteric tunnel and ligation of the urachus in the male sheep fetus [249], and the second has shown a link between VUR and the Angiotensin II receptor gene, in mice: the latter work is from a group who have found a 23% incidence of renal anomalies in the mouse model with the lack of this X linked Angiotensin II receptor. The latter group also found the multicystic kidney (MCK) and pelviureteric junction (PUJ) obstruction to be more common, associated with lack of apoptosis around the developing ureteric bud and renal mesenchyme. The gene, expressed in fetal life only, was found to have an A to G transition within intron one which they later found to occur in nine of the 10 men with the anomalies that had been identified in the rat model, but only 42% of 31 controls [250]. The suggestion that VUR and reflux associated nephropathy (RAN) are due to a bladder anomaly, on one hand, and possibly due to a field defect on the other, supports the concept that even "primary" VUR possibly represents a range of diseases.

Studies presented in the following pages show improved growth after the surgical management of high grade VUR, the development of dilatation in the collecting kidneys of refluxing fetal pigs and progressive changes in boys with high grade VUR discovered after a diagnosis of hydronephrosis pre-natally. This work also lends

support to the view that genetic predisposition to RAN, infection or obstruction are not the only factors which adversely affect kidneys into which VUR occurs.

STUDY 1

Surgical Management of Vesicoureteric Reflux

Patients and Methods

One hundred and eighty-five patients with primary VUR, treated by ureteric reimplantation at the Adelaide Children's Hospital from October 1990 to October 1993, were included. The clinical records and the radiologic studies were also reviewed and symptoms and signs were recorded. Patients with previous anti-reflux surgery were excluded from the study. Ureteric reimplantations were performed by one of two surgeons, 95 by the author (71 of those with completed follow-up), using the transtrigonal advancement (Cohen) technique [147].

Reflux Grading

Of the 185 patients, 126 had pre-operative and post-operative studies available for further assessment. Twenty-five did not have pre-operative studies available and 24 did not have a post-operative study available, these studies having been conducted at another hospital or misplaced. Grading was according to the IRSG five grade system [132,133]. The majority of the studies, both pre- and post-operatively were with an MCU; some patients had their VUR assessed with either an IRC or DIC.

Renal Growth Analysis

Renal growth was assessed with US measurements of kidney length, using standard views established in the Department of Ultrasound at the Adelaide Children's Hospital. Three measurements were recorded; pre-operatively, and three and 12 months after the surgery. Comparing the first and last investigations, sufficient information was available for the assessment of 81 patients or 132 affected kidneys. The remainder of cases were investigated in another hospital, incompletely followed, or the records were unavailable.

To compare the results of renal growth for the reflux-treated patients with a large normal population, the measurements of 1100 normal kidneys (720 Female, 380 Male) taken at the Adelaide Children's Hospital between 1988 and March 1994 were analysed. From these data, non-linear regression curves of renal length-for-age were obtained for males and females.

To assess whether the grade of reflux had any bearing on the change in renal growth, pre- and post-operative regression lines were obtained for three subgroups of severity of reflux. This manoeuvre was performed to achieve statistically meaningful data: the severe reflux group were those with grade V disease; moderate reflux included grades III & IV; mild disease was considered in those grades I & II reflux. The renal growth lines for each grouping of reflux severity were compared to the normal population.

Having compared the whole of the affected population pre- and post-operatively, individual changes in renal length were then observed. Age adjusted renal lengths were rated as deviations (measured as number of standard errors - SEs) of the normal regression line. This is equivalent to the standard deviation from the normal kidney size. Differences between the pre-operative and follow-up investigations were grouped as shown in Table 35.

Large increase	>2 SEs increase
Small increase	>1 SE, <2 SEs increase
Large decrease	>2 SEs
Small decrease	>1 SE, <2SEs
No change	<1 SE
Decreasing	(>1 SE) to above or into the normal range.

Table 35: Differences between the pre-operative and follow-up investigations were grouped as shown here.

The 'decreasing' category was included to separate those kidneys which were above or within the normal length range post-operatively, but they were decreased from their pre-operative size for age. This manipulation was to differentiate swollen pyelo-nephritic kidneys, which had returned to normal size, from kidneys which had become smaller because of progressive reflux nephropathy.

Results

The 185 patients consisted of 119 females and 66 males. One hundred and fourteen patients had bilateral reimplants, 39 had a left ureteric reimplant and 32 a right reimplant.

Presenting Symptoms and Signs

UTIs were by far the most common indication for surgery (74%). Other patients had wetting, pre-natal diagnosis, sibling VUR, and febrile convulsions as presenting symptoms and signs.

Technical Outcome

Of the 126 patients who had technically satisfactory reflux studies (both pre- and post-operatively), 27 left, 22 right and 77 had bilateral reimplants, giving a total of 203 refluxing units. The reflux grades are collated in Tables 36 and 37.

Grade	**I**	**II**	**III**	**IV**	**V**	**Total**
Left	12	27	34	21	10	104
Right	15	21	30	19	14	99
Total	27	48	64	40	24	203
% of 203	**13**	**24**	**31**	**20**	**12**	**100**

Table 36: Vesicoureteric reflux grades for those 126 patients having Cohen reimplant surgery and who had pre and post studies for review.

Grade	**I**	**II**	**III**	**IV**	**V**	**Total**
Left	3	15	18	16	9	61
Right	4	10	19	13	12	58
Total	7	25	37	29	21	119
% of 119	**6**	**21**	**31**	**24**	**18**	**100**

Table 37: The distribution of the grade of VUR for 71 patients with complete follow-up, and treated by the author.

Post-operatively, reflux was resolved in all but four of the 126 cases, all of whom had unilateral low grade VUR on follow-up. These figures equate to a success rate of 98% (122/126) for the patients and 97% (199/203) for the ureters. There was one case of contralateral reflux post-reimplant, but no vesicoureteric obstruction was observed.

Renal Growth Outcome

The information on renal growth was previously unavailable in the Adelaide Children's Hospital in a statistically valid form. The regression curve was found to be most accurately represented by the model: ***Renal Length = B0 + B1*** age, where B0 and B1 are both estimated constants; B0 representing renal length at birth, B1 being proportional to the growth velocity (Table 38). This model was then used to establish regression lines for the pre-operative and follow-up renal lengths. All measurements were adjusted for age, using the regression equations. The post-operative renal length and growth rate could be directly compared to the renal length and growth rate of both the pre-operative and normal populations.

The normal regression curves for both males and females are shown in Fig. 41. The estimated curves were found to adequately account for the variation. There was no significant difference found between the regression curves for the male and female populations.

Dividing the kidneys into the three groups of severity of reflux, some differences in kidney growth were observed. Pre-operatively, the severe (grade V) and mild (grades I & II) VUR kidneys were seen to have a slower growth rate than normal, and kidneys with moderately severe VUR (grades III & IV) had normal growth. Post-operatively, the growth rate of severe refluxing kidneys increased to the normal value (Fig. 42), whereas there was no significant growth rate change for those with mild or moderate VUR post-operatively (Fig. 43).

The individual changes in kidney size adjusted for age are summarised in Table 38. Thirty-seven showed improvement (26 >2SEs), 24 showed a decrease in size (19 >2 SEs), while 35 showed no change (<1 SE). Thirty-six kidneys were observed to decrease in size, but remained above or within the normal range.

Group	**Length**	**Velocity**
Normal males	$4.624 + 0.386\sqrt{Age}$	$0.193/\sqrt{Age}$
Normal females	$4.459 + 0.416\sqrt{Age}$	$0.208/\sqrt{Age}$
All Pre-operation	$5.019 + 0.345\sqrt{Age}$	$0.173/\sqrt{Age}$
All Post-operation	$4.748 + 0.298\sqrt{Age}$	$0.149/\sqrt{Age}$
Pre-op Grade I & II	$5.565 + 0.299\sqrt{Age}$	$0.150/\sqrt{Age}$
Post-op Grade I & II	$5.407 + 0.307\sqrt{Age}$	$0.153/\sqrt{Age}$
Pre-op Grade VI & III	$4.470 + 0.434\sqrt{Age}$	$0.217/\sqrt{Age}$
Post-op Grade VI & III	$4.108 + 0.446\sqrt{Age}$	$0.223/\sqrt{Age}$
Pre-op Grade V	$5.073 + 0.312\sqrt{Age}$	$0.156/\sqrt{Age}$
Post-op Grade V	$4.412 + 0.408\sqrt{Age}$	$0.204/\sqrt{Age}$

Table 38: Regression equations for renal growth. Length = $B_0 + B_1 \sqrt{Age(months)}$ B_0 = Kidney length @ one month. B_1 = Estimated growth parameter. Velocity = length change/age change = $B_1 / 2\sqrt{Age}$.

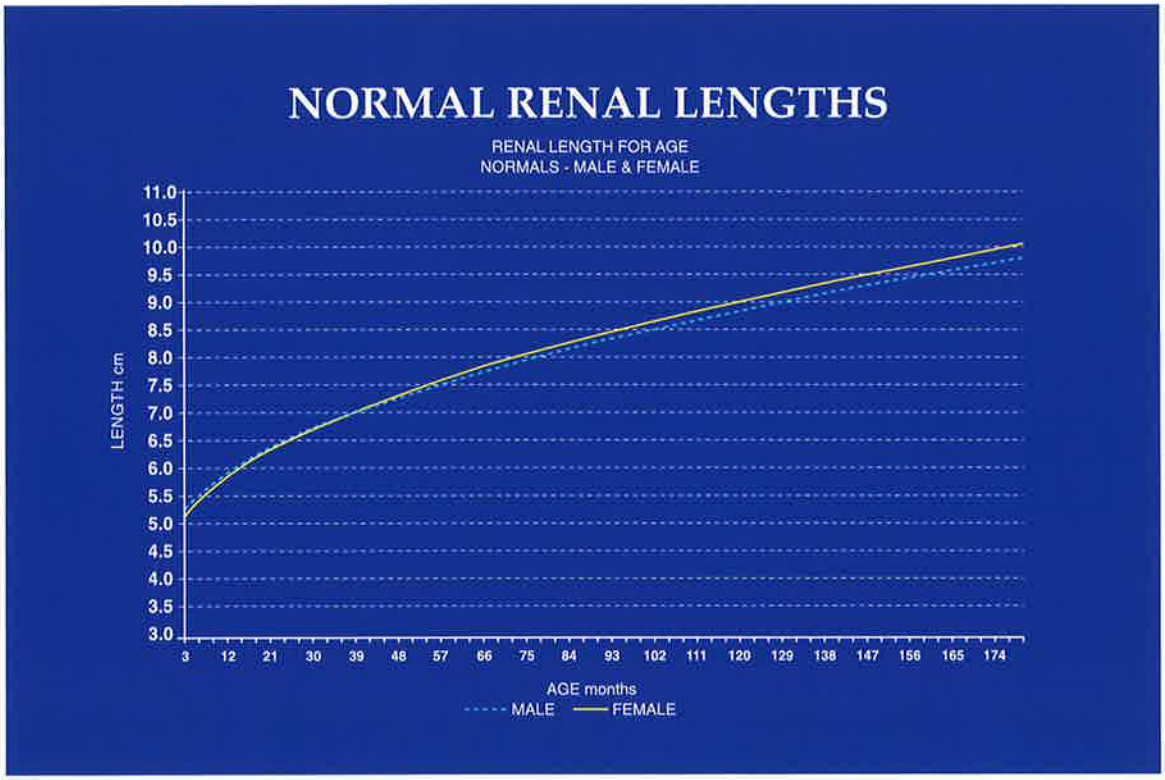


Figure 41: The normal growth curves for males and females. There is no significant difference between the sexes.

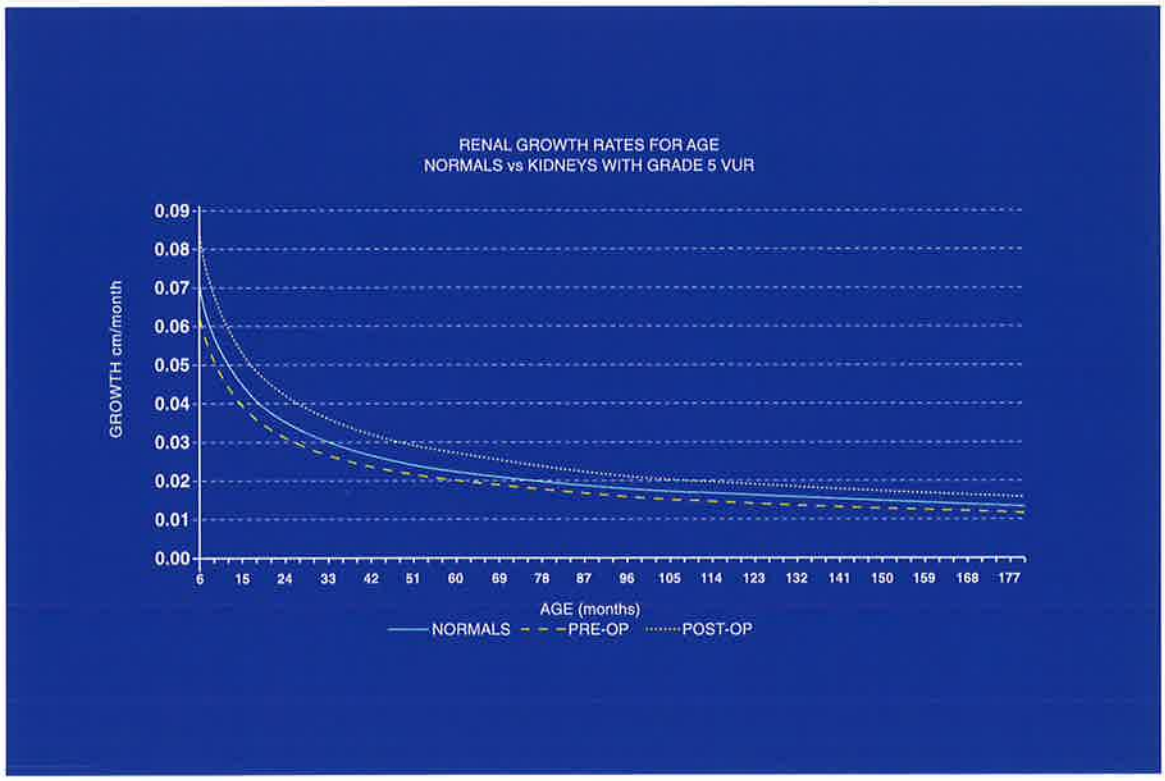


Figure 42: Renal growth rate is improved for kidneys with high grade VUR.

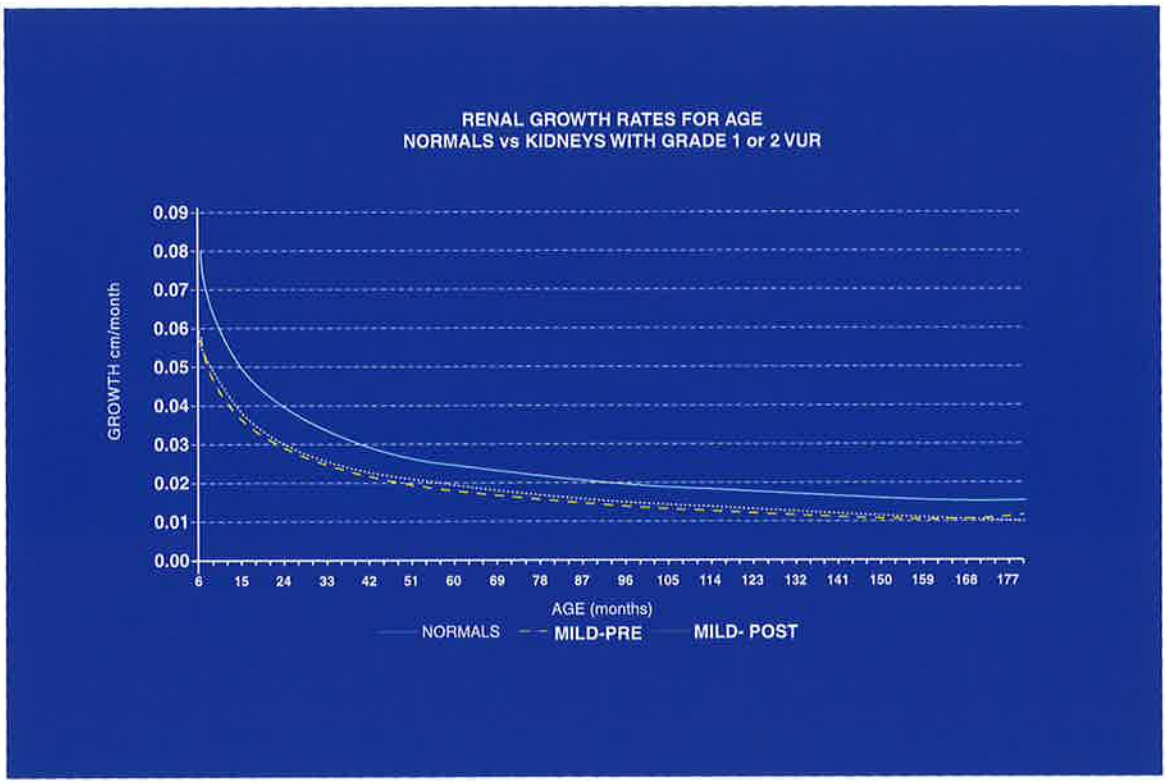


Figure 43: There was no significant growth rate change post-operatively in the mild reflux group.

STUDY 2

Progressive Antenatal Renal Deterioration Associated With High Grade Vesicoureteric Reflux

Introduction

To better understand the role of ultrasound in the management of the fetus with pre-natal hydronephrosis, the use of ultrasound in the investigation of the fetus with a renal anomaly will first be reviewed, particularly focusing on the role of intervention, to highlight how important it is to identify the high grade VUR group, to help avoid prenatal intervention in this group.

