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II. COMPLIANCE AND CONTRACTILITY ASSOCIATED WITH URINARY FLOW IMPAIRMENT

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RESPONSES TO THE REVIEWER'S COMMENTS: JPUROL-D-05-00227

The reviewer's comments are shown in *italics* and our responses are shown directly following each of his/her points. We appreciate the thoughtful comments provided by the reviewer and we have tried to answer these to our best ability. Please consider our responses for this paper alongside those for the accompanying paper (JPUROL-D-05-00239: Experimental short term fetal bladder outflow obstruction: I. Effects on morphology and cell biology). Major changes to the text in response to the referee's criticisms are underlined in the main text of the revised version.

This study is submitted in conjunction with the report that describes the morphological alterations of short term bladder obstruction in the fetal lamb. This study presents the alterations in compliance and contractility. As noted before, these studies would be better presented together with a summation of data to permit a more consolidated view of the data, rather than fragmenting them, leaving the reader uncertain as to their interaction.

The reviewer suggests that the two current reports could have been combined into one paper. While we quite agree that it is not appropriate to 'stretch-out' studies with only a few data sets over multiple papers, we feel that each of the current papers is already quite 'data-rich', with the morphology and physiology having been studied in some detail with a wide variety of methodologies. Thus, a combined paper would have a Results section with a large number of figures (many of which already have multiple frames within them) – this amount of original data is surely beyond the limit of a single article.

We have re-written the two Introductions to ensure that they are quite different, addressing different features of the experimental study: the first gives a brief history of use of sheep models and then summarises morphological/cell biology effects from our published work on the 30 days obstruction model, whereas the Introduction of the second paper talks mostly about the bladder physiology of humans with congenital BOO and also summarises the physiological effects of 30 days fetal sheep BOO.

With regard to the Methods sections, again these have been substantially re-written to ensure that overlap is minimised, and have made frequent reference to the accompanying paper to avoid such duplication. Combining the Introduction and Methods of the two papers would therefore save few words and result, in particular, in a very long Methods section.

The Abstract is also altered to reflect the changes in the text

Several of the figures are repeated and would presumably be placed in the same issue of the journal.

There is now no duplication of figures. The one that was duplicated previously has been omitted and reference is made to the accompanying paper, i.e. that obstruction increases bladder weight and the bladder-to-fetal weight ratio.

The authors need to provide a more mechanistic approach to their observations to permit further hypothesis-driven studies.

We do welcome the chance to try to provide a more integrated view of the current pair of studies, also linking them with our previous reports of longer periods of BOO, also starting at 70-80 days of sheep gestation: therefore, we have completely revised the

Discussion to outline how the fetal bladder differentiates morphologically and physiologically, comparing this with the altered trajectory caused by short- and longer-term BOO. In addition, we use this scheme as a springboard to suggest a strategy to determine when reversal of obstruction might prevent progressive bladder malformation and malfunction and might even permit recovery of anomalies.

1. It is unclear if the degree of obstruction is complete or partial. In the results section, it is stated that in the double ligation group, the urethra was obstructed proximal to the angle of the prostatic urethra. Does this mean it was a complete obstruction or partial. No mention is made of the amniotic fluid volume, although even complete obstruction for a short time may not induce oligohydramnios. This should be stated explicitly.

This is an interesting point. In the second half of gestation, if we presume that amniotic fluid essentially represents fetal urine, a complete bladder outflow obstruction (i.e. no urine flow through urachus or urethra) would be expected to lead to a complete lack of amniotic fluid. At autopsy in the current study, however, amniotic fluid was always seen in each fetus of the BOO (as well as the sham) groups. On the other hand, our impression was that the amount of fluid, although not formally quantified in this study, was often diminished in fetuses with urachal-only ligation and also in those when this procedure was combined with the placement of a urethral ring. These observations do indeed suggest that some urine must, at least initially, have been able to exit the bladder, probably via the urethra because the urachus in both intervention groups was confirmed to be ligated: hence we had most likely produced an ‘incomplete impairment of urine flow’. Indeed, we had anticipated that the ring placed around the fetal sheep urethra would not be completely occlusive

