Biopsy techniques

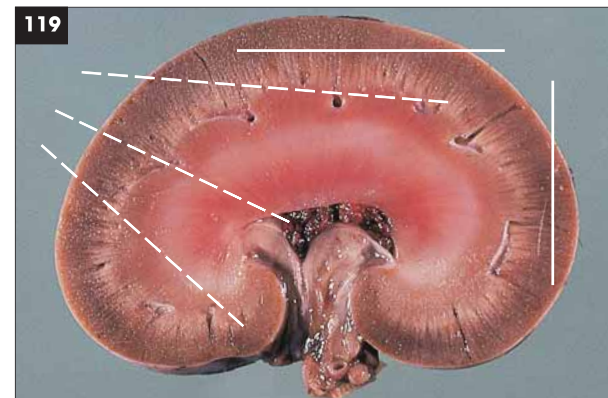
Indications

- To differentiate inflammatory and neoplastic lesions of the urinary tract (e.g. urethritis vs urethral carcinoma).
- To determine the nature of the disease present, more accurately to prescribe treatment and/or obtain a prognosis (e.g. malignancies, glomerular renal diseases).

Techniques

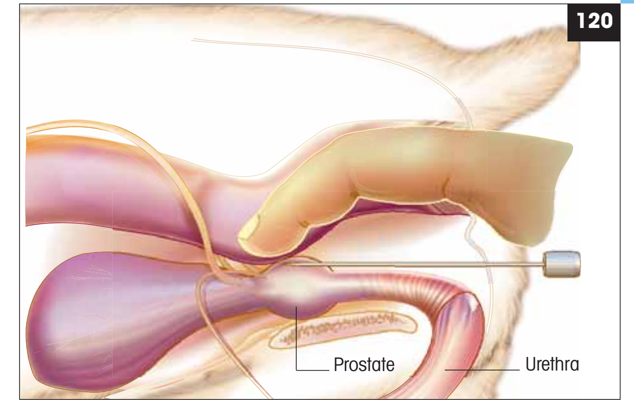
ALTHOUGH BIOPSY MATERIAL can be obtained by surgical exposure of the organ concerned, less invasive methods include needle biopsies and biopsies taken endoscopically (see Endoscopy, p. 55) or using a urethral catheter.

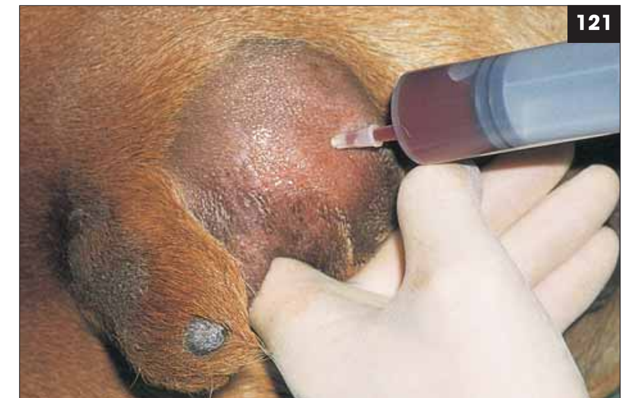
The kidney and prostate can be biopsied using a 'Tru-cut' disposable biopsy needle (Travenol) (**118–123**), preferably under ultrasonic guidance, while the catheter biopsy technique (**124–126**) is suitable for lower urinary tract urothelial lesions and the para-urethral prostate.