Renal tract anomalies are important in pre-natal diagnosis, representing half of all antenatally detected abnormalities and, thus, seen in 0.1-2.2% of pregnancies [251-253]. Minimal pelvicalyceal dilatation is the most common finding but cystic renal disease, hydronephrosis and bladder outlet obstruction are also frequently demonstrated.

Cystic renal disease, one of the better understood areas of fetal urology, can be detected pre-natally and includes the MCK, primary or obstructive cystic dysplasia and infantile polycystic disease. The MCK, on antenatal ultrasound, appears as a collection of variable sized, fluid filled cysts without the well defined intercommunications seen with hydronephrosis. The progress from a minimally hydronephrotic kidney to the typical "bunch of grapes" appearance with ureteric atresia, has only recently been recognised [254]. The subsequently abnormal kidney, if viewed before 18 weeks, will often be only minimally hydronephrotic or normal; an increase in renal size then occurs, associated with the development of cysts. The cysts subsequently increase in number and are variable in size. Ultimately, the cystic mass decreases in size such that only

75% of MCK are palpable at birth. The pathology is possibly a combination of renal parenchymal and upper ureteric ischaemia, with obstructive tubular changes secondary to the decreased ureteric blood supply [255]. The aetiology of the ischaemic injury may be the failure of apoptosis around the ureteric bud and renal blastema as seen in the Angiotensin 2 receptor, gene knock-out mouse; the failure of apoptosis is postulated to inhibit the ingrowth of vascularity to the pelvi-ureteric junction [250]. In the rare instance that the lesion is bilateral [256], oligohydramnios and failure of lung development will occur. The differential diagnosis of PUJ obstruction can be excluded by recognition of the open calyceal communication with the pelvis and the higher sodium content [257] of the MCK fluid, although aspiration is virtually never indicated. Pre-natal intervention for MCK is only required if the cystic mass interferes with lung growth, or is likely to cause obstructed labour [258], as the MCK usually decreases in size, and may be undetectable subsequently [259]. Post-natally, the affected neonate should be commenced on prophylactic antibiotics because of the 30-50% risk of associated anomalies, such as contralateral vesicoureteric reflux [260]. A cystogram, renal function study and ultrasound should be performed at four to eight weeks of age. The presence of areas of function, large size, failure to involute significantly in the first year, and the need for other surgical intervention are indications for removal [255]. The risks of leaving the kidney *in situ* are; hypertension [261], infection [262] and development of a Wilms' tumour [263], the risk of which is extremely low. Therefore, the main benefit of nephrectomy is significant reduction in the follow-up required.

One of the major difficulties in understanding the appropriate follow-up for patients with fetal hydronephrosis has been the lack of a satisfactory differentiation between a variation of normal and significant pathology. Dilatation of the fetal renal pelvis is a common finding, the classification of which is not yet agreed. Often authors do not give a definition of hydronephrosis when presenting their data [251] or the definition is only loosely related to gestational age. Some have attempted to be more precise,

suggesting a fetal renal pelvic diameter of >15mm after 20 weeks gestation [264], graded parenchymal thickness [265], the pelvic to parenchymal ratio [266], and the kidney to abdominal wall circumference as possible standards indicating significant pathology. The majority of first scans are performed between 17 and 19 weeks gestation; therefore, guide-lines for pelvic dilatation are more valuable at this gestation, but these have not been well established, because the majority of those with minor dilatation early in pregnancy resolve spontaneously. Mandell *et al.* [267] have suggested the diameters of 5mm at 15-20 weeks, 8mm at 20-30 weeks, and 10mm thereafter are kidneys which should be reviewed. Using these criteria, 37% of their group required surgery; others give a more conservative estimate [253]. However, as investigation of all these children, both before and after birth, imposes an enormous work load of doubtful cost effectiveness. Therefore, the guidelines should be further clarified, especially when up to 80% of those with persistent renal dilatation after birth may resolve spontaneously [268]. In contrast, 30% of those with significant obstructive renal tract pathology have a normal scan before 20 weeks gestation [269]. To further add to the confusion, post-natal PUJ obstruction, without loss of function, can recover spontaneously [270], and a PUJ obstruction is not always pre-natal in onset; both adults and children have been recorded to have significant renal changes secondary to a PUJ obstruction, having previously had a normal upper renal tract investigation, either pre- or post-natally. Also, those with a normal post-natal study, after having had an abnormal pre-natal ultrasound, can subsequently have progressive obstruction [271], and another 25% have VUR [2,243,245,246,248,249,272-279].

It can be seen that there are data which weigh for and against close monitoring of the pre-natally diagnosed hydronephrosis. From all this confusion, it is suggested that a pelvic diameter of >5mm before 20 weeks, and >10mm after 20 weeks should be monitored, and if the renal pelvic diameter is >15mm significant pathology is likely and ureteric dilatation is always significant [280]. Also, when the dilatation is marked the post-natal ultrasound should be on the first day after birth. If the distension is less

prominent, or if the scan on day one does not demonstrate the recently seen gross abnormality, the scan should be repeated on day seven of life.

More uncertain is the need for a cystogram in those who have only minimal hydronephrosis.

Factors which allow for improved interpretation of the pre-natal data include study of the echogenicity. However, establishing a standard for echogenicity is difficult, principally because the fetal renal parenchyma is relatively echogenic compared to children and adults. Estroff *et al.*, however, found that 80% of kidneys that were more echogenic than the liver had abnormal function at birth [281]; Kaefer *et al.*, in a study of 18 cases (six of whom had the megacystis-megaureter association) concluded that increased echogenicity of kidneys compared to the liver was 87% predictive of obstruction [282]. A more specific sign of renal dysplasia is the presence of parenchymal cysts, but less than half of the fetuses with dysplastic kidneys have cysts identified on ultrasound and early in gestation cysts are less likely to be detected in subsequently proven renal dysplasia [283].

The most conclusive study of fetal renal deterioration would be a series of scans of the same fetus which show *progressive changes* in the echogenicity, the development of cysts, or progressive thinning of the renal parenchyma.

Another consideration in the treatment of the fetus is whether there is a chromosomal anomaly, which is found in 2% of those with hydronephrosis [284]. Nevertheless, in the presence of unilateral mild hydronephrosis or upper tract obstruction, there appears to be no increased risk of a chromosomal anomaly over the normal population [285]; therefore, amniocentesis for minor pelvicalyceal dilatation, or unilateral PUJ obstruction does not seem justified [285]. If intervention is planned, or there is a co-existing abnormality on ultrasound or on serum screening, where the risk of an

aberration may be as high as 24%, chromosomal analysis should always be performed [284].

Pre-natal Intervention

The main benefit of pre-natal identification of the abnormal renal tract has been the improved understanding of renal tract pathology in children. Unfortunately, the anticipated nephron preservation have not as yet been widely achieved [286], mainly because of difficulty identifying those who will benefit from intervention, the short-comings of stenting the urinary tract and the risks of open fetal surgery, each of which will be addressed. However, animal experiments have shown that obstruction of the fetal urinary tract, at either the ureteric or bladder neck level, can cause renal dysplasia if created sufficiently early in gestation [287,288] and the affect of obstruction on renal development can be reversed by relieving the obstruction by vesicostomy [289] or shunt insertion [288]. The success in animals has validated attempts to consider intervention in the management of the obstructed fetal renal tract in humans [290,291]. A number of factors need to be considered in selecting a fetus for intervention, after chromosomal anomalies and other congenital defects have been taken into account, including the number of possible causes of fetal renal tract dilatation (Table 39).

Pelviureteric Junction Obstruction
Primary Obstructive Megaureter
Vesicoureteric Reflux
Neuropathic Bladder
Congenital Bladder Neck Hypertrophy - Marion's Disease
Posterior Urethral Obstruction
Bulbar Urethral Obstruction
Transient Urethral Obstruction
Urethral Atresia/Prune Belly Syndrome
Megacystis Microcolon Hypoperistalsis Syndrome
Cloacal Anomalies

Table 39: Differential diagnosis of significant pre-natal hydronephrosis

The importance of differentiating between these pathologies cannot be overstated, particularly as pre-natal intervention, with its maternal risks, is not warranted for transient obstruction, VUR, or for those with irreversible dysplasia.

An obstructive lesion is suggested by progressive pelvic dilatation, thinning of the parenchyma, decreased amniotic fluid volume, urinary ascites or urinoma, or in the case of a dilated bladder with a prominent proximal urethra and poor bladder emptying. These features indicate that an obstruction is probable; the question of prognosis with intervention should also consider the lung development and renal function potential.

For lung growth to be adequate the amniotic fluid has to be satisfactory early in pregnancy or lung hypoplasia will occur, even if the amniotic fluid volume has been improved subsequently [292]. Conversely, if intervention occurs soon after the late onset oligohydramnios, the prognosis for both lung and renal function is good [293]. In fact, a decrease in amniotic fluid should be regarded as a relatively late sign, suggesting that adequate amniotic volume should not be a contraindication to intervening; conversely, bilateral renal cystic disease is a contraindication to intervention. However, a definite bladder outlet obstruction with initially normal kidneys and amniotic fluid, followed by progressive dilatation but satisfactory biochemistry, is the ideal case for draining the fetal renal tract *in utero*.

Aspiration of the renal tract should only be considered where obstruction is present and should pre-empt early delivery or insertion of a drain. The fetus should be otherwise normal and further intervention should be expected to follow, if appropriate on all other selection criteria. While performing the bladder aspiration the intravesical volume and pressure should be measured [294], and the response of the upper tract dilatation to the bladder emptying should be assessed. Urine output can also be measured by leaving the needle in the bladder for a period of time. The fetus should be

paralysed, and amniotic fluid should be collected for microprotein analysis and karyotyping [286,295-299].

Fetal urine biochemistry has also undergone extensive investigation: Harrison's group looked at biochemical prognostic indicators in animals and then in patients [290], but did not provide data related to gestational age. Nicolini *et al.* have provided distribution tables of sodium for gestational age, showing that expected levels decline with gestation and increase with renal damage [300]. They recorded the normal sodium range at 16 weeks gestation as 60-130mmol/l and at 36 weeks, 10-60mmol/l. The sodium, amniotic fluid, and ultrasound changes have been found to be more reliable than the urine creatinine, potassium [290] or N-Acetyl-ß-D-Glucosaminidase (NAG) [286] in predicting the renal function outcome.

The microproteins β_2 and α_1 microglobulin have been recently explored as prognostic indicators and are thought to be better predictors of renal function than the sodium level. There is normally a progressive decrease in these levels toward the end of pregnancy [296], as for sodium. The microproteins are normally filtered through the glomerulus and reabsorbed in the proximal tubules. In the damaged kidney they are able to leak into the urine, and can then be measured in both the fetal urine and the amniotic fluid. The levels correlate well with fetal renal damage [286,295-299], as the microproteins are unable to cross the placenta [295].

In general, a fetal urine sodium of <50mmol/l and a $ß_2$-microglobulin of less the 2mg/l are indicative of good renal function at the time of sampling. A urine sodium of >70mmol/l and a $ß_2$-microglobulin of >15mg/l are virtually always associated with poor renal outcome [286,295-299].

The age and pregnancy history of the mother, the difficulty conceiving, the number of fetuses and the gestation are all important factors in deciding whether to intervene and

what the nature of the intervention should be. The risks of fetal loss and chorioamnionitis should be fully discussed before proceeding [290,301].

There are a number of fetal intervention alternatives available. Harrison *et al.* demonstrated the efficacy of percutaneously placed bladder catheters [288] and forming a vesicostomy [289] in sheep. Because of the problems with percutaneous shunts in humans, such as incorrect placement, dislodgement and ineffective drainage [301], a fetoscopic approach with both stent insertion [302], laser diathermy of the fetal abdominal wall [303] and percutaneous fulguration of the urethral obstruction and fetoscopic surgery have also been reported [126,304]. The majority of human fetuses treated with urinary tract diversion have had a percutaneous shunt inserted which, if required before 24 weeks gestation, usually has to be replaced. A small number of cases have undergone a fetal vesicostomy [291], but as yet the prognosis for those thus treated has not been satisfactory [286], usually related to an inability to identify which case has reversible renal damage.

Post-natal Management

To determine the correct post-natal management, it seems important to compare the images from each episode of pre-natal ultrasound with the post-natal findings, as the changes over time are more important than the findings on any one scan. In those patients with minor persistent hydronephrosis during pregnancy a follow-up scan should be performed at three months and, if normal, would appropriately be the last scan. For the infants with a moderate degree of renal pelvic distension, which is confirmed on a scan at the end of the first week, the infant should remain on prophylactic antibiotics until an MCU and a renal function study are performed at four to eight weeks of age. If VUR is present it is managed appropriately. Adequate scientific guidelines for the management of pre-natal hydronephrosis have not been established. However, where there has been a marked degree of hydronephrosis pre-

natally, the timing of the first post-natal scan should be different. There is no need to wait for the baby to be better hydrated before assessing the kidney, as the degree of change may be sufficient to warrant further action before the urine output increases - the classic examples would be the tense palpable unilateral hydronephrosis or the bilateral hydronephrosis with anuria. If on day one, the kidney is not dilated, and there have been marked pre-natal changes, a repeat scan should be performed at one week. If there is good function, but slow drainage, in a kidney that has *good* parenchyma, the delayed drainage will usually resolve. If there is significant parenchymal thinning at birth, early surgery is indicated, and should follow a function study. If the renal function is poor, a pyeloplasty should still be carried out, as neonatal kidneys may recover from an acute episode. If for some reason surgery is contraindicated, a nephrostomy tube can be inserted, accepting that cystic changes would preclude surgical treatment. The same approach to function and drainage applies to those infants where the drainage delay is at the vesicoureteric junction.

If the child does have urethral obstruction, treatment should occur early. Ideally a cystogram is performed through a suprapubic cannula and the obstruction disrupted with a resectoscope within 24 hours. Taking this approach has allowed the urethra to be endoscoped prior to catheterisation, and has identified the abnormality to be a Congenital Obstructing Posterior Urethral Membrane (COPUM) [305], rather than Valves in the posterior urethra as originally thought [306]. Nevertheless, the priority should be stabilisation of the patient, insertion of a urethral catheter and replacement of the post-obstructive diuresis fluid. Prophylactic antibiotics should be administered and long-term follow-up of kidney and bladder function is essential. Of those surviving after birth, approximately 30% will be free of significant bladder and kidney disease, 30% will have minor to moderate renal impairment and the remainder will require close monitoring for renal and bladder dysfunction. The following study of six patients, who had a diagnosis of hydronephrosis and subsequent deterioration, represents a selected subgroup of fetuses; the aim was to elucidate the relationship between VUR and renal

parenchymal anomalies. The six boys described here had early investigation in the perinatal period because the pre-natal US suggested that there was urethral obstruction.

Patients and Methods

Patients with antenatal hydronephrosis seen at the Women's and Children's Hospital, Adelaide, over a six year period to March 1996, were reviewed. Of this group of 28 patients, six were identified who had more than one antenatal ultrasound which demonstrated renal deterioration, but no subsequent evidence of urethral obstruction. Renal deterioration was identified on the basis of increasing pelvicalyceal dilatation and parenchymal thinning, and all the patients were male. All of these boys were referred to the author for co-ordination of the pre-natal investigation, through the pre-natal diagnosis and treatment group in the hospital.

Observed changes led to the provisional antenatal diagnosis of posterior urethral obstruction, which was the indication for repeated pre-natal scanning. Post-natally, all the infants were investigated with a renal ultrasound, an MCU and nuclear medicine studies. MAG3 was chosen as the preferred radionuclide, due to the known lower GFR in neonates, and the better excretion of the MAG3 compared to DTPA. Five infants completed these investigations prior to developing any UTIs. The sixth failed to attend for the nuclear medicine scan until 10 weeks of age by which time he had already had a UTI and was failing to thrive. The antenatal and post-natal films were collated and reviewed in conjunction with the Ultrasound Consultants involved in the pre-natal diagnosis clinic.

Results

The antenatal ultrasound scans all showed progressive deterioration in the degree of hydronephrosis, in at least one kidney in each child, with nine kidneys showing deterioration (Table 40: Figs. 44-46). The ipsilateral ureter was dilated for all nine kidneys, and the bladder was distended antenatally, in five of the six infants.