at the start. The reviewer suggests that we might term this a '*partial obstruction*': we, however, have a semantic difficulty with calling a functional impairment of urine flow 'partial' because functional flow impairment is either present or absent – in the same way, one can not speak about 'partial renal excretory failure' because the kidneys are either failing to excrete waste products or they are functioning in the normal range! On the other hand, we think it is more meaningful to us the phrase 'incomplete urinary flow impairment'. All these aspects are now covered in the revised first paper of this set.

2. The authors suggest that the preparations are near a threshold of decompensation, without any definition of decompensation, nor any suggestion as to the clinical significance. Indeed, in posterior urethral valves, the state of decompensation in the infant is unusual.

We agree that this was a poor use of the term that we have now removed all reference. Our earlier studies with longer periods of obstruction had clearly resulted in a flaccid bladder with poor unit contractile function. The aim of this study was to see if a shorter period of obstruction would produce an enlarged bladder but without evidence of muscle dysfunction.

3. In the assessment of contractility, the authors state that the dimensions and weights of the bladder strips were similar, yet the bladders are clearly thicker than normals; was this not seen in the strips? If so, then the effective contractility normalized to cross sectional area (the usual format, and indicated by the authors) would be less for the thicker obstructed sections. All the other data suggests the bladder walls are

thicker; it is unclear why this is not reflected in this parameter. Can the authors clarify this?

The experimental preparations used for the in vitro experiments were strips of tissue cut from the bladder wall. At the end of the experiments the muscle dimensions are measured and the cross-sectional area recorded. Muscle force is then normalised to cross-section area (the units are in mN per mm²). This normalisation therefore takes into account variations that occur in muscle strip dimensions. What you say about bladder cross-sectional area is correct, the thicker-walled bladder will develop more force per unit length in a vector tangential to the bladder wall due to laying down more parallel muscle fibres. The purpose of the experiments was however, to measure the unit contractile function of the muscle – i.e. was there muscle dysfunction and the normalisation procedure would enable us to do this. An additional sentence has been added in the relevant section of the Methods to emphasise this point. *In-vitro contractility measurements*. “Tension values are expressed as mN per unit cross-section area (mm²) of muscle to enable the normalised level contractility to be compared between specimens.” Please note that data values are now expressed non-parametrically (to be consistent with the sister paper).

4. As in the linked report, we do not have a mechanistic hypothesis in this paper, nor hypotheses that may be derived from these data. What is the mechanism of ultimate alteration in the mediators of contractility? Why does compliance change with increased severity or duration of obstruction? The changes are noted, but why? The authors need to develop their analysis for the reader to place all of this in some context and to permit rational progression of their studies.

We have tried to explain more clearly the aims and objectives of the study in the latter part of the Introduction. “The aim of this study was to generate a less severe form of obstruction, whereby bladder function would be sufficient to overcome the insult (i.e. a compensated state), and which could be potentially reversible following decompression. ... The objective of this part of the study was to characterise the functional properties of the bladder, to determine if any changes had occurred at this stage.”

One hypothesis-led analysis of the data is in the section regarding “*Compliance and wall stress*” to determine if increases to bladder weight and reflected in changes to the passive properties of the bladder wall. This is displayed in figure 1C

In addition in the Results, section “*Contractile function.*” We have emphasised the point by introducing the analysis of data by the statement “One hypothesis to be tested is whether bladder overgrowth is associated with contractile failure.”. This point has also been re-stated in the Discussion, paragraph 4,5.