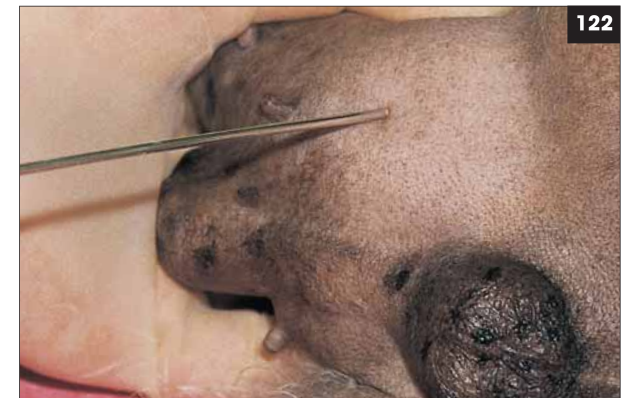


118, 119 (**118**) Renal cortical biopsies can be taken using a biopsy needle. A Tru-cut biopsy needle is being used to take a biopsy of this cat's left kidney. Accurate placement of the needle is necessary in order to obtain a meaningful biopsy while minimizing renal trauma. Therefore, although a biopsy can be taken from a sedated animal using local analgesia, the author prefers to perform the technique under general anaesthesia, if possible. Ideally, biopsy should be performed using an automated Tru-cut biopsy instrument under ultrasound guidance, since this makes the procedure less hazardous for the animal. The skin over the kidney (see **87–88**) is prepared for aseptic surgery and sterile ultrasound gel applied. In this illustration, surgical drapes have been omitted to facilitate orientation. The biopsy needle is inserted through a stab incision in the skin into the renal cortex under ultrasonic guidance and the biopsy taken, in this case using an 18Fr Biopty-cut needle and automatic biopsy 'gun'. If such facilities are not available and a manual Tru-cut needle has to be used, it is important to be familiar with the functioning of the biopsy needle before inserting it. This may seem obvious, but most veterinary surgeons perform needle biopsies infrequently and once the needle has been inserted, the effects of manipulations cannot be seen. The novice is advised to have two needles available; one to take the biopsy and the other in case he/she forgets what to do once the first needle has been inserted! The biopsy needle is introduced via a small stab skin incision and inserted into the kidney cortex, taking care to avoid the medulla or corticomedullary junction. In cats the kidneys are usually sufficiently palpable and mobile to be held through the skin and abdominal wall. The kidneys of dogs, on the other hand, may be difficult to locate and fix adequately and some authors suggest making a small flank incision through which a finger can be inserted into the abdomen, better to fix the kidney. In both species the left kidney is easier to biopsy than the right because of its more caudal location; therefore, this is the author's kidney of choice in cases of generalized renal disease (e.g. glomerulonephropathies). However, other practitioners prefer the right kidney, since this is less mobile than the left. The author prefers to hold the kidney in one hand (the left if one is right-handed) and to insert the needle with the other. Unless one has access to a biopsy 'gun', this means that an assistant is required to manipulate the needle in order to obtain a biopsy. Once the needle is withdrawn, it should be examined to ensure that the amount of tissue obtained is adequate for laboratory examinations (histology, electron microscopy and immunohistochemistry) and pieces of tissue should be transferred to suitable containers.

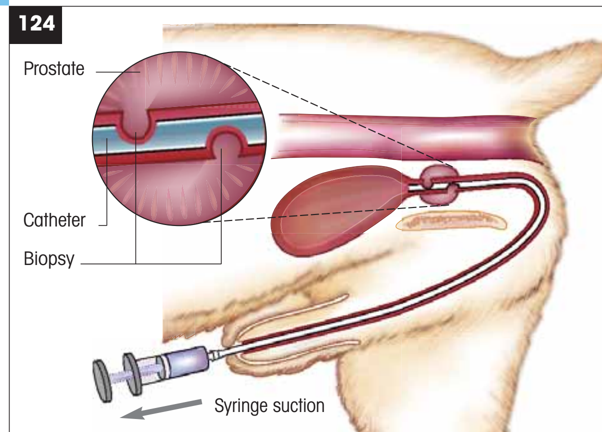
(**119**) Kidney specimen (from a dog with benign, idiopathic renal haemorrhage) illustrating the correct (solid lines) and incorrect (broken lines) positions of insertion of the biopsy needle.