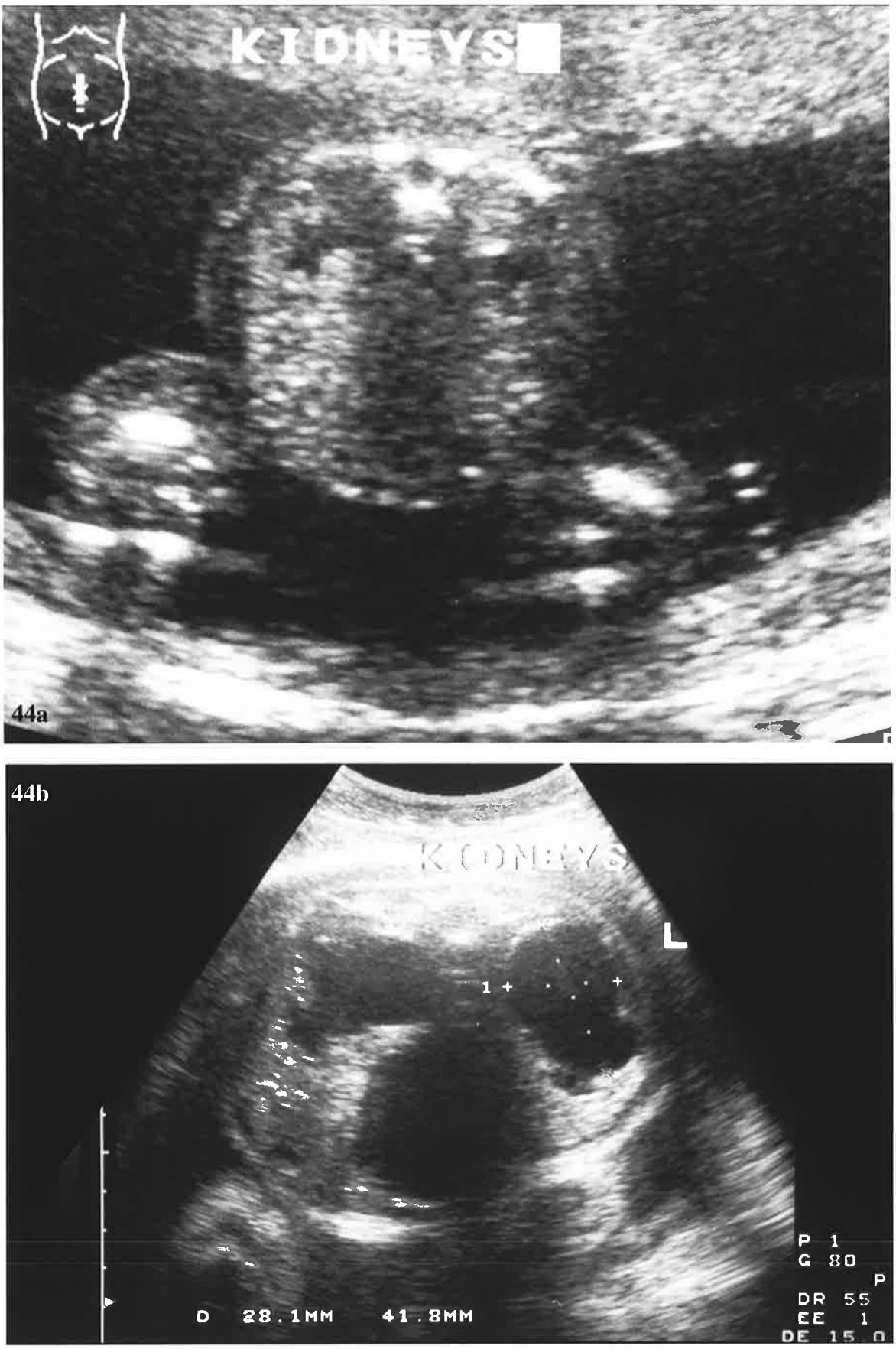


Figure 44: Antenatal scans for Case 3 at 19 weeks (A) and 34 weeks (B) gestation showing bilateral hydronephrosis with marked progression. The dilated bladder can also be seen.

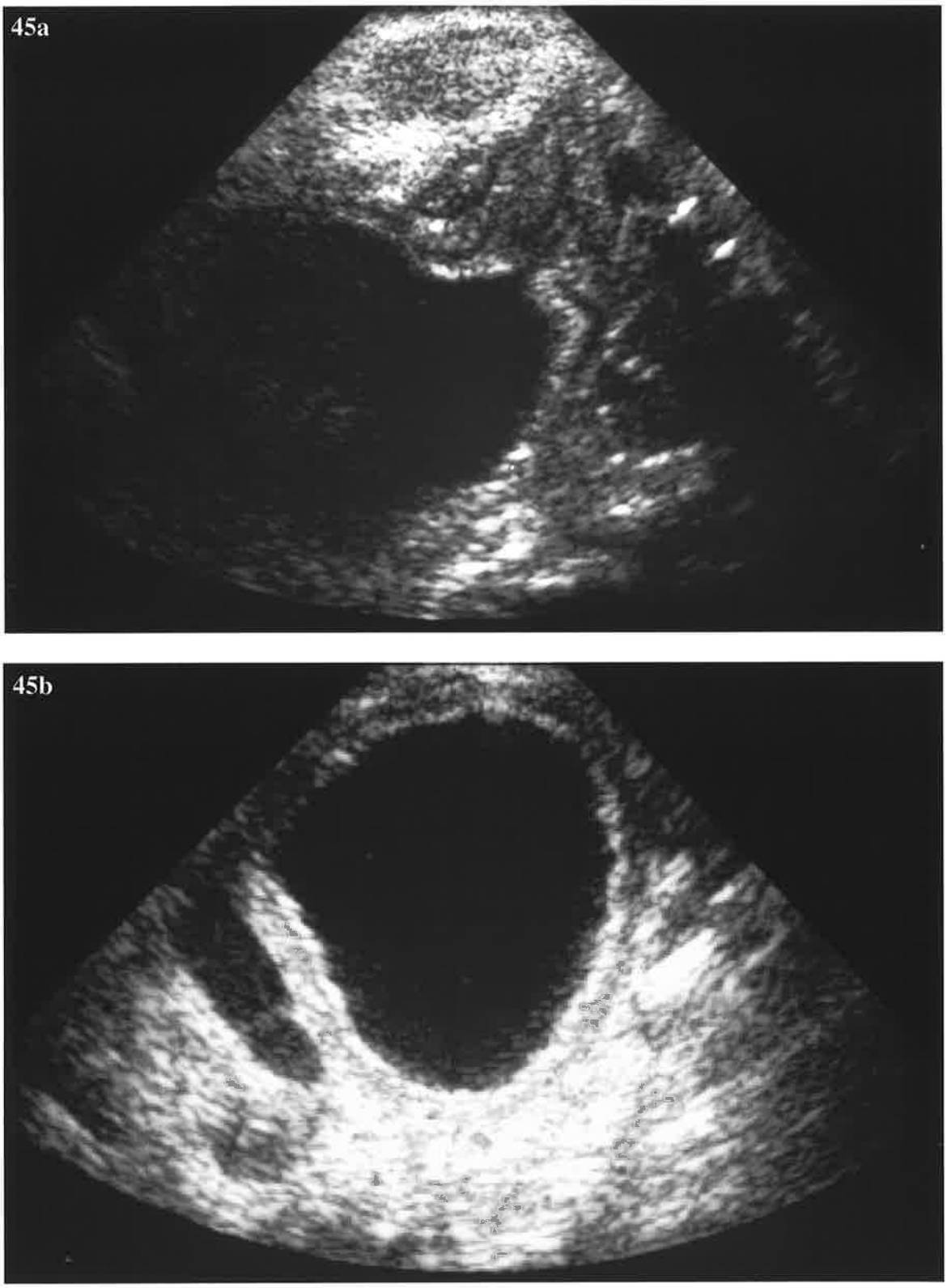
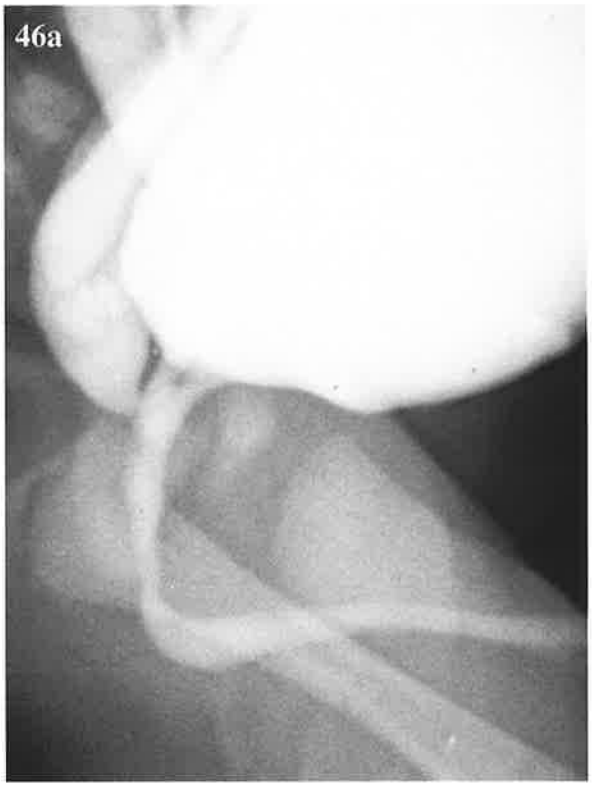


Figure 45: Case 3: A post-natal, perineal US shows the region of the bladder neck and urthra, without any evidence of urethral obstruction (A). The thick walled bladder and dilated ureter are seen on the bladder scan (B).



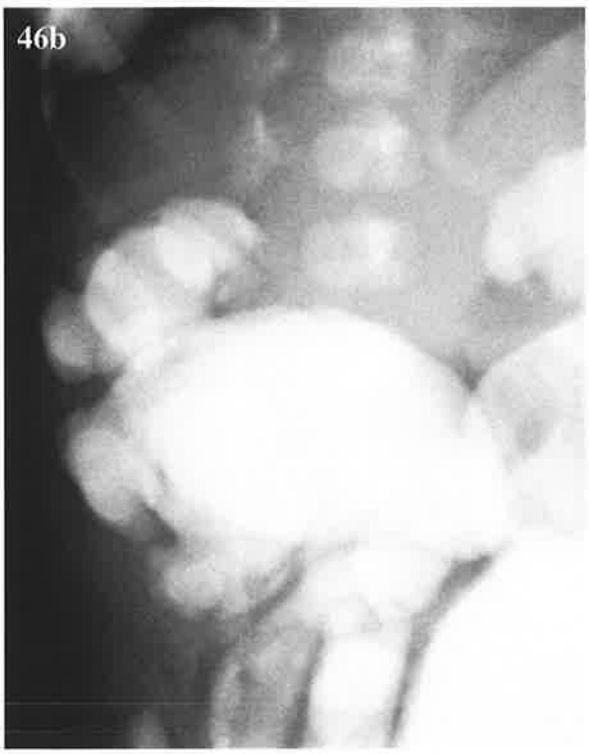


Figure 46: Case 3: The cystogram revealed bilateral high grade VUR (A) and a normal urethra. The ureters opened into the bladder, despite the appearance on the lateral view of the urethra (B).

Five of the mothers had antenatal scans performed at between 17 and 20 weeks gestation, all of which showed distension of the renal pelves of the kidneys which later increased; none had marked calyceal dilatation at that time. One mother had her first scan at about 30 weeks gestation at which time both kidneys were completely normal. All of the scans were repeated at least once prior to delivery and showed the development of parenchymal thinning and markedly increased hydronephrosis. In four fetuses these changes were seen in scans performed at around 30 weeks and all progressed on follow-up. Liquor volume was within normal limits in all cases.

Two infants were delivered early (cases three and five), with a provisional diagnosis of posterior urethral obstruction, based on the appearance of the antenatal films (Fig. 46). The fetus who had normal kidneys at 30 weeks (case two) had a unilaterally dilated kidney with parenchymal thinning noted at 38 weeks gestation when the scan was repeated for other reasons. Post-natal investigations confirmed the antenatal findings and ruled out urethral obstruction in all cases. A micturition cystourethrogram showed high grade VUR in seven of the nine kidneys which had deteriorated in utero. The MCU was technically inadequate for one child (case two) but the endoscopic findings of a large, widely-patent ureteric orifice, no intramural ureteric tunnel and a grossly dilated, tortuous ureter were felt to be indicative of high grade reflux. One infant (case five) had unilateral vesicoureteric junction obstruction associated with antenatal changes, however, his unobstructed (refluxing) kidney was more severely affected on antenatal US and was completely non-functioning on a nuclear medicine study performed post-natally. The non-functioning kidney was removed and showed diffuse lymphocytic infiltration with a germinal centre and metaplastic cartilage formation. The ureter also had changes of inflammation and fibrosis throughout its length; there had been no clinical evidence of UTI prior to the nephrectomy. The bladder was noted post-natally to be thick-walled and trabeculated in four of the six infants and distended but not particularly thick walled in one other (Table 40). These were the same five infants in whom the bladder had appeared distended antenatally and in whom some

degree of hydronephrosis was apparent in the mid-trimester scan. The nuclear medicine studies confirmed impaired function in all kidneys that had deteriorated on antenatal scanning. In those infants with unilateral changes antenatally, the differential function was much reduced in the affected kidney (Table 40). In the two infants with bilateral grade V reflux (cases one and three), the glomerular filtration rate (GFR) was impaired in both kidneys, but was asymmetric in the child in whom the antenatal changes were more marked on one side (case one).

Case No.	Gest. at deliv.	Gest. 1st US Scan	Side	Gestation Parenchyma deterioration noted	Grade of Reflux L:R	Bladder Appearance	Nucl. Med. Diff. funct. L:R
1	term	17/40	Both L>R	35/40	V:V	Distended	23:77 GFR decr
2	term	30/40	Left	38/40	V*:IV	Normal	Not done* early
3	34/40	19/40	Both	31/40	V:V	Trabec thick	49:51 GFR decr
4	38/40	20/40	Right	32/40	III:V	Trabec thick	69:31
5	36/40	17/40	Both L>R	29/40	V:0	Trabec thick	0:100
6	term	18/40	Right	30/40	III:V	Trabec thick	86:14

Table 40: Comparison of pre- and post-natal findings in six infants with progressive renal deterioration in utero. *Changes of high grade reflux were seen at operation only, as the MCU was technically inadequate (see text). This infant attended for nuclear medicine studies only after he had UTIs. International grading system for vesicoureteral reflux. Differential function on DMSA, DTPA or MAG 3 scan expressed as a ratio of left vs right kidney.
Trabec = trabeculated.

STUDY 3

Infant Pig Model of Vesicoureteric Reflux

Introduction

This study was conducted as a means of establishing and reviewing the technique of division of the ureteric tunnel in the post-natal pigs. The aim was to develop the technique for use in the unroofing of the tunnels of the fetal pigs.

The model used was initially established by Hodson *et al.*[307,308] and later used in Ransley's work on sterile, high pressure VUR [1,309,310]: particular attention was paid to the contention that the ureteric tunnel needs to be ablated, not just incised. The pig was chosen as the model because of the multirenulate multipapillate kidney.

Materials and Methods

Five infant female Kangaroo Island Small minipigs were anaesthetised with intravenous thiopentone, oxygen, halothane and nitrous oxide. The first two animals had the roof of the left ureteric tunnel excised, via a lower midline incision and an extraperitoneal approach to the bladder. The antireflux mechanism for the pig consists of a canopy of two abutting layers of urothelium which extends from the entrance of the ureter into the bladder wall, toward the bladder neck, rather than the human configuration of the ureter itself extending to the internal orifice; this canopy was the structure removed in these first two animals. The bladder was closed with a vicryl suture and the animal returned to the pen: Cotrimoxazole was added to the feeds in a dose of 8mg/kg/day. The remaining three animals also had the intramural ureteric orifice slit open, including the canopy of the intravesical extension of the ureter; this additional step was carried

out because the first two animals had not developed VUR. The procedure was otherwise similar; all animals had a methylene blue cystogram, via a suprapubic needle, with the animal under general anaesthetic, one month later. The bladder, ureters and kidneys were inspected after the animal was sacrificed with a lethal dose of barbiturate. Each kidney was examined histologically using Haemotoxylin and Eosin, and the urine was sent for culture at the time of sacrifice.

Results

All urine cultures were normal, the kidneys were histologically normal and reflux of methylene blue was seen to occur into all kidneys for which the ureteric tunnel had been obliterated.

STUDY 4

Ureteric Tunnel Incision in the Fetal Pig: a Model for Non-obstructive Pre-natal Vesicoureteric Reflux

Introduction

It has often been stated that reflux alone does not cause renal damage [1,308-312], however, it was recently shown in the fetal sheep model that obstruction of the urachus, together with unroofing the ureteric tunnel, induces more renal damage than does urachal obstruction alone [249] - work carried out subsequent to the model shown in this chapter. Earlier work had already demonstrated that sheep bladder outlet obstruction induces renal dysplasia in association with VUR [288,313,314]; ureteric obstruction in the sheep also causes renal loss [287]. However, reflux plus obstruction is already known to cause renal damage in humans, in the absence of infection. Interestingly, the sheep urachal obstruction model may represent the clinical circumstance in male infants who are born with high grade VUR. By creating VUR in the fetus it seemed possible to ascertain whether fetal renal abnormality is secondary to VUR or part of the primary embryological defect. Furthermore, it seemed appropriate to use the pig as the model, to further understand the changes in the multipapillary kidney.

Materials and Methods

After being withdrawn from food for 12 hours, four midgestation sows were sedated with xylazine (2mg/10kg) and ketamine hydrochloride (10mg/10kg). A 20 G Optiva cannula was placed in an ear-vein and anaesthesia was induced with thiopentone 2% (2 ml/10kg) intravenously. The sow was transported to surgery and a 12mm endotracheal tube was inserted using a stylet and a foal laryngoscope. Anaesthesia was

maintained with 3% Halothane and 2 l/min Oxygen. Throughout surgery 0.9% Sodium Chloride was intravenously administered as well as the antibiotics Carprofen and Amoxicillin. The sow was then placed in lateral recumbency and prepared for surgery.

An approximately 20cm long skin incision was performed in the right lateral flank and the abdomen opened using a muscle splitting technique, followed by the incision of the peritoneum. After the uterine horns were palpated for evaluation of the total number of fetuses, a section of the uterus containing one of the fetuses was exteriorised and placed on moist towels.

The uterus was incised along the antimesenteric border over the caudal end of the fetus, and special care was taken to avoid any major bleeding. The amniotic membranes were carefully opened to avoid loss of amniotic fluid. Approximately 50ml of amniotic fluid were aspirated into syringes and stored in the sow's abdominal cavity while surgery was performed on the fetus.