The further studies associated with this work are laid out in the final paragraph of the Discussion

“*Reversal of the effects of obstruction.* Implicit in this discussion is that reversal of the obstruction would, with the correct timing, permit recovery of function, or at least prevent irreversible decline. This has been addressed by others with respect to structural changes to the urinary tract, mainly measuring renal markers and have concluded that some reversibility is possible^{21,22}. Functional studies, particularly on bladder function, have not been systematically carried out and one of the key objectives is to determine the optimum timing, for functional recovery. The present study has shown this period does lead to some functional changes, i.e. passive bladder

wall properties, whilst others, contractile function, are intact. Future work will determine the duration of obstruction that is required to cause contractile dysfunction and how reversible such changes are on reversal of the obstruction.”

EXPERIMENTAL SHORT-TERM FETAL BLADDER OUTFLOW

OBSTRUCTION:

II. COMPLIANCE AND CONTRACTILITY ASSOCIATED WITH URINARY FLOW IMPAIRMENT

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ABSTRACT

Purpose. Posterior urethral valves (PUV) are the commonest cause of congenital bladder outlet obstruction. Despite valve ablation in the neonatal period, up to 70% of patients develop renal failure by their teen-age years, and progressive bladder dysfunction. This study forms part of a continuing project examining the relationship between severity and duration of obstruction and urinary tract dysfunction. Our objective here is to assess the result of short-term (nine-day) obstruction.

Materials and Methods. Fourteen male fetal lambs at 75 days gestation were assigned to three groups: urachal ligation; urachal ligation with partial urethral obstruction; sham-operated controls. Pregnancy proceeded thereafter for nine days. At autopsy, filling cystometry was performed with the urinary tract in situ and the bladder harvested for nerve counts using PGP 9.5 immunohistochemistry, or in vitro measurement of contractile function.

Results. Obstruction was associated with an increase of the bladder-to-fetal weight ratio (BFR). Compliance was variable in the obstructed bladders, but calculated wall stress per unit strain was either similar or less than control. Nerve-mediated or agonist-induced contraction magnitude, and nerve counts did not change, despite the increase of BFR in the obstructed groups.

Conclusions. Nine days of outflow obstruction at mid-gestation generated a bladder of increased weight but without evidence of contractile failure. However, an increase of bladder compliance as a function of bladder growth was observed even at this stage, and represents one of the initial responses to outflow tract obstruction.

INTRODUCTION

Posterior urethral valves (PUV) is the commonest cause of congenital bladder outlet obstruction (BOO) in boys¹. Despite advances in the management of this condition, recent outcome studies showed that whilst perinatal survival has improved, the rate of progression to end-stage renal failure has not changed: 80% of 116 patients, followed up for ten years, had urodynamic bladder abnormalities². Renal function impairment was significantly associated with poor compliance and detrusor overactivity, in addition to bilateral VUR and renal dysplasia. This raises the question if ante-natal decompression would ameliorate these pathologies. A meta-analysis of the 16 most relevant outcome studies of antenatal bladder decompression revealed a significant improvement in perinatal survival but any possible ameliorative effects on renal tract structure and detailed function were not assessed³. However, clinical studies overall have limited ability to address the status of the underlying pathology, and the gestational age at which decompression is carried out; factors which could later outcome. Such questions may only be addressed with a suitable animal model, examining how the severity and duration of fetal obstruction determine the structure, morphology, storage and emptying qualities of the normal and obstructed fetal bladder.

Our previous studies using a fetal lamb PUV model showed that thirty days of severe outflow obstruction at mid-gestation resulted in a grossly dilated, thin-walled, compliant and hypo-contractile bladder with reduced innervation and a disrupted detrusor^{4,5}. A bladder in this state would be unlikely to recover normal function following antenatal decompression. The aim of this study was to generate a less severe form of obstruction, whereby bladder function would be sufficient to overcome the insult (i.e. a compensated state), and which could be potentially reversible following decompression. The previous paper⁶ has shown that nine days of urachal ligation, with or without additional partial occlusion of the urethra, in the fetal lamb results in bladder overgrowth, but with apparent preservation of detrusor morphology. The objective of this

part of the study was to characterise the functional properties of the bladder, to determine if any changes had occurred at this stage.