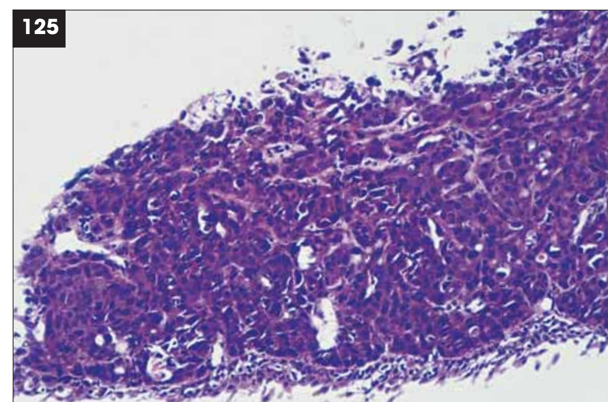


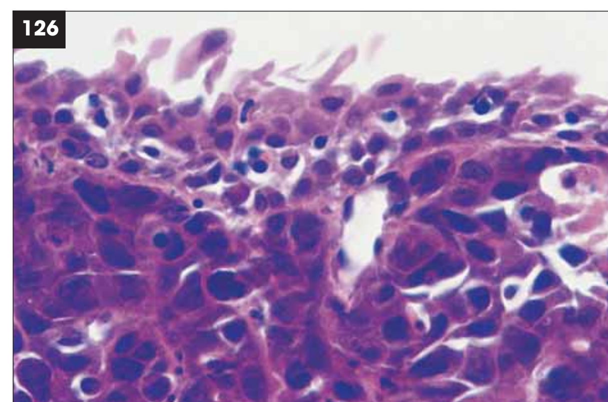


120–123 (**120**) A needle biopsy of the prostate gland is usually obtained through the perineum, as illustrated in the diagram, but a similar technique can be used prepubically to biopsy an intra-abdominal prostate gland. The prostate is fixed, in the case of trans-perineal biopsy using a gloved finger in the rectum. The finger is also used to guide the needle into the area of the gland to be biopsied. Care is taken to avoid the urethra.
(**121**) The rectum is evacuated, either by the administration of an enema or by digital removal of faeces. The anaesthetized dog is placed in lateral recumbency with the side to be biopsied uppermost. The perineum is prepared for aseptic surgery and sterile drapes applied, leaving the anus and perineum exposed (the drapes have been removed from this dog to facilitate orientation). The prostate is fixed with the index finger of the left hand (right-handed people). The biopsy needle or needle for centesis (as shown in this Boxer with a prostatic cyst extending caudally into the perineum) is introduced into the perineum adjacent to the anus and above it, as shown. This reduces the risk of contamination of the needle by faecal organisms, since any material leaving the anus will tend to fall downwards, away from the needle. Once the needle is inserted into the prostate, fluids can be withdrawn or a Tru-cut biopsy taken in an identical way to a renal biopsy. The centre of the gland is avoided to prevent iatrogenic prostatic urethral trauma and the biopsy needle is usually inserted into the dorsolateral region of the prostate.
(**122**) If the prostate is cranial to the pubic brim, it can be biopsied via a prepubic approach as shown (again, the surgical drapes have been removed to facilitate orientation). The prostate is fixed with the left hand (if one is right-handed) and the right hand used to introduce the biopsy needle, taking care to avoid the urethra in the centre of the gland.
(**123**) The resulting biopsy is illustrated. The prostatic enlargement, alopecia and feminization in the dog in **122** are due to excessive, prolonged oestrogen therapy, leading to squamous metaplasia of the prostate gland.