The rump of the fetus was exteriorised and the gender identified. Female fetuses were marked with a suture and immediately returned to the uterus. Male fetuses were placed in a supine position and a ventral midline, low abdominal incision performed. The urinary bladder was opened and the left ureteric tunnel was identified and slit opened with micro-scissors, in the manner determined to be appropriate from the earlier VUR Study 3. In one fetus (No 3-1) both ureteric tunnels were incised. The fetal bladder was closed with 6/0 polyglycolic acid, as was the fetal abdominal wall with 5/0 polyglycolic acid. Each fetus was marked with a suture and then returned to the uterine horn. After the amniotic fluid was replaced, the amniotic membranes and the uterus were closed with 4/0 polyglycolic acid.

In each sow three male fetuses underwent surgery. The uterus was replaced in the abdominal cavity and the abdominal muscle was closed with 3/0 polydiaxanone and the skin with staples.

The sow was closely observed throughout gestation and except for the fourth sow on which a Caesarean section was performed, all sows were allowed to deliver naturally.

Cystograms were performed on 11 male piglets, two of which were Piglet No. 3-1 (piglet one from sow three), which had the ureteric tunnels incised on both sides and Piglet No. 3-3, which had the left ureteric tunnel incised.

For the post-natal cystograms, the piglets were anaesthetised with Halothane and oxygen using a face mask. An Optiva cannula was inserted into the urinary bladder under ultrasonographic guidance. Ten ml Omnipaque contrast medium was injected into the bladder and radiographs were taken, after which the piglets were euthanized and the urinary tract removed. Gross examination of the kidneys was performed which were then studied histologically.

Results

From the first sow, who gave birth naturally, no marked fetuses were found. The second sow developed torsion of the uterus during delivery, and was euthanized. A post mortem was performed during which two marked, fully developed male fetuses were found dead and one marked male fetus was seen to be mummified. The third sow aborted one week before the end of gestation and all three male operated fetuses were found mummified. The fourth sow had a Caesarean section near term and all three operated fetuses were found to be mummified.

A cystogram was performed on the two developed marked fetuses (Nos. 3-1 and 3-3) from the second sow. Piglet No. 3-1, in which a bilateral incision of the ureteric tunnels had been performed, showed bilateral grade III reflux with dilatation of the pelvicalyceal system (Fig. 47). In Piglet No.3-3, in which only the left ureteric tunnel was slit opened, grade II reflux was present on the left.

Cystograms were also performed on nine control piglets, none of which showed VUR. The gross examination of Piglet No. 3-1 revealed hydronephrosis in both kidneys, with dilatation of the pelvicalyceal system bilaterally (Fig. 48). In Piglet No. 3-3 hydronephrosis with dilatation of the pelvicalyceal system was evident in the left kidney, the right kidney was normal (Fig. 49). Histologically, the kidneys were all normal. Neither cystograms nor pathological examination revealed hydronephrosis or dilatation of the renal pelvis in any piglet without VUR.

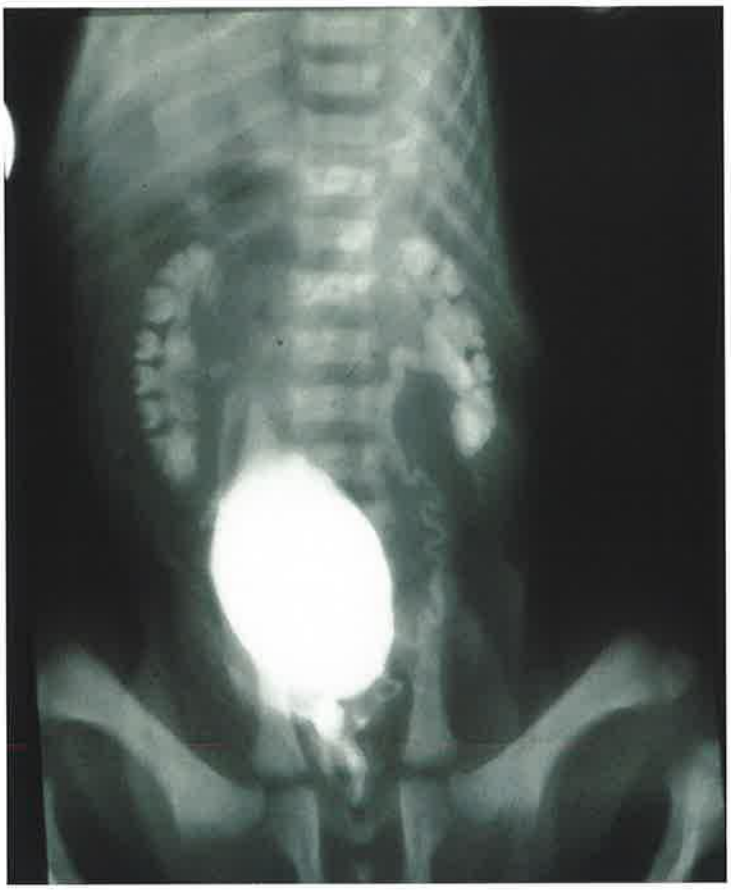


Figure 47: Cystogram in Piglet No. 3-1 with bilateral VUR and dilatation of the pelvicalyceal system after bilateral incision of the ureteric tunnel.

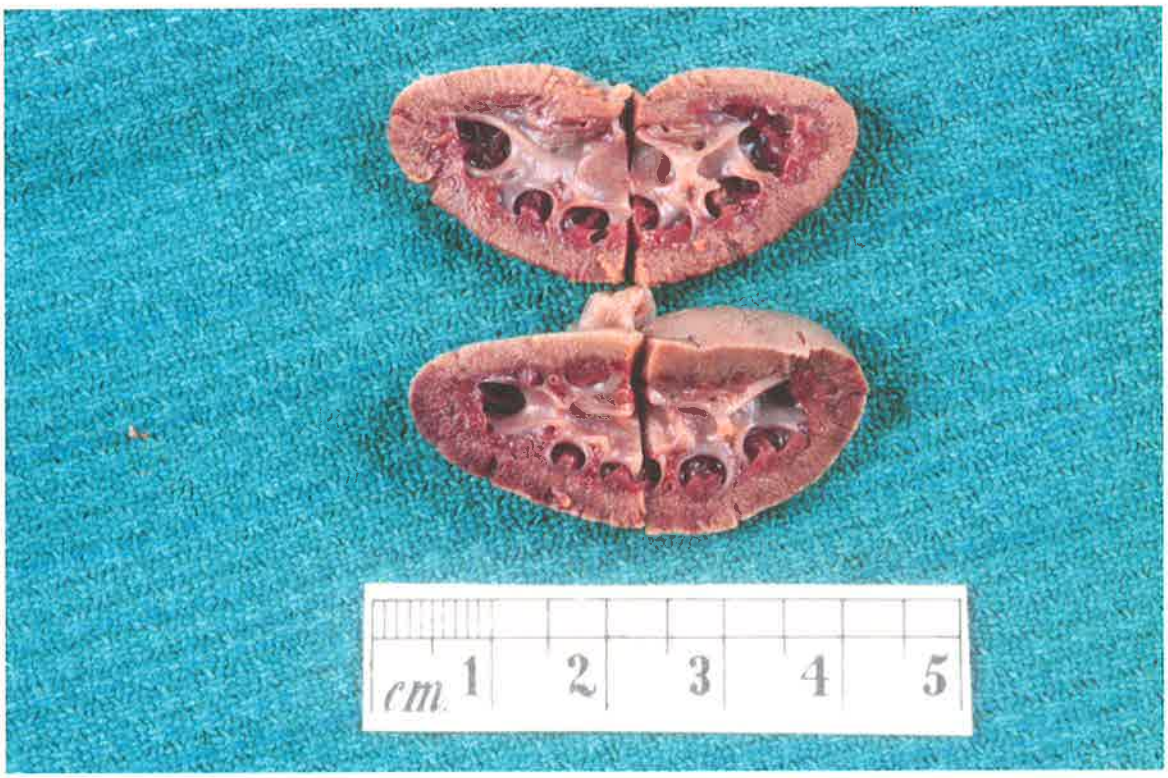


Figure 48: Comparative macroscopic view of the renal pelvis of the right and left kidney in Piglet No. 3-1, in which both kidneys were hydronephrotic, as suggested by the cystogram findings in the previous figure.

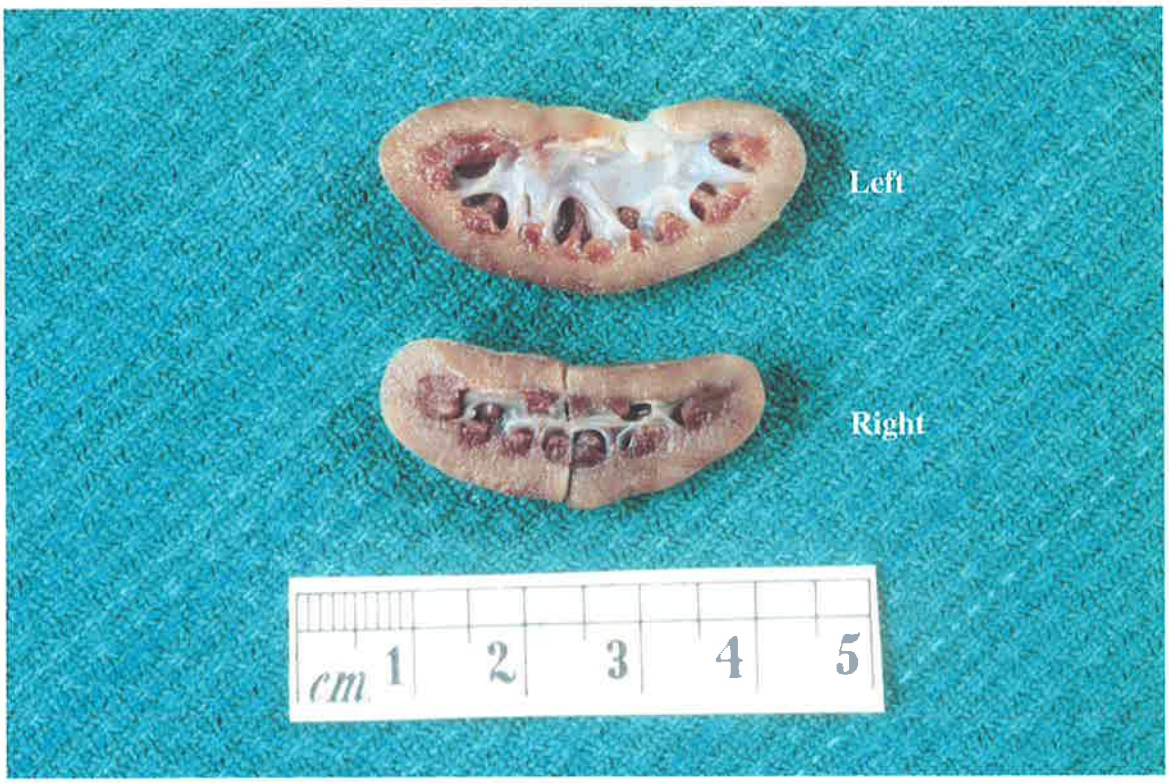


Figure 49: Comparative macroscopic view of the renal pelvis of the right and left kidney in Piglet No. 3-3. Hydronephrosis of the left kidney is seen after the left ureteric tunnel was obliterated *in utero*.

Discussion

Vesicoureteric reflux has been the subject of a many studies and a vast amount of literature, much of which make little contribution to our eventual understanding of the condition, its associations and effects, largely because the studies have been based on unsound assumptions. The best example of such an assumption is the "big bang" theory, which suggests that renal injury often occurs at the first infection; in fact, many kidneys, especially in male infants, are abnormal before birth [2,144,146,243,245,246,248,272,273,275-279,315-317]. Much is yet to be learned and it would appear, from the following review of the literature, that many of the lessons along the way have not been carried forward. In discussing the results of studies presented in this section, the author will present previously reported work on the anatomy of VUR, the history of the surgical management and radiological investigation of VUR and then discuss the complex issue of the interrelationship between VUR and abnormalities of the renal parenchyma.

Anatomy and Function of the Vesicoureteric Junction

In 1952, Hutch documented the history of study of the lower ureter, noting that Bell in 1812 [318] and Ellis in 1856 [319] described the muscle fibres that originate from the ureter and pass across and downward, under the mucosa, forming the lateral boundaries of the trigone. Bell states that *"The orifices of the ureters are not closed by the contraction of the muscular fibres around them. They are defended against the return of urine by the obliquity of their passage through the coats of the bladder ... as the bladder contracts the obliquity must be diminished ... these muscles, guard the orifice of the ureters by preserving the obliquity of the passage, and by pulling down the extremities of the ureters according to the degree of the contraction of the bladder generally"* [318]. Also, Soppig, in 1874, was credited with describing the ureteric

muscle as dovetailing with the bladder muscle for reinforcement of both the ureter and the bladder, and in 1903 Sampson further developed the idea of the oblique passage of the ureter through the bladder wall [320].

Stephens and Lenaghan *(1962)* studied the intravesical ureter, concluding that it is divided into an intramural and a submucosal segment, and that there are three kinds of anomaly to account for reflux-inducing impairment of the activated valve-flap mechanism of antireflux; congenital absence of the submucosal segment of the ureter, wedge segment defects of the ureteric muscle, or a combination. Spontaneous correction of the VUR was thought to be due to strengthening of the meagre amount of muscle with time [321]. Along a similar line, Tanagho and Pugh have shown that the ureteric muscle does not end at the ureteric orifice, without interruption it continues down as the superficial trigone to end at the verumontanum [322]. In addition, Waldeyer's sheath, which surrounds the distal end of the lower ureter, continues into the bladder as the deep trigone. Tanagho, Hutch and others provided a further study to support their contention, and experimental confirmation of the observations of Bell, Ellis, Soppig and Sampson; they showed that the competency of the ureterovesical junction is maintained by the integrity and the efficiency of the trigone muscle. Tanagho's team conducted a series of experiments in dogs, which involved either excision, incision, incision and suture or suture ligation of the trigonal muscle distal to the ureteric orifice. They showed that the pressure in the ureter rises as the bladder fills, due to increased trigonal tone and contraction. They also confirmed that there is clinical and histological evidence that the trigonal muscles and the distal ureteric muscle are poorly developed in cases of primary VUR [323,324]. The latter was from a study of 72 distal ureters in which there was a deficiency of the musculature, which correlated with the endoscopic appearance of the orifice and the degree of VUR [323]. In separate studies, Lerner *et al.* and Hanna have supported this view clinically [242,325].

Johnston, in his Hunterian Lecture, in 1962, provided evidence for the pathological nature of VUR from the study of 243 volunteers in four recorded series (Gibson 1949, Iannaccone and Pazironi 1955, Jones and Headstream 1958, Leadbetter *et al.* 1960), and by citing Young's experiment in 1897, in which post-mortem bladders were distended to bursting point without creating VUR [326]; the view that VUR does not generally occur from the normal human bladder was again supported by Pranther, in 1944 [327]. The usual lack of reflux has also been identified by Lich *et al. (1964)* in over 3000 MCUs, with VUR only seen in association with current or previous urinary infection; their paper included 26 normal newborn infants, none of whom had VUR [328]. Likewise, Peters *et al. (1967)*, found no VUR in 66 premature infants having a cystogram via a suprapubic puncture [329].

Ambrose and Nicolson *(1962)* suggested that there are five factors involved at the vesicoureteric junction to prevent VUR: (a) The length of the ureteric tunnel; (b) diameter of the intramural ureter; (c) flexibility of the intramural ureter; (d) anchoring of the end of the ureter; and (e) intravesical pressure. They considered lateral ectopia of the orifice to be the most common cause of VUR, which they felt could result from ectopia of the ureteral bud or from poor development of the fibres which attach the distal ureter to the trigone. They supported their argument by highlighting that VUR is unilateral in 44% and that hydronephrosis and hydroureter often resolve after ureteric reimplantation [330,331]. However, there have been a number of schools of thought about the mechanisms by which the lower ureter and bladder prevent VUR. Hutch's theory of maturation *(1961)* is based on the premise that the intravesical ureter gains length with age, thereby providing competence [332] and Paquin believed that the diameter-to-length ratio of the intracystic ureter is the most significant factor in ureteral valvular competence [333]. Whereas Lyon *et al. (1969)*, in a follow-up of the ureteric orifice of 330 girls, described the A, B, C position of the orifice (medial to lateral) and the volcano, stadium, horseshoe and golf-hole figuration of the ureteric orifice. They found that all golf-hole orifices refluxed, none of which had reflux

resolution in one year, and the horseshoe orifice was more likely to have a poor prognosis if it were more laterally placed; concluding that the position and configuration of the orifice was a better predictor of VUR resolution than the intravesical length of ureter [334], a view later supported by others [335,336] and suggested earlier by Jewitt, in 1955 [337]. Brock and Kaplan also correlated the appearance with the likelihood of spontaneous VUR resolution, but also noted low grade VUR to be associated with small ureters on IVP, and a normal ureteric orifice on endoscopy; conversely high grade VUR ureters are dilated on an IVP and are likely to have a golf-hole orifice and not to resolve spontaneously [335].

However, despite the range of views, it is generally accepted that a congenitally short submucosal tunnel will prevent the largely passive ureterovesical valve mechanism from closing the intravesical ureter when the intravesical pressure increases [320,321,326,331,332,337,338]. Witherington *(1963)* provided evidence for this view in a study of VUR in dogs in which a tunnel of at least 8mm long produced a VUR cure rate was 82.4% which was only 69% if the tunnel was only 5-6mm [339]. Pranther, in 1944, presented a table of the relative length of the rabbit, cat, dog, pig and human ureteric tunnel and the incidence of VUR for each group, indicating that the shorter the tunnel the higher the incidence of VUR (rabbit - 80%, cat - 65%, dog - 30%, pig - 2%) [327]. A detailed study by Carr *et al.*, of 177 Landrace pigs, showed the increase in the tunnel length with age, from a mean length of 5mm at birth and 36mm at maturity, and an increase in orifice width from 0.5mm at birth to 3.9mm in the adult pig [340].