MATERIALS AND METHODS

Fetal surgery and sample collection. The study was performed in accordance with the United Kingdom Home Office Animals (scientific procedures) Act 1986. Details of the anaesthetic and surgical procedure have been detailed previously^{4,6}. For this study, 14 pregnant Romney Marsh ewes at 75-82 days gestation (Royal Veterinary College, Potter Bar, UK) underwent laparotomy and hysterotomy. Male fetuses were assigned to one of three experimental groups: complete urachal ligation alone (U, n=4), urachal ligation and partial constriction of the urethra (UU, n=6) or sham (S, n=4). Fetuses were returned to the maternal abdomen and pregnancy continued for nine days, with ultrasonography on post-operative days one and eight to confirm fetal viability and monitor any urinary tract dilatation. At autopsy, filling cystometry (see below) was performed in situ. The bladder was then removed and separate sections stored in 10% formaldehyde (for histological analyses), Ca-free HEPES Tyrode's solution (for in vitro physiology studies), or snap-frozen (for biochemical analysis).

Filling cystometry. The fetal urachus, ureters and proximal urethra were ligated, and urine aspirated and its volume recorded. The bladder was catheterised with a 5-French double-lumen tube, inserted through an incision in the proximal urethra, and connected to a PDCR 75 water-filled transducer (Druck Ltd, Groby, UK) placed level with the bladder. One lumen filled the bladder, intravesical pressure was measured via the second using a Lectromed chart recorder (Letchworth, Hertfordshire, UK). Pressure was recorded at baseline and during intermittent filling with Ca-free Tyrode's solution at room temperature, during stepwise increments of 0.1-0.5ml. Filling was stopped when initial bladder volumes were reached or when the bladder became tense on visual observation.

Compliance curves were constructed using raw pressure-volume data. To standardize the curves, circumferential wall stress (σ) was calculated to account for disparate bladder volumes and any

variability in wall-thickness. A modified Laplace relation $\sigma = P.r_1/2.d$ was used, where P is luminal pressure, r_1 the bladder inner radius and d wall thickness; r_1 was calculated from bladder volume (V) using the relationship $V = (4\pi/3).r_1^3$. Tissue volume (W) of the bladder wall (bladder weight/density, where density = 1.05 g.ml⁻¹) was used to calculate d, assuming that tissue formed a concentric sphere around the bladder lumen, using the formula $d = \left(\sqrt[3]{\frac{3W}{4\pi} + r_1^3} \right) - r_1$. Plots of wall stress, σ , versus bladder volume were linear and the slope was calculated as a measure of normalised wall stiffness.

In-vitro contractility measurements. Bladder dome strips, with the mucosa removed, and less than 1mm in diameter were mounted in a horizontal trough between a fixed hook and an isometric force transducer, and superfused at 4 ml.min⁻¹ with Tyrode's solution at 37°C. Electrical field stimulation (EFS) used 3 second tetanic trains (1-60Hz; 0.1ms pulses) every 90 seconds. Tetrodotoxin (TTX, 1 µM) and atropine (10 µM) were added to Tyrode's from 10 mM aqueous stocks, during EFS, to achieve the final concentrations. Carbachol (10 µM), α - β methylene-adenosine triphosphate (ABMA, 10 µM) were added to the Tyrode's in the absence of EFS. A high-K (80 mM) solution was made by adding solid KCl to Tyrode's solution, no osmotic correction was made. At the end of the experiments, the preparation diameter and weight were recorded. Tension values are expressed as mN per unit cross-section area (mm²) of muscle to enable the normalised level contractility to be compared between specimens.