124 Diagram illustrating the catheter biopsy technique of Melhoff and Osborne (1977). The catheter tip is introduced to the level of the tissues to be biopsied (in this case, the prostatic urethra), as judged from previous clinical and radiographic investigations. A syringe is attached to the catheter and used to apply suction. Pieces of tissue are sucked into the side holes of the catheter and dislodged by moving the catheter to and fro. The catheter is then withdrawn and the pieces of tissue flushed out of the catheter (using formol saline) into a container of formol saline. Experience suggests that it is easier to obtain biopsies from diseased (e.g. neoplastic or inflammed) tissues than from normal urothelium and its surrounding tissues. The small pieces of tissue obtained are aggregated by centrifugation, fixed, sectioned, stained and examined (see **125, 126**). The pieces are not always large enough to allow orientation of the sample by a veterinary pathologist, but this is often not essential. In most cases the differentiation of neoplastic from inflammatory tissue is sufficient for diagnostic purposes. Tiny fragments may be treated as cytology samples and smeared on a slide to be examined microscopically.



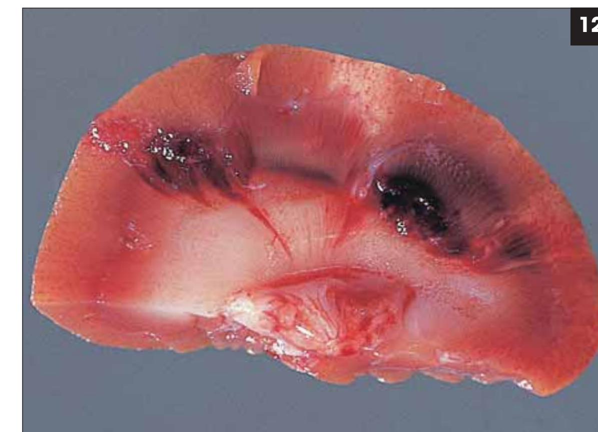


125, 126 Histological appearance of material obtained from a catheter biopsy: H&E x100 (**125**) and x300 (**126**). This catheter biopsy was from a nine-year-old castrated male German Shepherd Dog with a prostatic urethral carcinoma.

Problems

THE MAIN PROBLEMS with needle biopsy techniques are haemorrhage from or into the organ biopsied and iatrogenic trauma to this or adjacent organs (**127, 128**). In addition, attempts to biopsy infected (especially abscessed) organs may result in dissemination of infection. The risk of introducing infection with the biopsy needle itself can be minimized by careful attention to sterile technique.

The catheter biopsy technique shares the problems associated with urethral catheterization described previously, but otherwise is free of complications.



127, 128 (**127**) Postmortem appearance of a feline kidney, one week after a Tru-cut biopsy. Note the pale areas associated with renal infarction.
(**128**) A section through the kidney demonstrates that the biopsy needle had been introduced too deep, damaging the renal arcuate vessels at the corticomedullary junction and leading to thrombosis and infarction.

Blood and urine analyses

Indications

HAEMATOLOGICAL AND BIOCHEMICAL examination of blood are indicated in animals with dysuria or haematuria. They are of less value in incontinent animals (unless the animal's incontinence is related to polydipsia/polyuria). Haematology is mainly of use in assesssing the degree of anaemia (in animals with severe haematuria or renal disease), dehydration (for example, the vomiting dysuric patient) and toxaemia/bacteraemia (e.g. acute prostatitis or prostatic abscessation). Biochemistry can be used to evaluate the degree of pre-renal, renal or post-renal azotaemia in dysuric animals, but it is also valuable in assessing renal function before nephrectomy or nephrotomy is performed. In cases of suspected bladder rupture, comparison of blood and ascitic fluid creatinine levels is a useful diagnostic aid. If uroperitoneum is present, ascitic fluid levels of creatinine will be markedly higher than those in the blood (comparisons of urea levels are less reliable, since urea diffuses readily across the peritoneum and thus blood and peritoneal fluid levels tend to equilibrate).

Urine biochemistry may provide further information on the nature and degree of renal damage, but it is mainly of value (along with urine cytology) in detecting the presence of microscopic haematuria. Urine bacteriology is mandatory in most animals with urological signs; even if urinary tract infections are not the main cause of the clinical signs, they are frequently present as secondary complications and, if possible, should be eliminated before urological surgery is carried out. This is particularly true in animals in which ureteral transplantation is to be performed (see Chapter 8, p. 134). Dogs with prostatic infections frequently shed organisms into the urine and, therefore, urine bacteriology is valuable in such cases, particularly if it is not possible to sample prostatic secretions or abscesses directly.