Hutch *(1961)* further developed the tunnel length theme in a study of 50 dissected *human* bladders, and found the intravesical ureteric length to be 0.5cm at birth and 1.3cm in the adult. This work was effectively a more detailed version of Gruber's earlier work *(1929)* [341,342]. Hutch reiterated Pranther's emphasis that animals with a poorly developed trigone have a high incidence of VUR [332,341] and added the

contrary finding that the intravesical pressure in the newborn is roughly the same as the adult, intimating that tunnel length and intravesical pressure are not the only factors in VUR development [332]; this view is supported by the infrequent occurrence of VUR on an MCU in newborns [328] and premature babies [329], despite their short ureteric tunnel.

Other anomalies adjacent to vesicoureteric junction have assisted in the understanding of the anatomy of the region; Hutch *(1952)* noted a paraureteric out-pouching was seen in 60 of 94 refluxing ureters, from 300 cystograms [320]. Detailed dissections of diverticula were also conducted by Stephens [343] and Johnston [326], who concluded that saccular formation is due to a deficiency of the *muscle of Bell* and *Waldeyer's fascia*, further supporting the concept that these structures are important in the prevention of the development of VUR.

Radiological Investigation of Vesicoureteric Reflux

The first diagnostic study of VUR appears to have been conducted by placing blue dye in the bladder; the subsequent efflux from the ureter was observed endoscopically, by Sampson, in 1903 [344]. Thereafter, cystograms were used by Beer in 1933 [345] and delayed cystography was introduced by Charles M Stewart, in 1948. Hinman and Benjamin went on to develop serial cystography and cinefluorography in 1954 and 1955, as stated by St Martin *et al.*, who, in 1956, studied 60 children radiologically over 10 months and found VUR in 50% of patients having a voiding cystogram: St Martin *et al.* concluded that *"investigation of the Pediatric urological problem is incomplete without cystography"* [346]. The role of VUR in the aetiology of acute and chronic pyelonephritis in young children eventually became established (Williams *et al.* 1961 [347]; Hinman and Hutch, 1962; Johnston, 1962 [326]); and Hutch *et al. (1963)*, identified the association of VUR with hydroureter and hydronephrosis.

The first cystograms were delayed single-film studies, then St Martin *et al.* used a two film cystogram [346], followed by the work of Gross and Sanderson *(1961)* and Hutch *et al. (1963)* who used cineradiography and found demonstrable reflux in 47 and 48% of comparable groups of children. Similar experience was reported from England, by Hodson and Edwards *(1960)* [348] and Rosenhaim *(1963)*; these advances are summarised by Tanagho *et al.* in 1966, who stated that the MCU, IVP and cystoscopy are all tools for the diagnosis of VUR, with the combination of studies giving information on the anatomy of the disease, its severity and likelihood of resolution [349].

Various classifications of VUR have since been suggested, the most significant of which have been the three grade system used in the Birmingham study [146], and the International Reflux Study Group (IRSG) five grade system [132,133]. The latter was broadened, in 1985, to include a comment on the degree of ureteric dilatation within each of the groups (a, b and c subgroups); with the grade being determined by the most severe VUR, which usually coincides with peak voiding [350]. A better grading system would produce a score for each of: (a) the US views of the lower ureter; (b) the width of the lower ureter during various phases of voiding; (c) the impression of the length of the tunnel on the oblique view of the bladder base at each level of bladder filling; (d) recording whether the VUR occurred during bladder filling or voiding; (e) the association with bladder instability; (f) the age; (g) the size of the individual; (h) the pre-natal hydronephrosis degree; (i) the endoscopic appearance of the orifice, and (j) the ureteric tunnel length endoscopically. Such a system would be difficult, but would potentially allow for a better understanding of VUR and the collection of better quality scientific data for analysis of outcome. Obviously, the addition of information on the state of the upper tracts would also be a necessary part of the prognostication algorithm. Thus, the DMSA scan should be discussed.

The DMSA scan was first introduced in 1974 by Lin *et al.* [351] and is well established as the most sensitive investigation for renal parenchymal changes in VUR, in comparison to both the IVP and the US. Rushton *et al.* compared DMSA to histology for the kidneys of 22 piglets, nine at one week and 13 at two weeks after creating reflux and infecting the urine; they found the scan was 87% sensitive, 100% specific and 94% predictive of individual acute pyelonephritis scars confirmed histologically [352]. Similar results have been obtained from pig studies by Risdon *et al.* [353] and Arnold *et al.* [354]. The clinical studies supporting the accuracy of the DMSA for scars include those by Webb and Britton [355], and Elison *et al.*; the latter group conducted a prospective study of 208 patients, which showed more anomalies seen on DMSA than IVP, with good correlation of the DMSA findings with the degree of VUR [356]: Monsour *et al.* assessed 150 children with IVU, US and DMSA, finding 1.92±1.92 scars on IVU, 2.79±1.87 scars on US and 3.82±1.71 scars on the DMSA; they suggested an eight part grading system for the renal scarring based on the DMSA appearance [357]. Although the DMSA scan is obviously accurate in assessing discrete cortical defects, Mclorie warned that diffuse parenchymal injury may not be obvious when the kidney is small without focal scarring [358].

Recently, the DMSA scan, when performed within 72 hours onset of an acute UTI, has been found to record pyelonephritic changes in children without VUR [359,360]. Also, subsequent resolution of the initial changes is common in those with VUR. These data suggest that not all pyelonephritis is associated with VUR and that transient changes can occur with VUR associated pyelonephritis [152,359-362]. Smellie *et al.* was one of the first groups to make the latter point in a study of 54 patients in 1988 [363]: Goldraich and Goldraich *(1995)* added tubular dysfunction, hypertension, the use of Captopril for renovascular hypertension, and duplex kidneys as other potential pitfalls, adding that an abnormal DMSA scan during a febrile UTI, while it may not indicate permanent scarring, may allow the identification of children at risk of developing renal scars [362].

Nuclear medicine studies, in addition to identifying renal parenchymal anomalies, have taken on a role in the detection of VUR, and was first used by Winter, in 1959 [364]. It would appear that, while nuclear cystography does not give anatomic information, it is the more sensitive of the VUR investigations, as shown in a study by Dikshit *et al. (1993)*, who conducted an MCU and a DIC on 48 patients. Twenty-two had VUR on a DIC, 20 on MCU; three had high grade VUR on DIC but not on MCU; those in whom VUR was missed on DIC, had only low grade VUR [336]. Gelfand *et al.*, supported this view with paired MCU and DIC studies in 68 children. They also found an initial negative study was followed by the demonstration of grade I to III VUR in 20% of cases, independent of the type of test performed, indicating the superiority of the DIC in identifying VUR, but the variability of cystography results [365].

Merrick *et al.*, in a study of 3646 children with at least one UTI, studied an alternative radionuclide reflux-detection investigation and found it was both a more sensitive and more specific test than the MCU; the IRC they used is performed by asking the patient to void the radionuclide, once it has filled the bladder via renal clearance of an intravenous injection. Kidneys with VUR on an IRC were 13.5 times more likely to deteriorate than those in which no reflux was observed, recognising that grade I VUR may be missed, and higher grade VUR will not be detected if the kidneys are not cleared of the radionuclide, either because of obstruction or poor function. In contrast, kidneys with VUR on MCU were only four times as likely to suffer progressive renal damage [152]. However, it is generally accepted that the IRC is only applicable to children over the age of four and a half years of age, usually as a follow-up study. This topic is discussed earlier in relation to the follow-up patients treated by Teflon injection.

In a study of 100 children with a UTI, 55 had an MCU, 17 of which had VUR, 14 of whom had a normal US; this represents the common clinical view and, therefore most would now recommend both an MCU and an US for children younger than five years

of age who present with a UTI and a DMSA scan is added if VUR is found [366]. The DIC and IRC are generally used to document VUR persistence or resolution, and US is used to follow renal growth.

History of the Surgical Treatment of Vesicoureteric Reflux

The earliest attempt at ureteric reimplantation was reportedly by Nussbaum in 1876 [345]. Then, in 1903, Sampson conducted a number of dog experiments on ureteric reimplantation and urinary infection: he also described the case of a woman who had renal pain with a full bladder in whom he reimplanted the left ureter via an extraperitoneal approach on 20 July, 1903 and the right on 8 September, 1903 - using local anaesthesia [344]. Up to 1932, the ectopic ureter, paraureteric diverticula and 'congenital' were reasons given to proceed to ureteric reimplantation [345], and in his paper in 1933, Beer stated, *"Considerable doubt exists as to whether it is worthwhile attempting to conserve a kidney by reimplanting its ureter in the bladder, provided the other kidney was functioning normally"*. Beer also described the use of indigo to assess the function of the kidney and the advent of the IVU to better study these kidneys; his 40 cases were not operated on for VUR but for ureteric injury or cancer. Six of his patients had an MCU post-operatively and none had VUR: he used an operative procedure which simply placed the ureter directly into the bladder. During discussion at the meeting, Doctor V. Vermooten reported a series of experiments in dogs in which he reimplanted the ureter in a slit in the muscle, over which he closed the mucosa [345].

A large number of techniques have subsequently been suggested, including drawing the ureter through the original ureteral channel and fastening it to both the inside and outside of the bladder (Patton -1939) [367] or, alternatively, bringing the ureter directly through the bladder wall, splitting and securing the ureter by sutures placed through the bladder with knots on the outside (Toulson -1939) [368]. Stevens and

Marshall *(1943)* modified the approach of the latter two authors, producing a report of 10 patients in whom they performed a ureteric reimplant. They used a technique which involved reinsertion of the ureter into both a separate muscle and mucosal orifice, adding a submucosal tunnel [368]: alternatively, division of the transvesical bar with an hour-glass deformity of the bladder was thought to be curative in Pranther's case, in 1944 [327].

Before 1950, many of the operations for reimplanting the ureter were for disease other than VUR. Once VUR could be treated surgically and was recognised to be associated with renal injury, interest in the surgical management expanded; thus, it is not surprising that Hutch's contribution is frequently recorded as the first effort to surgically reimplant the ureter into the bladder for VUR [320,369].

The Hutch-I procedure was first used in May 1950; the operation consists of *intravesical* mobilisation of the ureter, leaving the attachment to the mucosa intact and the ureter ultimately came to lie over the mucosa. The initial presentation was of nine paraplegic patients operated on over one year, following which six were cured of their VUR [320]. The Hutch-II incorporated the Jewitt principle and, thus, closed the mucosa over the transplanted ureter [369]. Jewitt, in 1955, suggested that a wide open ureteric orifice needs an alternative approach and therefore he developed a second technique for wide ureters. One of his innovations involved dividing the ureter outside the bladder and bringing the ureter through a tunnel to lie near its initial orifice; the distal ureter was sutured over the top of the bladder mucosa. The second Jewitt technique was intended for non-tortuous ureters and was similar to the Hutch-I technique; ie, intravesical mobilisation, with the orifice being left intact; it differs from the Hutch procedure in that the bladder muscle was closed under the ureter, but the mucosa was closed *over* the distal ureter [337]. Fuqua, in 1958, presented a small series of cases in which he used a minor modification of the Hutch operation, with only 80% success [370].

Several new concepts then appeared; Grey *et al.* in 1957, discussed the results of a series of dog experiments in which the ureter was made to be dilated by tying it around a fine cannula; they then conducted three different types of ureteric reimplant: (a) a hook was used to intussuscept the ureter after which it was reimplanted into the dome of the bladder; (b) the ureter was mobilised inside the bladder, a catheter was placed into the ureter and transfixed, facilitating intussusception of the distal ureter *in situ;* (c) an intussuscepted valve was created part way up the ureter. The latter operation was used in two cases without success: Grey's techniques have not been otherwise been reported [371].

The first widely used and still popular ureteric reimplantation technique was introduced by Politano and Leadbetter, who reported a series of patients in 1958. The procedure involves dissecting the ureter from the bladder, and then passing the ureter through a more cranial part of the bladder, through a submucosal tunnel and then suturing it to the original mucosal insertion site. A total of 21 ureters were presented in 1958; 14 patients had their VUR resolved, but one ureter was obstructed [372]. Hendren *(1968)* performed the Politano-Leadbetter operation on 465 ureters, stopping reflux in 99%, with a 3% obstruction rate [373]; Deweerd *et al. (1969)* had upper tract deterioration in 24 and VUR in six of 144 renal units of 121 patients [374]: Price *et al. (1970)* had no patient with VUR after 191 Politano-Leadbetter reimplants. However, six had worsened hydronephrosis after the operation [375]; Warren *et al. (1972)* had a 96% success rate [376], and Bellinger and Duckett *(1984)* had a high rate of VUR resolution, but seven of 207 cases with obstruction [377]. This technique has maintained a degree of popularity, particularly for transplant kidneys, during bladder augmentation and for Mitrofanoff, intermittent catheterisation conduits. Unfortunately, the Politano-Leadbetter operation does appear to have the potential problem of kinking at the entry into the bladder muscle when the bladder is full.

Glenn and Anderson *(1967)* provided a method of elongating the tunnel without inserting the ureter into the bladder dome; they advocated a tunneled advancement toward the bladder neck, which they trialed on dogs and used in eight patients [378]. This advancement concept is occasionally a valuable adjunct to ureter reimplantation of most techniques of surgical intervention.

Paquin *(1959)* used a method which involved an incision on the posterior aspect of the bladder, after which a tunnel is developed, once the ureter has been mobilised outside the bladder. His technique also involved the formation of a nipple on the end of the ureter, by taking a wedge from the distal ureter [333]. Woodard and Keats reported the only subsequent series; they used the procedure in 217 ureters with a 96% success rate with only one case of transient obstruction [379]. Many Paediatric Urologists have used the nipple formation as an option, although only rarely, because of the added risk of obstruction.

Bischoff and Busch *(1961)* provided the first paper which involved tailoring of the ureter; the operation involved extravesical dismemberment with resection of the dilated ureter: the VUR was treated with a U-shaped incision over the ureter, forming an extension of the ureter, *in situ,* forming the distal ureter from the floor of the bladder. The procedure is similar to the Duplay tube procedure for hypospadias repair [380,381] and was termed an "ostioplasty"; if both sides were to be treated for VUR they were dealt with at different operative sessions [382]. Witherington *(1963)*, studied the Bischoff procedure in the laboratory and found that an adequate tunnel length (>8mm) was required to achieve success [339]. He added the alternative procedure of raising a flap of mucosa from cranial to the ureteric orifice to provide half the distal neo-ureter; an operation similar in concept to the Mathieu hypospadias repair [383].

Johnston *(1962)* is one of the few to report the use of the Hutch-I operation, with poor results (five of 16 cured); he suggested that Bischoff's technique may be better for the

large, thick walled ureter [326]. Hutch *et al. (1968)* were obviously disillusioned with the Hutch innovation as, in reporting on 245 ureterovesicoplasties on 140 patients between 1954 and 1966, they used six different procedures with variable results, including combinations incorporating the Leadbetter-Politano procedure [369]. Likewise, in London, Williams and Eckstein *(1965)* indicated their discontent with early operative techniques. They operated on 276 cases using a number of operations, including one described by Turner-Warwick, in 1961. Their success rates were as follows: Turner-Warwick - 86%; Bischoff - 23%, Hutch - 83%, Paquin - 80% and Politano-Leadbetter 96% [384]. It is useful to consider that antireflux surgery was developed with the belief that 95% of patients have an associated congenital bladder neck obstruction [375,385]. Thus, surgery was almost exclusively bilateral, as the problem seemed to be of a bilateral nature.

The continuing development of alternative techniques is of no surprise: the 'gold standard' came with the transtrigonal ureteric reimplant, first used in 1966. Cohen subsequently published his technique, and the results of 189 reimplants, in 1975. He recorded VUR resolution in almost 99%, with complications in only six patients (3.2%) [147]. Other early series supported the subsequent enthusiastic adoption of the Cohen technique [386], which has largely superseded the Politano-Leadbetter procedure, with a reduced risk of kinking [373], less post-operative ileus, less perivesical drainage and more rapid recovery [387]. Both the Glassberg *et al.* [388], and the Scholtmeijer and Griffiths [389] groups did not have post-operative obstruction, as was the case for the patients treated in Adelaide.

One argument against the use of the Cohen, transtrigonal approach has been the difficulty of retrograde pyelography post-operatively; Lamesch provided a solution in his article in 1981. He devised the technique of inserting a trocar into the bladder percutaneously and, under cystoscopic control, inserting a ureteric catheter via the trocar. The technique was applied successfully to five of Lamesch's cases [390].

Modifications of the Cohen procedure have included the pull-through technique, in which the ureter is divided outside the bladder, then reimplanted through the same orifice; the bladder is opened in the midline first; Ahmed *(1980)* reported the technique, with favourable results for 69 ureters, in 1983 [391]: the added step of dissection outside the bladder has limited its popularity. Canning *et al.*, in 1993, described the cephalotrigonal reimplant for bladder exstrophy [392] and Kondo & Otani *(1987)* suggested the ureters be crossed, in the submucosal tunnel, to enhance the ureteric compression effect of the tunnel [148]. For wide ureters, the Starr procedure of ureteric imbrication [387,393], the Hendren plication [373] and the Glassberg *et al.* procedure of incision of the bladder muscle superiolateral to the orifice during the Cohen procedure, have been suggested [388].