Solutions. Tyrode's solution contained (mM): NaCl, 118; KCl, 4.0; NaH₂PO₄.2H₂O, 0.4; NaHCO₃, 24, MgCl₂.6H₂O, 1.0, CaCl₂, 1.8; Na pyruvate, 5.0; glucose, 6.1; gassed with 95%O₂-5% CO₂. Ca-free solution contained (mM): NaCl, 105; KCl, 3.6; NaH₂PO₄.2H₂O, 0.4; HEPES, 19.5; NaHCO₃,

22.3; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.9; glucose, 5.4; Na pyruvate, 4.5, pH 7.1 adjusted with 1 M NaOH. All chemicals were from Sigma-Aldrich Co Ltd, Poole, Dorset, UK.

Immunohistochemistry. Paraffin sections of each bladder were stained with polyclonal rabbit anti-PGP 9.5 antibody (DakoCytomation, Glostrup, Denmark) at a concentration of 1:200 to visualise nerve bundles. Slide preparation and immunostaining techniques were described as previously^{4, 6}. The numbers of nerve bundles were counted in six fields under x25 magnification and the average value was used for analysis.

Statistics. Data are expressed as median values [interquartile ranges] and groups were compared using the Mann-Whitney test to account for small sample size. The null hypothesis was rejected at $p < 0.05$. Correlation between different variables was assessed by calculation of a correlation coefficient, r , estimation of a t-value from $t = r\sqrt{(n-2)/(1-r^2)}$, to allow estimation of p .

RESULTS

The previous paper⁵ has demonstrated that the obstructed groups had a greater bladder:body weight ratio. Bladder wall thickness (measured when the bladder was empty) was significantly increased in both obstructed groups (U; 0.77 [0.68,0.84] mm: UU; 0.64 [0.58, 0.84] mm) compared to control (0.41 [0.34,0.46] mm). Urine volume on excision of the bladder was also greater in the obstructed groups (U; 4.7 [1.1, 11.5] ml: UU; 2.5 [1.3, 3.5] ml) compared to control (1.4 [1.2, 1.9] mm).

Compliance and wall stress. Figure 1A shows ex vivo pressure-volume curves for bladders from the three groups. The curves from sham-operated bladders were similar and are shown as averaged data; pressures did not exceed 7 cm H₂O at maximum volumes of 2 ml. Corresponding curves from obstructed bladders were more variable: two bladders (one from each intervention group) had low compliance, and the remainder had a similar or greater compliance compared to the sham group.

Part of this variability may be due not only to differences in the unit elasticity of the bladder wall, but also its thickening in response to obstruction. Wall stress, normalised to unit cross-section area, was calculated as a function of filling volume (see Methods) to compensate for the increase of wall thickness. Figure 1B shows that the transformation linearised the curves, the abscissa has been restricted to 2 ml for clarity, but all curves were linear up to the volume limit in part A (5 ml).

Curves from the obstructed groups were either similar or had reduced stiffness compared to control. The slope of the line gives a normalised value of wall stiffness and median values were not different between the three groups (S; 2.10 [1.84,2.56]: U; 0.78 [0.57,1.42]: and UU 1.01 [0.53,1.35]

mN.mm⁻².ml⁻¹). However, the scatter of data may hide any trend that may develop with obstruction.

The previous paper⁶ has shown a good correlation between bladder:fetal weight ratio (BFR) and either bladder protein or DNA content, indicative of bladder overgrowth with obstruction. The question arises if normalised wall stress is a function of increasing bladder weight with obstruction.

Figure 1C plots this estimate of wall stiffness as a function of BFR, irrespective of the interventional group. The data show a significant negative correlation between BFR and wall stiffness, with the exception of a single datum point for one of the obstructed bladders, with a correlation coefficient of -0.76 ($p < 0.01$). Thus overgrowth of the bladder is associated with a loss of elasticity of the wall.