A guide to normal values

THERE IS A VAST ARRAY of commercial laboratories offering a service for the analysis of veterinary samples, although the increasing availability of desk top analysers means that even those practices without practice laboratories are now perfoming routine laboratory examinations. The analytical techniques required vary between analysers and laboratories and will not be described here. A few basic principles are worth mentioning, however.

It is important to know the range of normal values for the variables being assessed for that particular laboratory or analyser. Most commercial laboratories will supply details of their 'normal' values, as may the manufacturers of some analysers. However, in the latter instance, experience with the use of the analyser will lead to a knowledge of the ranges of normality that can be expected. Variations in normality should also be considered. For example, young animals often have a lymphocytosis and lower packed cell volume (PCV) and haemoglobin levels: fit, working dogs (e.g. Greyhounds, Whippets) normally have PCVs that would be considered high for other dogs.

Table 8 **Normal biochemical ranges**

Analyte	*Units*	*Dog*	*Cat*
Albumin	g/l	32–38	24–35
Globulin	g/l	20–35	21–51
A:G ratio		0.6–1.5	0.4–1.3
ALT	u/l	20–60	15–45
Ammonia	μmol/l	0–50	0–50
Amylase	u/l	450–1,000	450–1,000
Lipase	u/l	0–300	0–300
AST	u/l	20–35	0–20
Bile acids	μmol/l	0–20	0–20
Calcium	mmol/l	2.3–2.6	2.3–2.5
Cholesterol	mmol/l	3.5–7.0	4.0–10.0
Triglycerides	mmol/l	0–1.0	0–1.0
CK	u/l	75–230	50–150
Creatinine	μmol/l	70–110	110–160
Glucose	mmol/l	3.5–5.0	3.5–5.5
Phosphate	mmol/l	0.75–1.25	0.95–1.55
LDH	u/l	35–450	130–470
Lipase	u/l	0–300	0–300
Magnesium	mmol/l	0.8–1.1	0.8–1.1
Potassium	mmol/l	3.5–4.5	4.0–5.0
ALP	u/l	0–110	15–60
Sodium	mmol/l	135–150	149–157
Total bilirubin	μmol/l	0–10	0–10
Total proteins	g/l	63–71	77–91
Urea	mmol/l	2.0–7.0	6.5–10.5

Table 9 **Normal haematological values**

Estimation	*Units*	*Dog*	*Cat*
PCV	l/l	0.35–0.45	0.25–0.48
RBCs	x10^{12}/l	5.4–8.0	5.5–10.0
Haemoglobin	g/l	120–180	80–150
MCH	pg	22.0–25.0	12.5–17.0
MCHC	g/l	350–370	300–350
MCV	fl	65.0–75.0	40.0–55.0
Platelets	x10^9/l	170–500	200–700
WBCs	x10^9/l	5.5–17.0	4.9–19.0
Differential WBC counts			
Neutrophils	x10^9/l	3.0–11.5	2.4–12.5
Lymphocytes	x10^9/l	0.7–3.6	1.4–6.0
Monocytes	x10^9/l	0.1–1.5	0.1–0.7
Eosinophils	x10^9/l	0.2–1.4	0.1–1.6
Basophils	x10^9/l	0–0.1	0–0.1

Table 10 **Normal urinalysis values**

Estimation	*Dog*	*Cat*
pH	5.0–7.0	5.0–7.0
Specific gravity	1.015–1.045	1.015–1.060
Protein	negative	negative
Blood	negative	negative
Haemoglobin	negative	negative
Glucose	negative	negative
Ketones	negative	negative
Bilirubin	negative/trace	negative/trace
Urobilinogen	negative	negative
Urine production	24–40 ml/kg/day	22–30 ml/kg/day

The reference ranges of normal values used in the clinical laboratories at the Bristol Veterinary School are given as a guide (*Tables 8–10*).