The latest of the open, intravesical techniques to be developed is that reported by Gil-Vernet [394]. A transverse incision across the cranial aspect of the trigone is closed longitudinally, including both muscle and mucosa; this pulls the ureter medially across the trigone, thus increasing its intravesical length. The method was published in 1984, with a report of two failures in a total of 20 children and 18 adults [394]. Subsequently, little has been published on the technique, apart from attempts in the USA and Japan to perform the procedure percutaneously. Okamura *et al.(1995)* used the Gil-Vernet technique with a minimally invasive approach, combining urethroscopic visualisation with percutaneous manipulation via three ballooned trocars. Their study involved 12 patients who each had a long hospital stay (12.3±4.7 days) after protracted operations (178±52 hours); all had their reflux resolved [395]. Cartwright *et al.* used the Okamura *et al.* technique on 22 children in 1996; the success rate for VUR resolution was a disappointing 62.5%, with three major complications of vesicovaginal fistula, hyponatraemia and perivesical fluid collection [396]: such results are obviously unacceptable.

Many have felt that the intravesical surgery is best avoided. Thus, in 1961, Lich *et al.* developed a procedure described as follows; *"through the open bladder a size 4FG ureteral catheter is introduced cystoscopically into the ureter as a guide. The ureter is then gently separated, outside the bladder, from its contiguous bladder muscle (using the tips of a mosquito forceps as a dissector) until the mucosa attachment is reached. The bladder muscle is then incised in the normal direction of the ureteric bed for a distance of three to four inches and this muscle, at its submucosal junction, is undermined to create a muscle trough that can be easily approximated over the ureter which is placed in the submucosal bed* [397]." A further similar report came from Gregoir and Van Regemorter *(1964)*, who reported good results for 31 of 35 patients, with no need to open the bladder or use indwelling catheters, and with a short operation [398]. The Lich-Gregoir procedure, as it has come to be named, is in effect a Bischoff procedure from outside the bladder and is the most popular technique for the implantation of the renal transplant ureter. Hendren *(1968)* used the Lich-Gregoir procedure in 43 ureters and had success in only 18 [373]. Nevertheless, the procedure is now the "flavour of the month" as it is able to be performed virtually on a day-case basis and is potentially amenable to the laparoscope [399].

Outcome of the Surgical Management of Vesicoureteric Reflux

The ability of reimplantation surgery to resolve VUR is documented both in the literature and in the series of patients managed by open operation in Adelaide. Obviously, resolving VUR is the primary aim of reimplantation surgery, but the outcome should also be measured by any impact on UTI incidence, GFR, urine biochemistry, renal growth, renal scarring, renal pain, hydronephrosis, hydroureter, somatic growth, incontinence and general well being. The difference in outcome between operative and non-operative management will be discussed later, firstly some

of the claims about the outcome of surgical intervention will be discussed, beginning with the development of contralateral VUR.

The development of VUR into the contralateral kidney has been considered an adverse outcome of reimplantation surgery with a variable incidence of up to 16% [376,377,400-402], leading some authors to advocate bilateral reimplantation for unilateral VUR [375], while others have selectively reimplanted according to the endoscopic appearance of the ureteric orifice, a previous history of VUR on the contralateral side or the presence of contralateral scarring [376,377]. Distortion of the trigone by surgery is often suggested as the cause of contralateral VUR, however, as the contralateral rate is no less with the use of the STING [403], other factors, such as the pressure release via the more severe side pre-operatively and variation from one reflux study to the next, may prevent the detection of VUR on the contralateral side. Warren *et al. (1972)* added a report which highlighted the variable outcome for the contralateral ureter [376]. Also, others have recorded the lack of correlation between the original grade of the VUR and prospect of developing contralateral disease [403]. The approach used in the patients presented in this manuscript, including those managed endoscopically and by the Cohen procedure, was to treat the contralateral ureter with low VUR, if scarring was present or the orifice was abnormal. Using this approach the post-operative contralateral VUR incidence was negligible.

Ureteric obstruction is a more sinister complication of ureteric reimplantation: this complication did not occur in the Adelaide patients. For this group of 203 patients the VUR resolution rate was 99%, however, kidneys associated with low grade reflux, for which growth failure was the major indicator for surgery, showed a poorer overall growth rate than the normal patient population, both before and after the operation, indicating that it was the length change-over-time expected for these children, rather than due to the VUR (Fig. 43). This conclusion is also supported by the renal growth velocity being little changed by the procedure (Table 38). These findings are

noteworthy as a significant proportion of the operations were for grade I or II VUR (51 of 203).

Two significant confounding factors may be responsible for this surprising result. Firstly, kidney size was often larger before the operation due to pyelonephritic swelling. This swelling can be responsible for increasing kidney size as drastically as 150%, and usually subsides soon after surgery. Hence any real renal growth that occurs in the post-operative period in these kidneys would be totally encompassed within the shadow of subsiding swelling. Secondly, scarring most often occurs at the site of intrarenal reflux (IRR) at the poles of kidneys. Thus, vertical growth is inhibited and growth in the coronal plane preferentially occurs. This pattern of growth could not be detected by interpretation of renal length, the commonly used indicator of renal growth.

Converse to the low grade VUR kidneys, a post-operative increase in growth of grade V disease was demonstrated. This encourages surgical management of slow growing kidneys with a high grade of reflux. The reverse is also true; no benefit in terms of renal growth was observed for those kidneys with low grade of reflux. It is interesting that the kidneys with either grade I or grade II reflux were slow growing before the operation and showed no change afterwards. Hence, in the absence of additional indications, surgery may not be indicated for the slow growing, low severity of reflux kidneys.

The literature reports mixed results on the issue of renal growth after ureteric reimplantation. Babcock *et al.* in 1976, found no change in renal growth when compared with the contralateral non-refluxing kidney [404], Decter *et al.* compared of kidney length and bipolar thickness and showed that the bipolar parenchymal thickness was significantly less in the eight with persistent VUR [316], and Smellie *et al* found poor growth in those with persistent VUR and scarred kidneys [405]. Shimada *et al.*

(1988) measured renal length pre- and post-implant in 68 kidneys that were either within the normal range or below. Growth velocities of these kidneys were as expected of normal kidneys, although some kidneys with no scarring did show accelerated growth [406]. Willscher *et al. (1976)* also demonstrated accelerated growth post-reimplant, in kidneys that had no history of pyelonephritis, as assessed by IVP [400]. McRae, Belloli *et al.*, Sutton and Atwell, and Ginalsik *et al.* reported accelerated growth after successful surgery, particularly for high grade VUR and when the surgery was performed at a young age [407-410]. However, these increases in growth velocity could merely be a reflection of accelerated somatic growth seen after successful antireflux surgery. Nevertheless, Merrel and Mowad *(1979)*, recorded growth curves of 35 consecutive paediatric patients with an average age of 5.7 years, finding the average pre-operative length centile was 27% which increased to 49% post-operatively for those with resolved VUR [411].

In future study of renal growth after anti-reflux surgery, some effort should be made to take into account the two confounding factors mentioned previously. Firstly, those kidneys with pyelonephritis and swelling at the time of operation should be excluded. Secondly, renal volume should be measured if it is at all feasible. Unfortunately, renal volume is not measured routinely, and as long as this is so, will always be a problem of retrospective methodology. There are, however, other indications for surgery which should be included in any further research, namely pyelonephritis, UTIs and renal scarring.

Further data which favour surgical treatment of reflux include:

(a) documented improvement in renal function [408,412,413];

(b) improved urinary concentrating ability [309,414,415];

(c) decrease in the incidence of pyelonephritis [369,408];

(d) resolution of hydronephrosis and hydroureter [330];

(e) anecdotal, improved well-being of surgically treated children.

In summary, ureteric reimplantation has been demonstrated to be technically successful. Also it can be concluded, tentatively, that there is an increase in renal growth rate in grade V refluxing kidneys after this surgery, and surgical intervention in the management of low grade refluxing kidneys is probably of no benefit. It is well known that kidneys in patients with VUR are often significantly smaller than normal and exhibit renal parenchymal scars with associated decline in renal function [334]. Thus it could be expected that removal of reflux would lead to an improvement in these parameters.

Laboratory Studies of Vesicoureteric Reflux

Laboratory studies of VUR have focused on the development of a post-natal model and the influence of VUR, infection and obstruction on the histological, radiological and biochemical changes to the kidneys. The model of fetal VUR described on page 183, is the first attempt to investigate non-obstructive VUR *in utero.*

The pig has been the most favoured animal for the investigation of the effects of VUR on the upper renal tract, because the anatomy of the pig kidney is more analogous to the human than any other common species of animal, including primates. Also, the pig has a multirenulate, multipapillate kidney with true calyces [1,416], has similar renal physiology to the human [416] and does not have VUR as a normal phenomenon [340,341]. For ease of handling, the dog [311,417-419] and the monkey [420-422] are also frequently used for the study of VUR, but not rodents, as they have a high rate of VUR without renal disease.

King and Idriss *(1967)* reported a study of 20 female dogs in which VUR had been created; eight animals were followed for 17 to 20 months without UTI and no abnormality was noted in the kidneys. Three were followed for 14 to 20 months after the development of UTIs and marked changes in the parenchyma were noted, leading the authors to conclude that VUR was only harmful to kidneys after the development of infection [418]. The same group went on to study VUR in puppies, finding no macro or micro difference between the refluxing and non-refluxing kidneys and no difference in the split GFR. However, only five animals were followed, of which three had contralateral VUR (King and Sellards 1971) [419]. Likewise, Lenaghan *et al. (1972)* found no histological difference in the renal parenchyma with sterile VUR, although they did record dilatation of the refluxing ureter [311], and again confirmed that renal injury occurs with the addition of infection. They also added the observation that distal obstruction alone did not produce VUR [312].

In the monkey, Roberts *et al. (1982)* found similar results to the above, ie that obstruction plus *sterile* VUR had no affect on renal function, even in the presence of IRR [421]; whereas ureteral inoculation of bacteria induced pyelonephritis, delayed excretion of radionuclide and induced areas of decreased uptake in the kidney [420].

Hodson *(1975)* was the first to put forward the idea of IRR from a pig model [308], and Ransley and Risdon identified that the compound papillae were those in which IRR was more likely to occur, and are in the areas of the kidney that were more likely to develop scarring [1]. Whitaker and Cuckow have since invented a method of recording the IRR: kidneys are retrogradely injected with 50% solution of Batson's resin in methyl methacrylate which is mixed with a promoter, catalyst and colouring and poured into a 50 ml luer-lock syringe which is attached to the pelvic cannula. Twenty, 40 and 60 mm of water pressure is used for the injection and the tissue is then dissolved in potassium hydroxide [423]: casts of the IRR were thereby produced.

There is considerable controversy whether scarring occurs following IRR of urine [424] or whether urinary infection is an essential prerequisite [1]. Ransley's group have undertaken a number of other studies; in 1978, the "big bang" theory, ie it is the first infection which causes the damage, was suggested by the observation that most cases of RAN have scars already established when the child is first seen. To test this theory, four groups of animals, were followed for 95 -161 days by Ransley *et al.*; the antireflux mechanism was obliterated by ablating the roof of the ureteric tunnel in males which were used because of the long urethra and the low risk of spontaneous infection. High pressure bladders were produced with the Hodson ring and results were compared with animals with a low pressure bladder. Scarring occurred in all kidneys subject to VUR plus infection, whether they had low or high pressure VUR, but if there was no infection there was no scarring [1]. Another study was undertaken to see how quickly parenchyma damage and scarring might occur in the presence of reflux. Unilateral VUR was established in 43 suckling piglets between two to four weeks and urine infection established. Changes were never seen on the non-operated side and more rapid deterioration of the parenchyma occurred in the presence of obstruction [425].

In contrast to their earlier work, Ransley *et al.* did show changes in the kidneys with high pressure, non-infected reflux in subsequent studies, particularly when they looked at more sensitive indicators of renal injury. Their 1984 pig-model study showed that, in the presence of sterile VUR and raised peak voiding pressures, significant renal parenchymal lesions develop when bladder decompensation occurred. In these circumstances acute tubulointerstitial damage ensued after only a few days and progressed quickly to scar formation with interstitial collagen formation and tubular destruction. Extravasation of Tamm Horsefall Protein into the interstitium, indicative of tubular rupture, was noted in many but not all of these lesions [310]. Further work, published in 1987, reported a one-kidney model in the growing minipig over a period

of approximately five months, in which a significant association was found between VUR and reduced urinary concentrating ability [309].

Similar to the results from the dog work, some pig researchers have shown renal parenchymal changes and others have not: Heptinstall and Hodson, excluded animals with infection and concluded that sterile focal scarring in their pig model is in the same distribution as the scarring from IRR of infected pig urine. In some animals there is an acute lesion which occurs as the result of IRR, which lasts as a focal swelling for 10-15 days, and then undergoes increasing contraction until few parenchymal elements remain. In other animals in the early stages, a mechanism akin to obstructive atrophy appears to be at work in the areas subjected to IRR; interference with the blood supply is the probable pathogenetic mechanism [307]. This view was partly supported by Orr *et al. (1971)* who found that renal blood flow, in the dog, falls during a rise in bladder pressure; 11 to 18.5% greater on the VUR side than on the control side [417]. Also, Greenfield *et al.* who found reduction in blood flow in the VUR kidney was specifically to the juxta-medullary and inner cortical regions [426]. From the fetal pig model it would appear that changes can occur in the fetal kidney with VUR; the degree of change to the biochemical parameters and it effect on predisposition to infection need to be further studied.

Interesting additional information from pig-model studies has been provided by a study of infection in the contralateral, non-refluxing kidney by Arnold *et al.* In an experimental study of female piglets with surgically created unilateral VUR, E.Coli were inoculated into the bladder and, at later sacrifice, bacterial culture was undertaken of the renal parenchyma from the refluxing and non-refluxing kidneys. Positive cultures of the same E.Coli were obtained from 33% of refluxing kidneys with pyelonephritic scars, 23% of refluxing kidneys without scars and 21% of non-refluxing kidneys. These findings suggest that VUR plays a role in the pathogenesis of renal scarring over and above a means whereby pathogens gain access from the lower

urinary tract to the renal substance. They added the interesting postulate that renal injury is necessary to include the injury by the bacteria [427]; do the kidneys have an embryological defect, a genetic predisposition to become infected, or both?

Sheep models of bladder outlet obstruction have shown renal dysplasia in association with the concurrent development of VUR [288,313,314], and ureteric obstruction in the sheep also causes renal loss [287]. It has often been stated that reflux alone does not cause renal damage, however, it was recently shown in the fetal sheep model that obstruction of the urachus, together with unroofing the ureteric tunnel, induces more renal damage than does urachal obstruction alone [249]. This may be the animal model equivalent to the six Adelaide boys born with high grade VUR.

Previous attempts to induce hydronephrosis by obstructing the urethra in the fetal pig were unsuccessful [288]. It is possible that the ureterovesical junction is competent in these obstructed pigs, but not in humans with bladder outflow obstruction, because the ratio of length of the pig intravesical ureter to its width is higher than the ratio seen in humans [340]. In Section III, VUR was induced by slit-opening the ureteric tunnel, thus avoiding the additional effect of obstruction. Histologically, there were signs of renal dysplasia in the refluxing kidneys but, as the porcine kidney is considered to be fully developed at 55 days gestation, it seems likely dysplasia will only occur if the VUR is induced at an earlier stage than in the current study. This is upheld in the fetal sheep with urethral or ureteric obstruction; if the obstruction is induced at an early stage of gestation, dysplasia occurs; when obstruction is induced after midgestation, hydronephrosis is produced [288,314].

Although the attempt to develop a fetal pig model for investigations into VUR was successful, there was a high fetal loss. That the sows tolerated surgery well, recovered soon after and the number of unoperated mummified fetuses was very small compared with the high loss of operated fetuses, shows clearly the extreme sensitivity of this

species of fetuses to exposure and the necessity to improve the technique. As two of the operated fetuses were normally developed and histology of the kidneys revealed the number of nephrons as usually found in newborn piglets, it seems likely that reasons other than the opening of the ureteric tunnel led to fetal death.

During prolonged surgery, we observed that the temperature of the fetuses and the uterus was observed to decrease. As hypothermia was previously identified as a major complication resulting in fetal cardiac depression and distress in porcine fetal surgery [428], it seems necessary to take greater care with temperature control during this procedure and to reduce the number of operated fetuses per sow, to reduce the length of the procedure. With the establishment of the fetal pig model it will be possible to further study the nature of VUR. It may also provide a uniform group of animals, born with VUR, which will allow comparison of therapeutic modalities.

Clinical Data on Vesicoureteric Reflux

Childhood UTIs affect 3-5% of girls and 1-2% of boys, with VUR being found in 30-50% of cases [317,429-431]. Polarising statements are often made in the debate of the relative worth of the medical management versus the surgical treatment of VUR. For instance, Dwoskin, in 1984, wrote, *"When an operation is over 90% successful and takes no more than 90 minutes, its use may be the conservative form of management"* [432]. Fortunately others have tried to be more constructive, as was an American Urological Association committee *(1997)* which reviewed the subject, and provided a set of guide-lines, concluding, from a meta-analysis of VUR studies, that there was no consensus for grade I/II management for all ages and either sex, but most should not have surgery. For grade III and IV, the recommendation was for surgery if VUR persists beyond five years, or if bilateral. Whereas, for the management of grade V

disease, they suggested surgery after one year, if bilateral, or after five years if unilateral [433].

However, the role of surgery should be to reduce the secondary affects of VUR, which are infection, renal scarring, hypertension, renal pain, general ill-health and the complications during pregnancy, without added risk; the ideal would be to establish criteria by which those likely to have complications of VUR can be identified and operated on before adverse events occur. Importantly, the success of surgical management must be balanced against the prospect of resolution of the VUR spontaneously and the relative risk of the surgery. A number of animal and human studies have suggested that VUR is a benign disorder that does not cause any renal damage, as long as UTIs are prevented [243,244,434-436]. However, determining how best to manage patients with VUR has been made more difficult since the identification of fetal hydronephrosis, which has given us a wealth of new information to add to the formula used for evidence based medical practice for VUR.