Contractile function. Dimensions and weights of detrusor strips were similar from all groups. Contractions elicited by electrical field stimulation (EFS) were blocked completely by the neurotoxin TTX, indicating they were nerve-mediated. Atropine abolished completely nerve-mediated contractions in detrusor strips from all groups. This was not due to a lack of response of the detrusor to ATP that may be released from nerves, as application of 1 μ M ABMA generated transient contractures in all preparations, of similar magnitude in control ($n=4$) and obstructed ($n=5$) groups (1.46 [0.92, 1.97] vs 3.00 [1.39, 5.27] mN.mm⁻²). The data from the two obstructed groups have been combined because of the relatively small data sets. In the presence of atropine, EFS generated small transient relaxations in nearly all preparations from both sham-operated and obstructed bladders.

One hypothesis to be tested is whether bladder overgrowth is associated with contractile failure. Plots of BFR as a function of tension generated by various interventions did not show a significant correlation: nerve-mediated stimulation at 40 Hz ($r = 0.02$, $p > 0.05$), 10 μ M carbachol ($r = 0.14$, $p > 0.05$) or an increase of extracellular [KCl] ($r = -0.04$, $p > 0.05$). Figure 2 shows plots of the median data for the three interventions, because of the similarity of data in the two obstructed groups, they have been combined, and they show no difference compared to the sham-operated group.

Innervation. Nerve bundles were present in the detrusor from all three groups, as identified by PGP 9.5 immunostaining. These were situated mainly between the detrusor layer and the serosa, with some neurovascular bundles interspersed between muscle fascicles. Nerve bundles in experimental groups were visualised by immunohistochemistry (Figure 3). However, there was no significant relationship between PGP nerve count and either BFR ($r=-0.42$, $p>0.05$) or tension generated by electrical field stimulation at 40 Hz ($r=0.50$, $p>0.05$). These correlations are consistent with the observation that bladder overgrowth at this period of obstruction is not associated with denervation or reduced contractility. Wall stress also showed no correlation with PGP counts ($r=0.44$, $p>0.05$). However, it is of interest to note that if the single point associated with the very high wall stress observed in figure 1C was omitted, a strong negative correlation was observed ($r=0.74$, $p>0.01$).

DISCUSSION

This study has shown that nine days of bladder outflow obstruction in the fetal lamb at mid-gestation generated a heavier and thicker bladder, with a well-preserved detrusor architecture and hydronephrotic kidneys⁶. The detrusor maintained normal contractile function despite the tendency for compliance to increase. Furthermore, urine osmolality was not significantly perturbed⁶. These findings contrast with the effects of 30-days obstruction which resulted in the generation of a flaccid, over-compliant bladder, accompanied by detrusor contractile failure; in addition, the prolonged period of obstruction was associated with uniform renal morphological damage, with a disrupted nephrogenic cortex and subcortical cysts, the failure of urinary dilution^{4,5,7}.

Several other groups have employed animal models of fetal obstructive uropathy to investigate different aspects of kidney and bladder development with several different models, but none has attempted to combine morphological and functional observations. Compliance changes, similar to those observed by us, have been seen in a rabbit model of obstruction⁸, but this have generally been abandoned in favour of the sheep model⁹. The use of bladder outflow obstruction in fetal sheep is now the widely accepted model and initially focussed on renal dysplasia and lung development^{10,11}. Later studies demonstrated developmental structural changes to the obstructed kidney¹²⁻¹⁴ and bladder^{15,16}. Our series of studies are to our knowledge the first to document morphological and physiological changes to the upper and lower and urinary tract in the same fetuses, and shows changes at two time-points on a continuously evolving obstructive picture.

Changes to passive and active detrusor contractile properties. A change to the passive properties of the bladder wall, as assessed by measurement of normalised wall stress, precedes functional changes: reduction of wall stress correlated well with bladder-to-fetal weight ratio (BFR). The implications of this observation are several-fold. Bladder filling would be facilitated and may

ameliorate the consequences of urinary retention in the obstructed bladder by minimising intravesical pressure changes and reducing the possibility of raised upper tract pressures. However, reduced passive stiffness would lessen the ability of tension developed by muscle cells to be transmitted through the tissue mass, and so reduce the ability of the bladder to raise pressure. However, because tissue contractility was not related to the decrease of wall stress, the latter would not seem to be a problem at this stage of the development of bladder overgrowth. Whether wall stress recovers after a period of de-obstruction remains a further question to address.