Problems

If blood and urine samples are to be posted, they should be prepared and packed in an appropriate way. Most laboratories prefer blood to be clotted and the serum decanted and submitted for analysis rather than whole blood. Apart from the obvious problem that the sample may haemolyse, serum levels of potassium in whole blood tend to increase during transit (as potassium leaks from blood cells). This may lead to a misdiagnosis of hyperkalaemia and inappropriate therapy.

If urine is posted to a laboratory for bacteriological culture, there may be overgrowth of organisms of low pathogenicity during transit, masking the significant bacteria. This can be prevented by placing the fresh urine sample in a receptacle containing boric acid powder in the proportion of 20 ml of urine to 200 mg of boric acid powder. There is increasing evidence that urine bacteriology should be quantitative as well as qualitative and that low counts of bacteria are of no clinical significance. Opinions vary on what is meant by 'low counts', but in general, if a sample is obtained by aseptic catheterization, counts of $<1,000$ and <100 organisms per ml are taken to be due to contamination in dogs and cats, respectively. If the sample is taken by cystocentesis, this figure is <100 organisms per ml in both species.

If samples are posted to a commercial laboratory, it is a legal obligation that the post office guidelines are followed for their package and labelling. Apart from the illegality of inadequate packing and labelling and the health hazards to handlers, urine and blood samples squeezed from sodden envelopes are of little diagnostic value.

Urodynamics

Indications

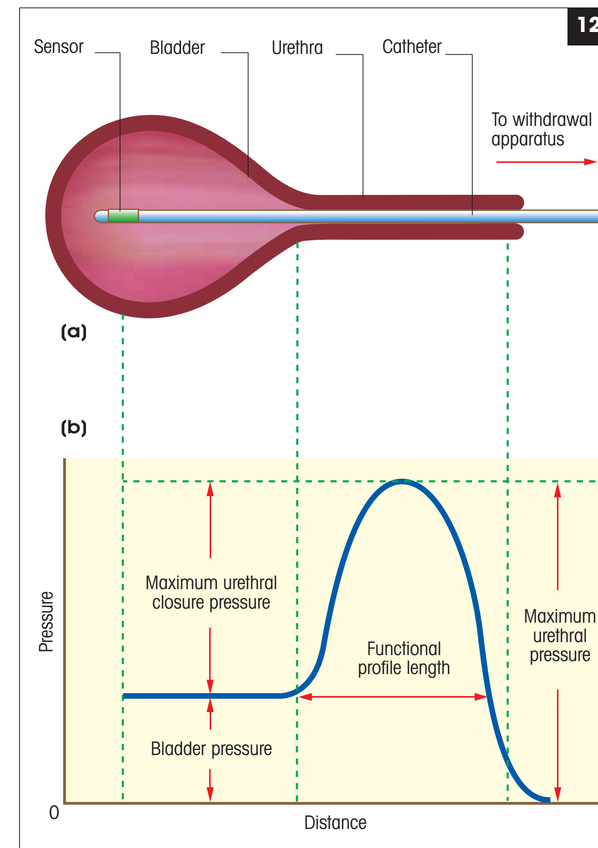
Suspected abnormal functioning of the lower urinary tract such as urethral sphincter mechanism incompetence, detrusor instability, reflex dyssynergia and neurogenic abnormalities.