In 1975, Booth *et al.* documented fetal VUR during an attempt at fetal blood transfusion [437] and Marra *et al.* later identified that mild dilatation of the pelvis might be an expression of potentially severe VUR [243]. A number of studies have since identified the incidence of VUR subsequent to a prenatal diagnosis of hydronephrosis [247,274]: a review of the literature finds that 10-15% of prenatal dilatation is due to VUR and 30-50% have reduced renal function in the affected kidney, at birth [144,244,246,247,252,253,272-275,321,434,438]. Thomas *et al.* support this view and added that calyceal dilatation may be a more reliable marker of pathology than distension of the renal pelvis alone [439]. Therefore, with the improvement of pre-natal diagnosis of renal anomalies, it is now known that pre-natal hydronephrosis and VUR in the neonatal period is most often seen in males [2,144,146,243,245,246,248,272,273,275-279,315-317] who, on average, present with more severe reflux than girls [2,245,248,273,275,276,279,440,441]. I would

therefore hypothesised that different types for primary VUR exist; minor disease in older girls with UTIs and bladder instability, severe VUR in neonatal boys and an intermediate group of boys and girls [2].

Overall, girls are affected by VUR more than boys [146,331,375,436,441-444] which may relate to bladder neck and urethral dysfunction in girls. Therefore, urethral dilatation and anticholinergic medication are often advocated as part of the management of these children [317,375]. Of 3505 patients investigated by Shopfner, who presented with a UTI, twice as many girls as boys were investigated; 29% of boys with a UTI had VUR and 14% of girls had VUR [430]. Secondary reflux due to a neuropathic bladder, lower urinary tract obstruction [146], and voiding dysfunction [445] are well known associations, and should be treated in addition to the treatment of VUR, either medically or surgically. However, the role of the bladder has probably been under-represented in the past; it was recently recorded to be important in a study of 310 children from the International Reflux Study Group; after excluding neurogenic bladder and overt dysfunctional voiding, 18% were found to have urgency, staccato voiding, fractionated and incomplete voiding and voiding postponement. Also, there was a strong correlation between recurrences of UTIs and bladder abnormalities, as well as a negative correlation with the disappearance of VUR [446]. Sillen *et al.* suggested that incoordination between the detrusor and the sphincter may explain both the VUR and the increased bladder wall thickness seen in infants presenting with pyelonephritis; their view was supported by recordings of voiding pressures above 100cm H_20 and associated uninhibited bladder contractions [447].

Sommer and Stephens expanded the understanding of renal changes associated with VUR when they studied kidney and ureter morphogenesis, in 32 autopsy and 11 surgically excised renal tracts from 32 infants [448]. Thirty-one kidneys had a ureteric orifice of the lateral ureteric ectopia group, as categorised by Mackie and Stephens [159] and Lyon *et al.* [334] classifications, either cystoscopically or by measurement of

the bladder specimen at autopsy. The more lateral the ureteral orifice position the more blunted the calyces, the greater the degree of hydronephrosis and more dysplasia was seen. They concluded that kidneys with VUR exhibit preformed grades of hydronephropathy, which are determined embryologically [448], a view which they supported by demonstrating the interrelationship between lateral displacement of the ureteral orifice in the boys with urethral obstruction and the degree of associated renal dysplasia [449]; they also studied renal dysplasia of the associated renal segment of the ectopic ureter from duplex kidneys and reached a similar conclusion [159].

The embryopathic perspective of reflux nephropathy is also supported by histological studies by others: Hinchcliffe *et al.* reviewed 86 nephrectomy specimens [450], and Cussen and MacMahon collected 46 VUR kidneys, all from newborns [435], which showed a strong association of hypoplasia and VUR, and minimal scarring. On-the-other-hand, Risdon, Yeung and Ransley found segmental scarring to be similar in males and female, but dysplasia to be confined to male patients: dysplasia was evident by the recognition of primitive ducts, lined by columnar epithelium and surrounded by mantles of mesenchyme: other features included foci of metaplastic cartilage, and primitive glomeruli and tubules, as well as occasional fibrous walled cysts. They supported the possibility of distal obstruction playing a part in the aetiology of VUR in males [451]. John Duckett's group also believed the lateral ectopia of the VUR orifice in the urethral obstruction male was a secondary phenomenon, as is the renal parenchymal abnormality. They coined the term "VUR and dysplasia" or "VURD syndrome" [452,453]. However, Ambrose had earlier collected 37 nephrectomy specimens and found dysplasia in only six patients, none associated with primary VUR. He also found pyelonephritis in 51 of the VUR specimens and concluded - *"Our review indicates a high correlation among abnormal, incompetent ureteral orifices, reflux and pyelonephritic scarring, and indicates that pyelonephritis is the major cause of renal atrophy with reflux"* [454]. Berstein and Arant examined 25 complete and partial nephrectomy specimens from VUR cases and concluded that the identification of acute

disruptive ductal lesions in several of the specimens indicates that the abnormality had continued to evolve, despite initial severe renal damage and atrophy. This evidence suggested that a number of "little bangs" culminate in a gross scar which continues the process of renal atrophy. They believed that the small, sublobar scars result from continuing intrarenal reflux with ducal disruption and microvascular damage [455]. The marked difference between the views of the authors may be explained by recent studies taking specimens from neonates and not older children, as were examined in the earlier reports. Importantly, there also appears to be difficulty in the interpretation of the histology, with poor interrater concordance [455]. However, the nephrectomy specimen from the prenatal deterioration group presented in section III showed diffuse lymphocytic infiltration with germinal centre and metaplastic cartilage formation, plus the finding of inflammation and fibrosis of the ureter; despite no clinical evidence of UTI prior to the nephrectomy, supporting the prospect that refluxing urine may induce an inflammatory response.

Pelvicalyceal changes seen in the fetal pig study and the finding of progressive changes in the Adelaide boys who had VUR as the cause of their prenatal hydronephrosis suggests that the VUR itself may have a secondary effect *in utero*. It would seem, however, that progressive renal deterioration associated purely with vesicoureteric reflux in the antenatal period has not been previously documented in humans. The renal pelvis is usually protected from high pressures, not only by the valve mechanism of the vesicoureteric junction but also by the peristaltic activity of the ureters. When the ureter is grossly dilated and the peristalsis is no longer efficient, the combination of a high pressure bladder with free vesicoureteric reflux may subject the renal parenchyma to high pressures and effectively "obstruct" the kidney. It is interesting that changes observed in the single resected kidney from a boy in section III, included findings consistent with obstruction [272,456] and that all of the radiologically abnormal kidneys were associated with antenatally detected ipsilateral ureteric dilatation, five of the six bladders were abnormally distended, and four were also thick-

walled and trabeculated; changes usually associated with urethral obstruction or abnormal bladder innervation. Transient urethral obstruction early in the pregnancy has been proposed as an explanation for the high incidence of vesicoureteric reflux in male infants and this would also explain the bladder changes seen in this group of infants as well as those in other series [248,315,440,447]. Ineffective bladder emptying in the setting of gross reflux may lead to a type of "high output bladder failure" characterised by distension and muscle hypertrophy analogous to that seen in the left ventricle with mitral valve regurgitation. If sterile high-grade reflux *per se* causes renal damage *in utero* then it would presumably continue to do so in infancy and childhood.

Many reports suggest there is little difference in outcome between conservative versus operative management of VUR [146,457,458] and others have shown infection and obstruction to be more significant factors in renal scar production in children [71,309,421]. The issues that are generally raised are the rate of VUR resolution, the incidence of breakthrough infections, the risk of scarring and hypertension, and the development of complications of pregnancy; each of these aspects will be dealt with in turn.

Reflux resolution is variably recorded [248,275,321], but poorly related to the age of the patient, the age at diagnosis and even less well related to the bladder function and anatomy of the lower ureter. A better grading system, which was mentioned earlier, would produce a score for each of: (a) the US views of the lower ureter; (b) the width of the lower ureter during various phases of voiding; (c) the impression of the length of the tunnel on the oblique view of the bladder base at each level of bladder filling; (d) recording whether the VUR occurred during bladder filling or voiding; (e) the association with bladder instability; (f) the age; (g) the size of the individual; (h) the upper tract findings; (i) the pre-natal hydronephrosis degree; (j) the endoscopic appearance of the orifice, and (k) the ureteric tunnel length endoscopically. Some

studies have looked at reflux resolution related to some of these parameters and documented spontaneous resolution of high grade reflux in infants [248,275,321], however, Elder found that only 20% of neonates with grade IV or V reflux had spontaneous reflux resolution by age two years [247]. Similarly, McLorie *et al.* [459] and Scholtmeijer + Griffiths [389] concluded that high grade VUR only resolves in a minority of patients over a protracted period; the latter groups' figures are noteworthy: of those in the observation group with grade IV VUR 83% still refluxed at two years and 70% had VUR at five years [389]. Also, only 33% of grade IV patients ceased refluxing spontaneously in the group followed by Bellinger and Duckett for three years, and only 2/13 with an abnormal orifice ceased VUR when treated non-surgically, while 32 of 64 (50%) with a moderately abnormal orifice ceased refluxing during the average three year follow-up [377]. The Birmingham reflux study group recorded that half the patients treated medically still had VUR for five years, and VUR was more likely to persist if bilateral [146]. Skoog *et al.*, in a study of 545 children with 844 refluxing units found that VUR was more likely to resolve in the first 12 months and grade II was significantly more likely to resolve than grade III [457]. The parameters which predict VUR persistence need to be better defined.

Breakthrough infections occur both before and after the resolution of VUR. Cooper and Atwell's group of patients had an infection rate of 37.6% in 96 girls and Matrinell *et al.* studied infection in 87 females, followed for a median of 23 years, and found the UTI rate in females is highest during the first year of life, with a gradual decrease to the lowest rate at 11-15 years of age, increasing in the late teens and extending through the early twenties at 0.4 per year. The proportion of infections having a pyelonephritic character decrease with age and number of infective episodes, but not in females with severe renal scarring. The first infection was the most likely to be associated with pyelonephritis, and pyelonephritis was most likely to occur in the first year of life [460]. Matrinell *et al.* looked at the presence of VUR, whereas the IRSG reports compared surgical management with non-operative care and found no difference in the

infection rate. However, they did find significantly more episodes of *pyelonephritis* in the medically treated group, particularly in males. Recurrent infections were related to age, sex and treatment centre, and were common in boys and girls entering the study under one year of age but were less common in girls, and even rarer in boys entering after one year of age: UTIs developed during the first five year follow-up period in 59 patients in the medical group (38%) and 59 of the surgical group (39%) but the incidence of pyelonephritis was higher in the medical group (21%) than in the surgical groups (10%) ($p<0.01$) [442,461-464]. In the study by Elder, 25% of the boys developed a UTI while on antimicrobial prophylaxis [247], while Gonzales *et al.* recorded the generally held view that boys usually have a lower incidence of infection, with and without VUR [441].

Goldraich and Goldraich, in a study of 202 South American children, appropriately separated the groups and found breakthrough infections were more common in girls [400,465], usually limited to cystitis [400], although the severity of symptoms is not always a good indicator of renal injury [466]. The other major study to compare the medical and surgical management of VUR, the Birmingham Reflux Study Group had a 28% breakthrough infections rate in the Birmingham study [146] and Dunn and Smith reported a rate of 34% [143]. So that, while Stephens and Lenaghan believed *"in the absence of infection it has seemed that no serious deterioration of function occurs"* [321], breakthrough infections are a frequent event and, when VUR is still present, infection of the kidney is more likely than after surgical cure.

Renal scarring: Several studies have reported the interrelationship between VUR and RAN [146,308,321,348,389,406,424,429,442-444,450,463,467-475], which cause the renal disease of 6.5% of those requiring renal transplants in Australia [470]. Hodson and Edwards were among the first to suggest the association, in 1960; they presented a review of the evolution of the relationship of VUR to chronic pyelonephritis and stated: *"Our first experience of unexpected reflux occurred while carrying out*

cystography on Case 4, a youth with small kidneys, uraemia and skeletal changes of rickets and hyperparathyroidism. It was considered that lower urinary tract obstruction might be present, and after filling his bladder he was asked to micturate while under fluoroscopic observation. Normal emptying occurred but urine was also noted to flood back to both ureters as far as the renal pelves and calyces causing considerable distension of the upper urinary tracts" [348]; indicating that upper tract changes are seen in association with VUR, but the secondary affects, and whether scarring can be reduced by intervention, were still to be studied.

Polito *et al.* suggested that scarring is an unfavourable outcome of VUR, from a study where they compared 156 children with VUR and a normal GFR with 156 age and sex matched controls. They noted that those with bilateral VUR and scars had significantly lower height standard deviation scores (-0.5±1.4 vs 0.05±1) and a weight for height index which was below the controls (100.6%±16% compared to 108%±12%). In contrast to controls, and the other VUR groups, those with bilateral VUR and scars had a decreased relative height and normal weight for height index, suggesting that renal scarring has a significant adverse systemic effect [476].

However, the accepted principle, now, is that there is no difference in the renal scar outcome for the surgical and non-operatively treated groups, based particularly on the IRSG figures, therefore implying that surgical intervention is of no advantage over non-surgical treatment. To review this perspective, let us look at the literature and conclude with discussion of IRSG papers.

Merrick *et al.*, in a study of 3646 children with at least one UTI, claimed that VUR was not documented in almost half of the children who suffered progressive renal damage and was, therefore, not a risk factor for progressive renal damage in boys over one year and in girls at an age [152]. Nevertheless, Skoog *et al.* showed the increased risk of abnormal renal parenchymal with increased grade of VUR (I - 5%; II - 6%; III -

17%; IV - 25%; V - 50%) [457], as did Jakobsson *et al.* [443]; and new scars do occur during the non-surgical care of VUR patients, leading Rolleston *et al.* to report incidental and progressive renal injury in a study of 91 males and 84 females: they only observed new scar formation with gross VUR [424], and more scarring in the presence of IRR. Intrarenal reflux was not observed in patients over five years old, and "atrophic pyelonephritis" was seen in 13 of the 20 kidneys showing IRR and in 12 of the 13 the damage corresponded to the area of IRR [472], confirming the importance of IRR in the genesis of renal damage. Later studies by Bailey and Rolleston on 350 children with UTI, indicated that the critical periods for renal damage are infancy and early childhood, and that the damage was confined to those kidneys with severe VUR [429]; features also identified by Jean Smellie [431,477] and others [308,450]. In contrast, Shimada *et al.* found the mean age of new scar formation to seven years and 10 months [406] and other have found that new scars can develop in previously normal kidneys [475,478]. However, some adults have VUR without scarring [479]. The follow-up work of Bailey *et al.* on the long-term outcome for 31 infants with gross VUR, concurred that almost half had RAN at the time of presentation, and that infants with gross VUR who presented within the first year of life appear to do well if free of severe bilateral RAN [469].

Others have also reported the development of new scars during non-operative management of VUR [389,406,473,478], including Dunn *et al.* who reported that 96 of 134 cases had "failed" conservative treatment and 13 children developed new scars during non-operative treatment [444]. Also, in a study of acute pyelonephritis, Jakobsson *et al.* found that 56% of VUR affected kidneys ultimately show permanent scarring whereas only 19% of kidneys without VUR [443]. Smellie *et al.*, in a study of 74 children, highlighted that 74 kidneys were initially normal and 13 previously scarred, diagnosis and effective treatment were delayed in 45, and 58 suffered a further urine infection between the baseline IVP and the first showing new scarring; the group advocated early diagnosis, prompt effective treatment, investigation and long-term

supervision of children with urinary infection [475]. With regard to renal scarring after surgery, Cooper and Atwell found no new scars in their patients [467] and although many studies have shown the development of scars after surgical treatment [146,424,436,468,471,474], it may be that many of these defects are due to the evolution of renal change in areas of parenchyma injury prior to the surgery.

The IRSG [442,468] has followed 532 children (401 in Europe and 131 in USA) [442,468]. It is important to note that the study included only grade III and IV randomised patients, and used the IVP to document the renal scarring. Also grade V VUR was excluded from comparison of surgical and non-surgical treatment. Of the European and American children 48% and 54% had renal scarring at entry and 17% and 14% had parenchymal thinning respectively. There were interesting differences between the Europe and America population profiles: in Europe 24% were boys and 77% bilateral; in America 11% were boys and 57% bilateral [474]. In assessing the results, it is important to recognise that 4.6% of the surgical cases in the European limb were obstructed by the surgery [468] - whereas none of the Adelaide cases had the same complication. This means that of the 20 cases of new scarring after the surgery, six had obstruction as a complication of the surgery - a complication which increases the risk of renal injury significantly, and only 93% had a successful outcome, compare to 98% in the Adelaide series. In the IRSG kidneys, more new scars developed in the children with parenchymal thinning at entry than in those with scarred or normal kidneys at entry [436], and younger patients had a higher frequency of new scar formation (less than two years - 19.8%; two to four years - 9.8%; >four years, 4.6%) [436].

In the Birmingham study [146], VUR was classified in only three grades, and they did not use the "gold standard" for diagnosis of renal scars, i.e. the DMSA nuclide scan, all of which must throw at least some confusion into the interpretation of their results.

The follow-up for the non-operative cases in both the IRSG and Birmingham studies needs to be beyond the resolution of their VUR, to enable appropriate comparison of the treatment modalities. This comparison would be best achieved by the use of the fetal pig VUR model, with a surgical and non-operative treatment cohort.