Our major hypothesis was that a short period of obstruction would cause overgrowth of the bladder with out failure of function. This would represent a stage of compensation to allow the bladder to develop additional power to overcome the increased outflow resistance. The lack of association of nerve counts or contractile function with increased BFR implies that such a compensated stage was achieved. In addition, two modes of contractile activation were tested, i) via the cholinergic motor nerves, ii) by direct activation through depolarisation with elevated [KCl] or with muscarinic or purinergic agonists. This implies that the functional nerve-supply to the detrusor and the ability of detrusor itself to generate force were not related to bladder overgrowth. The importance of these observations is that antenatal decompression of the obstructed bladder at this time would, we hypothesise, allow recovery of normal function, but that a longer period would be not allow such a functional reversal. Future work is required to test this hypothesis, and also to define more accurately the period of obstruction that would permit recovery of function.

Analysis of the data sets indicates that this period of obstruction generates an evolving pattern of changes. Variability of the measured parameters was generally greater in the obstructed groups than the control set. For example, the variance ratio of normalised wall stress values in the obstructed and control groups (about 147 - see figure 1B) was, as indicated by Fisher's test, highly

significant. This indicates that there was a natural variation in the extent of obstruction and this led to a spectrum of responses. This made comparison of group data sets difficult, as the large natural variation may have masked any trends. For this reason the different variables were assessed as a function of the BFR, and in any group representation of data the two obstruction data sets were combined. The implication also is that in this model urachal obstruction alone causes a similar functional obstruction to that when combined with partial urethral obstruction. In fact, the obstructive nature of fetal urachal-only ligation has been previously noted¹⁷. This observation has practical implications because the urachus has previously been used to access the bladder lumen and monitor intravesical pressures^{18,19}. Such measurements may reflect those in an acutely obstructed bladder rather than one that is normal.

Reversal of the effects of obstruction. Implicit in this discussion is that reversal of the obstruction would, with the correct timing, permit recovery of function, or at least prevent irreversible decline. This has been addressed by others with respect to structural changes to the urinary tract, mainly measuring renal markers and have concluded that some reversibility is possible^{20,21}. Functional studies, particularly on bladder function, have not been systematically carried out and one of the key objectives is to determine the optimum timing, for functional recovery. The present study has shown this period does lead to some functional changes, i.e. passive bladder wall properties, whilst others, contractile function, are intact. Future work will determine the duration of obstruction that is required to cause contractile dysfunction and how reversible such changes are on reversal of the obstruction.

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LEGENDS**Figure 1. Pressure-volume relationships.**

A. The median [25,75% interquartiles] data for the sham-operated animals are shown as closed squares. Data from the bladders undergoing urachal ligation (open circles) and urachal ligation with urethral occlusion (open squares) are shown for individual animals. B. Transformed data from part A showing calculated wall stress as a function of filling volume. The sham-operated data are shown as median [25,75% interquartiles] and the thicker fitted line. Other data as in part A. C. The relationship between normalised wall stress (slope of lines in part B) and bladder-to-fetal weight ratio: symbols the same as in parts A and B.

Figure 2. Contractile function of isolated detrusor.

A. Contractions evoked by 10 μ M carbachol in strips from sham-operated and obstructed bladders. Data from the two obstructed groups have been combined – see text.

B. Contractions evoked by electrical field-stimulation at 40 Hz or raised superfusate [KCl]. Median values with 25% and 75% interquartiles.

Figure 3. PGP 9.5 immunostaining.

Nerve bundles are preserved in the urachal ligation (B) and urachal ligation with urethral occlusion (C) groups compared to sham (A).

Figure 1

C

Figure 2

A

B

Figure 3