Techniques

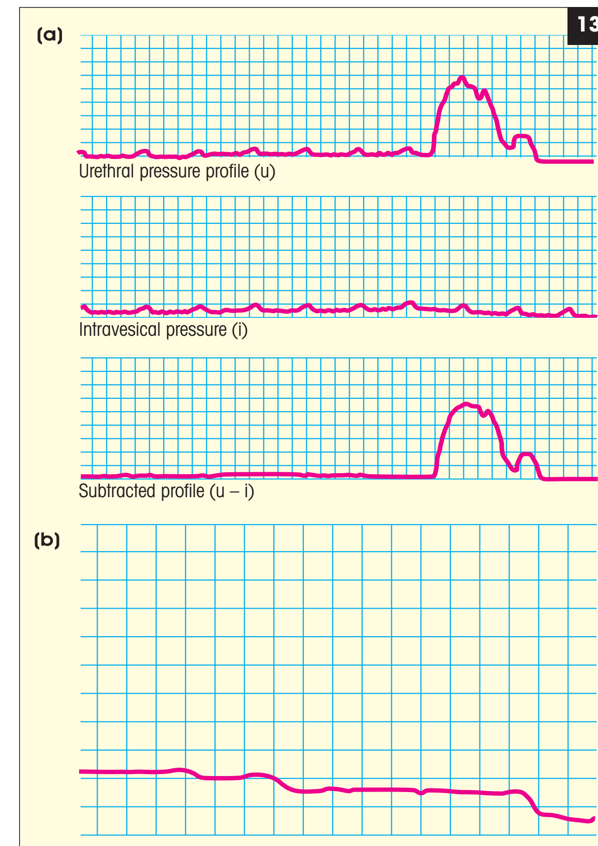
Techniques included within urodynamics are:

- Urethral pressure profilometry ('resting' or 'stressed').
- Cystometry.
- Electromyography.

In small animal practice, most work has been done on resting urethral pressure profilometry and indications for other urodynamic techniques are uncommon. However, all of these techniques are subject to a wide variety of artefacts and variations in the results obtained. Apart from the fact that they are not available in most veterinary practices (and unlikely to be so in the foreseeable future), they have proved to be of less diagnostic value than was first hoped. Therefore, a brief description only will be given of urethral pressure profilometry (**129**, **130**). There is additional information in the Further Reading section at the end of this book.



129 During urethral pressure profilometry, a catheter with a pressure-measuring device at or near the tip is inserted into the bladder and then withdrawn along the urethra (**a**). The pressure can be measured by the resistance to outflow of fluid from catheter side holes (perfusion profilometry), pressure changes within balloons or using microtip pressure transducers. The latter are preferable but are expensive. As the catheter passes along the urethra, a graph of pressure against distance is produced on a recorder: this is the resting urethral pressure profile (**b**). A number of variables, defined by the International Continence Society, can be measured from the graph.



130 Subtracted urethral pressure profile recordings from a continent bitch (**a**) and an incontinent bitch (**b**). A 'subtracted' profile makes allowances for variations in intravesical pressure such as those due to respiration (regular small peaks). Subtracted profilometry involves the simultaneous measurement of intraurethral and intravesical pressures. The intravesical pressure is continuously subtracted from the intraurethral pressure to give a more accurate representation of the urodynamic event, the subtracted urethral pressure profile. This is summarized in **a**, but **b** shows only the subtracted profile. The profile from the

continent animal conforms to the previous diagram (**129**), but urethral resistance in the incontinent bitch is less than intravesical pressure throughout the profile. It would be nice if all profiles were as diagnostic of good or poor urethral function as these two examples, but, unfortunately, this is not the case.

Problems

In order to minimize variations, it is imperative that a standard technique is used. Following study of variables such as the position of the animal, orientation of the catheter within the urethra, method of chemical restraint, size of catheter, catheter material, catheter withdrawal rate and degree of bladder filling, the following standards have been suggested:

- The animal should be positioned in right lateral recumbency.
- The catheter should be orientated so that the side hole (if a perfusion technique is used) or microtip transducer is facing dorsally within the urethra.
- The catheter should be as narrow and soft as possible; in practice, this usually means a 3–4Fr polyurethane catheter in cats and a 6–8Fr silicone rubber catheter in dogs. Softer catheters are available, but they are more difficult to insert and, if microtip catheters are used, more liable to damage.
- A standard method of chemical restraint should be used in all cases. However, a perfect anaesthetic or relaxant (i.e. one that has no effect on the urodynamic variables being measured) has yet to be discovered.
- The catheter should be withdrawn at a speed of 1 mm/second or less.
- The bladder should be filled with sterile saline or emptied of urine to achieve a resting intravesical pressure of 5 cm H_2O.