Hypertension develops in a proportion of VUR patients, with reflux nephropathy recognised as the commonest cause of hypertension in childhood [480]. In the series reported by Cooper and Atwell, 23.1% of those with bilateral and 4.2% with unilateral scars developed high blood pressure [467]. Two studies which have looked at the long-term follow-up in adults with reflux nephropathy, 18.5% had hypertension with bilateral scarring and 11.3% when the nephropathy was unilateral (>140/90 mmHg; mean age 19 years) [481,482]. The alternative perspective is provided by Goldraich *et al.* who identified VUR in 31% of children with mild hypertension, 30% of those with severe and persistent hypertension and 15% of children with renal failure [483]. Hypotension is therefore, important to consider in VUR patients.

The effect of persistent VUR on pregnancy is often debated, but poorly studied, particularly the significance of grade I VUR. However, Cooper and Atwell collected data on 96 girls to determined the late outcome, including their post mature height and weight, hypertension, progressive and new scars, UTI's and pregnancy outcomes. The majority of pregnancies were uneventful, but UTI occurred in 36.4% - higher than normal - and three of 20 offspring had VUR [467]. El-Khatib *et al.*, from Kincaid-Smith's group, reviewed the outcome of 345 pregnancies in 137 women with reflux nephropathy. Complications occurred in 39% of pregnancies, with a fetal loss rate of 18% if the Creatinine was greater than 0.11mmol/L and only 8% if the creatinine was lower than 0.11 mmol/L at the start of the pregnancy. Maternal complications were increased by the presence of bilateral scarring over unilateral, but there was no statistical difference in the risk of infection, except for a trend to an increased incidence of pyelonephritis in those with VUR [484]. Overall, it seems appropriate to cure VUR

if it appears that the woman will enter her child-bearing years with VUR, even grade II disease, particularly given the results of Matrinell *et al.* who found the infection rate for women with VUR was 0.4 episodes per year, and the proportion of infections having a pyelonephritic character does not decrease with time in the presence of severe renal scarring [460].

Biochemical Assessment of Renal Parenchymal Injury

Proteinuria has long been used as an indicator of renal injury, however it is now evident that the presence of albumin in the urine is a late sign of renal injury, thus other new predictors of parenchymal dysfunction have been increasingly studied in recent years [485]. In 1994, Tomlinson *et al.* developed the normal ranges of $ß_2$-microglobulin, retinol-binding protein, α_1-microglobulin and urine protein-1 measured by using ELISA in children, rather than immunodiffusion [486]. The group subsequently reported urinary retinol binding protein and N-acetyl-ß-D-glucosaminidase (NAG) levels from 143 children with renal scarring, finding significantly elevated levels in 51% of those with bilateral scarring and 7% with unilateral scarring, more-so with high grade VUR and severe scarring; elevated urinary protein levels were only found in 10 patients, leading the authors to conclude that tubular dysfunction in children with renal scarring precedes any glomerular protein leak [487]. Carr *et al.* quoted results as International Units (IU) of NAG/mg of Creatinine in the urine from a study of 31 children without VUR and 32 with various grades of VUR. They found the control group had levels of 10.63 IU/mg, whereas the VUR group had levels of 16.47 IU/mg of Creatinine in the urine: of particular note were the two patients with intrarenal reflux and NAG levels of 52 and 48 IU/mg of Creatinine [488]. Other authors have found similar value in the use of these indicators of tubular injury in VUR and other renal disease [489,490]. Additional markers have been

studied, but have not been found to correlate with the degree of renal parenchymal abnormality [491].

As well as being used to assess renal injury in VUR, NAG can be used for the monitoring of renal transplant patients and for the early detection of renal disease. N-acetyl-ß-D-glucosaminidase is widely distributed in mammalian tissue, and is present in lysosomes isolated in human kidney, entering the urine in high levels after renal tubular injury. Tucker *et al. (1975)* developed an automated measurement system which they showed gives the same results as the routine method [492], thus making the test more widely applicable. Of the other biochemical substances suggested for the monitoring of early parenchymal injury, $ß_2$ microglobulin has shown a similar correlation to renal injury to that of NAG, but retinol binding protein may be a more practical tool because of its greater stability [493].

More extensive use of the biochemical indicators will allow for more detailed interpretation renal events with VUR and greater understanding of the parenchymal changes in the fetal VUR model.

Hereditary aspects

Multiple studies have shown a high incidence of VUR amongst relatives of index cases [494-499]. In a prospective study of 104 siblings of 78 patients, Jerkins and Noe found 34 had VUR (32%) of whom 25 had no UTIs or bladder dysfunction. If the patient had renal damage, the sibling was more likely to be similarly affected, nevertheless only half of the siblings of those with renal damage had renal injury [494]. The incidence of sibling VUR in a retrospective study of 242 families was found to be 2.2%, but in a prospective study of 24 families, eight of 50 (16%) had VUR [497] which indicated the difficulty of obtaining accurate data. Kenda and Fettich used the

isotope cystogram to find VUR in 47 of 105 asymptomatic siblings (aged four months to 6.3 years), 10 (23%) of whom had scars on a DMSA scan, and others have recorded high grade VUR in up to 50% of those siblings less than one year of age [498]. Noe found a similar incidence in 354 siblings; 75% of those affected were asymptomatic and there was no correlation with the index patient grade of VUR, sex or established renal damage. The incidence of renal damage was significantly less in the siblings than in the index patients and female siblings of female index patients have a significantly higher rate of VUR than the male siblings [499].

Burger suggested that the congenital anomaly of the ureteric tunnel length is the inherited factor [338], although a polygenic type of inheritance seems likely, with VUR being multiple diseases, findings which question the certainty of embryopathy in the aetiology of renal parenchymal anomalies in the index cases. An alternative view was incorporated with a presentation of reflux families; Frye *et al.* contend that a single gene inheritance is possible, with variable expression and occasional lack of penetrance, and no sex linkage [500]. Ultimately it will probably be shown that 'primary' reflux represents the clinical expression of multiple diseases.

Eccles *et al.* draws an association between VUR and a number of clinical scenarios, including duplex systems, PUJ obstruction, nocturnal enuresis, detrusor sphincter instability, hypospadias and undescended testes [495]. Also, they record an association of VUR with Hirschsprung's disease, prune belly syndrome, a syndrome of ectodermal dysplasia and cleft lip/palate, coloboma-ureteral-renal syndrome and Robinow dwarfism. In addition, they note that chromosomal abnormalities of the short arms of chromosomes 8 and 10 have been reported in two studies where VUR was associated with developmental disabilities, skeletal anomalies, hypostonia, rectal atresia, horseshoe kidney and malrotation of the intestine, and MCK, hearing loss, heart defects, growth retardation and psychomotor delay. Importantly they contend that the

problem of analysis is the spontaneous resolution and therefore recording of all infants at birth is required to assess the genetic status.

Several studies have produced weak evidence of a linkage with several histocompatibility antigens - these were HLA-B12 [501,502], HLA-B15, HLA-AW32 [503], HLA-A3, HLA-A9 and the haplotypes A9-B12 and A2-B8 [495]. Torres *et al.* added to the list of HLA linkages HLA-B8 in combination with either HLA-A9 or HLA-BW15 in the male patients, and HLA-B8 in combination with HLA-B12 in both sexes [501]; Eccles *et al.* draws the conclusion that PAX2 is a good candidate as a major gene for VUR [495], and Hon *et al.* suggest an association with 10p. The propositus identified in their study was monosomy for the distal region of 10p and severe psychomotor delay, growth failure, congenital heart defect, MCK and grade V VUR [504]. A sex difference in the inheritance was also suggested by a family of a father and three sons and one daughter affected by VUR and scarring [505]. Also, girls are more likely to be dysfunctional voiders and only 20% of siblings of index patients with VUR plus dysfunctional voiding demonstrate VUR, compared to 38% of siblings of refluxers without symptoms of bladder dysfunction [496]. Overall, the genetics, like much of the "truth" about VUR and RAN would appear to be far from resolved!

Conclusion to Part III Discussion

A greater understanding of the nature of VUR will hopefully be gained from this work, which will contribute to the debate of medical versus surgical management of grades III and IV reflux. It is generally accepted that grade V reflux should usually be treated surgically [457] and that grades I and II virtually never require operation. Grades III and IV reflux can be associated with the development of new scars, and pyelonephritis occurs more frequently if the children are treated medically. However, there is no

difference in the scar formation rate between the medical and surgical groups. By having a uniform group of animals that are born with VUR, the outcome of the different regimens can be assessed. The greatest difficulty in interpretation of the clinical data on VUR has been the lack of a uniform group of children to be compared (because of the great variation in the nature of the disease). Once the renal dysplasia model has been further tested in this study, the relevance of the treatment options will be explored.

OVERVIEW OF LABORATORY AND CLINICAL STUDIES

Part I of this manuscript has presented a review of prospectively collected data on the management of VUR from the centre where the initial development of the Teflon injection treatment took place. The studies included a large group with primary VUR and a smaller group of spina bifida children treated by Professor Guiney, children with duplex kidneys and a group of infants, all of whom were treated in Ireland. The results were satisfactory and compared well with the published figures, which are also presented.

Subsequently, data was prospectively collected on patients treated with endoscopically injected Teflon for VUR in South Australia (all patients were managed by the author); both long-term and short-term results were collated, with the outcome for the patients being equal to or better than most published series. An interesting aspect of the Adelaide study was the recording of all the endoscopic procedures on video and then correlation of the endoscopic appearance with the clinical outcome, thus providing data to improve the reliability of the procedure.

While working with the STING development team, a number of surgeons outside Ireland raised concerns about the use of the injectable plastic for the treatment of VUR in children. Thus the literature on the use of plastics in medicine was reviewed and studies commenced on the inflammatory and migratory outcome from the injection of particulate PTFE under the skin of the rat, into the venous system of the rat and into the brain of the sheep. Silicone was included as a comparative substance, because of the suggestion that the larger particles would solve the problems caused by PTFE-containing Polytef paste. The results would suggest that the injectable plastics are probably safe to use and, if anything, the larger particle plastics may be the less safe - the data is, however, not sufficient to make any definitive recommendations.

The work which followed was an attempt to put the use of the injectable plastics into perspective. The literature has shown that plastic migrates from infusion-pump tubing when used at high flow rate; the infusion pump study showed that this also occurs at rates used for the treatment of children over short periods of time, and also occurs with implanted silicone devices. The skin migration of Teflon was included to highlight potential adverse outcomes when these new technologies are used unadvisedly.

The next logical step was to develop a fetal model of VUR, with the prospect of having animals with which one could ultimately use endoscopic methods to treat the neonatal animal, to look for differences in the outcome for the kidney. It seemed important to review the outcome for the current surgical approach, thus, the third part of the manuscript presents assessment of the outcome for a group of patients having surgical management of VUR, showing that renal growth is improved for kidneys with high grade VUR. Also, fetal kidneys can be abnormal at birth, and VUR *in utero* alone does seem to have an affect on the appearance of the kidney, as suggested by the six boys reported here. To what extent urethral obstruction and VUR impact on the outcome for these fetuses is still conjecture, but the findings of the fetal VUR study in pigs are certainly significant and worthy of further study. Do progressive antenatal changes in the small group of patients reflect possible urethral obstruction, progressive changes secondary to a congenital anomaly of the kidneys, or does the bladder and the kidneys become abnormal because of the VUR? These are questions still to be answered.

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16. Dewan PA, Byard RW. Histological response to injected Polytef and Bioplastique in a rat model. *Br. J. Urol.*, 1994 73:370-376.

17. Dewan PA, O'Donnell B. Endoscopic correction of vesicoureteric reflux in infants under 2 years of age. *Pediatr. Surg. Int.*, 1994 9:73-75.

18. Dewan PA, Henning P, Juriedini KF. Vesicoureteric reflux and the surgical perspective. *J. Pediatric. Child. Health*, 1994 (letter) 30:82-83.

19. Dewan PA, Byard RW. Plastic particle migration. *Br. J. Urol.*, 1993 (letter) 71:112.

20. Dewan PA, Guiney EJ. Endoscopic correction of primary vesicoureteric reflux in children. *Urol.*, 1992 39:162-164.

21. Dewan PA. Is injected Polytetrafluoroethylene (Polytef) carcinogenic? *Br. J. Urol.*, 1992 69:29-33.

22. Dewan PA, O'Donnell B. Polytef paste injection of refluxing duplex ureters. *Eur. Urol.*, 1991 19:35-38.

23. Dewan PA. Endoscopic management of vesico-ureteric reflux. In Bailey R R ed; *Proceedings of the second CJ Hodson symposium on reflux nephropathy* pp. 65-68. Christchurch, Sandoz 1991.

24. Dewan PA, Guiney EJ. Endoscopic correction of vesicoureteric reflux in children with spina bifida. *Br. J. Urol.*, 1990 65:646-649.

Conference Paper Presentations

European Urology Meeting, Salzburg, Austria, April 1998.

1. IgG development in response to plastic.

Urology Society of Australasia, Melbourne, March 1998.

2. Fetal vesicoureteric reflux.

Royal Australasian College of Surgeons, Victorian State Meeting, Bendigo, October 1997.

3. Plastic migration from medical devices.

Bangladesh Society of Surgeons, Chittagong Chap. Symposium, Bangladesh, Sept 1997 *(Visiting Professor)*.

4. Vesicoureteric Reflux.

Australasian Association of Paediatric Surgeons, Christchurch, August 1997.

5. Plastic migration from a plastic device.

International Endourology Conference (Faculty Member), November 1996.

6. Correlation of the radiological and endoscopic view of the external urethral sphincter.

Australasian Society of Ultrasound in Medicine, Symposium on pre-natal diagnosis, Flinders Medical Center, Adelaide, October 1996.

7. Follow-up of fetal uropathy **(Invited)**.

SAPMEA General Practitioner Conference, Barmera, May 1996 *(Invited)*.

8. Vesicoureteric reflux and bladder instability.
9. Pre-natal hydronephrosis.

South Australian Urology meeting, October 1995.

10. Silicone embolisation from IV infusions.

International Paediatric Surgery and Radiology meeting, Melbourne, March 1995.

11. Malignancy risk of injected plastics in a rat model.

Asian Association of Surgeons, Bali, March 1995.

12. Malignancy risk from injected plastics.

Paediatric Urology Symposium, Surabaya, March 1995 *(Invited)*.

13. Fetal Urology: diagnosis and therapy.

Paediatric Urology Symposium, Semarang, March 1995 *(Invited)*.

14. Vesicoureteric reflux.

Mildura Medical Society Conference, Mildura, November 1994 *(Invited)*.

15. Vesicoureteric reflux and Bangladesh.

South Australian Urology meeting, October 1994.

16. Malignancy risk of plastics in a rat model.

International Urology Society meeting, Sydney, September 1994.

17. Migration and malignancy from plastics in rats **(Invited)**.

Royal Australasian College of Surgeons meeting, Adelaide, August 1994.

18. Plastics in a rat model and clinical implications.
19. Correlation of the endoscopic and clinical outcome for the STING procedure.

Indonesian Perinatal Society, Annual meeting, Djakarta, June 1994 *(Invited)*.

20. Interventional fetal Urology.
21. Neonatal non-obstructive Urology.

South Australian Urology meeting, October 1993.

22. Endoscopic and clinical outcome for the STING procedure.

Bangladesh Paediatric Association, Annual meeting, Dhaka, April 1993.

23. Endoscopic management of vesicoureteric reflux.

Urology Society Australasian meeting, Hobart, March 1993.

24. Polytef and Bioplastique in a rat model.

Royal Australasian College of Surgeons, GSM, Canberra, May 1992 *(Invited)*.

25. The endoscopic management of vesicoureteric reflux.

Australian Urology Society Meeting, Adelaide, March 1992.

26. Polytef and Bioplastique in a Rat Model.

Paediatric Urology Club, Melbourne, November 1991 *(Invited)*.

27. Problems of endoscopic management of vesicoureteric reflux.

South Australian Urology Meeting, Burra, October 1991.

28. Polytef and Bioplastique in a Rat Model - early results.

Australian Paediatric Surgeons, Perth, May 1991.

29. Analysis of the endoscopic management of vesicoureteric reflux **(Invited)**.

Westmead Hospital Reflux Seminar, March 1991 *(Invited)*.

30. Current status in the endoscopic management of vesicoureteric reflux.

CJ Hodson Memorial Seminar, Christchurch, February 1991 *(Invited)*.

31. Overview of the endoscopic management of vesicoureteric reflux.

Basle Endourology meeting, Switzerland, September 1990 *(Invited)*.

32. Endoscopic management of vesicoureteric reflux.

European Association of Urologists, July 1990.

33. STING in Duplex systems.

Asian Surgical Association, Penang, May 1989.

34. STING in infants.

Glossary

Bioplastique - Injectable form of silicone; combined with hydrogel carrier
COPUM - Congenital obstructive posterior urethral membrane
DIC - Direct isotope cystogram; performed via a urethral catheter
DMSA - Dimercaptosuccinic acid; a test for renal scarring
EDXA - Energy dispersive x-ray analysis
ELISA - Enzyme linked immunosorbent assay
ESR - Erythrocyte sedimentation rate
H&E - Haematoxylin & Eosin
IU - International units
IRC - Indirect radionuclide cystogram; via an intravenous injection
IRR - Intrarenal reflux
IRSG - International Reflux Study Group
IVU - Intravenous urogram
MAG3 - Mercaptoacetyltriglycine; a test of renal function
MCK - Multicystic kidney
MCU - Micturition cystourethrogram
NAG - N-Acetyl-ß-D-Glucosaminidase
Polytef - Injectable form of Polytetrafluoroethylene; combined with glycerin
PTFE - Polytetrafluoroethylene
RAN - Reflux associated nephropathy
SE - Standard errors
SEM - Scanning electron microscopy
Si - Silicon
Silicone - Silicone the plastic, not silicon the element
STING(s) - Submucosal Teflon injection
STINGer - Endoscopic instrument for performing Submucosal Teflon injection
Teflon - Trade name for Polytetrafluoroethylene
US - Ultrasound
VUR - Vesicoureteric reflux