Once the method has been standardized, it is important to determine the range of normality using that method, since this will vary from centre to centre. Meaningful conclusions can only be drawn when normal values are known.

Belt driven catheter withdrawal devices are unsuitable, since they do not prevent the weight of the catheter or its lead from pulling the catheter from the urethra. It is preferable to use a rod driven device, which clips directly on to the catheter (**131**).

The use of a single sensor on the catheter makes the assumption that intravesical pressure remains constant as the sensor passes along the urethra. This problem can be overcome by the use of two sensors – 'simultaneous' urethral pressure profilometry (**130–132**).

131 The problems of inadvertent pulling of the catheter from the urethra and catheter rotation are overcome by the use of a rod driven device that clips directly onto the catheter.

132 Double sensor catheter used to measure (simultaneously) intravesical and urethral pressures. The microtip transducer at the tip of this silicone rubber catheter remains in the bladder throughout profilometry, while the second sensor starts in the bladder but then passes along the urethra during withdrawal. An electronic subtraction unit in the recorder continuously subtracts the intravesical pressure from the urethral pressure, resulting in a more accurate 'subtracted' pressure profile (see **130**). In most animals in which urethral pressure profilometry is indicated (i.e. incontinent large breeds of female dog with suspected urethral sphincter mechanism incompetence), the tip and second sensors should be separated by 8 cm to allow the tip sensor to remain in the bladder throughout profilometry.

Endoscopy

Indications

Although laparoscopy can be applied to small animal urological cases, the most commonly used endoscopic technique is urethrocystoscopy (**133, 134**). The indications, therefore, are suspected lesions of the bladder or urethra. In humans, surgical procedures (e.g. transurethral prostatic resection) can be performed endoscopically. Unfortunately, the smaller size of the canine urethra means that endoscopic procedures are usually limited to obtaining biopsy material (unless the endoscope is inserted via a cystotomy), since the diameters of resectoscopes are usually too great to permit introduction into the urethras of small animals. Smaller, paediatric resectoscopes are becoming available, which may allow resection techniques in small animals in the future.

The main advantage of urethrocystoscopy is the ability to visualize, and thus take an accurate biopsy from, urethral or bladder lesions. If lithotrypsy equipment is available, endoscopic fragmentation of vesical calculi can be performed. The cystoscope can also be used to guide catheters into the ureters, thus allowing sampling of urine from individual kidneys (e.g. in suspected cases of idiopathic renal haemorrhage) as a less traumatic alternative to cystotomy.

Techniques

A variety of equipment is available and most is designed for use in humans rather than animals. It is also rather expensive. Urethrocystoscopy technique will not be described in this book; rather, the reader is referred to the more extensive descriptions of the apparatus available and its use in the sections on urethrocystoscopy by Brearley *et al* (1991), Senior (1999) and McCarthy (2005).

Problems

Urethrocystoscopy is associated with the same potential problems as introduction of other instruments into the lower urinary tract (see Urethral catheterization, p. 38) and is contraindicated in the presence of acute bacterial infections, which it may exacerbate.

133 Urethroscopic appearance of a urethral carcinoma in a bitch. This bitch was dysuric and the urethra can be seen to be almost completely occluded by abnormal, white, vascular tissue. This lesion was readily detectable on retrograde positive contrast vagino-urethrography and the diagnosis was confirmed by means of a catheter biopsy.

134 Urethrocystoscopy does allow more accurate collection of biopsy material if the lesion is more discreet, as in this case of localized, diphtheritic cystitis. In this German Shepherd Dog bitch, double contrast cystography had revealed a proliferative lesion near the bladder neck ('skylined' in this illustration). It would have been difficult to obtain an accurate biopsy using the catheter biopsy technique in this case.