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# Urinary bladder blood flow

## I. Comparison of clearance of locally injected $^{99m}$Technetium pertechnate and radioactive microsphere technique in dogs

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**Summary.** The blood flow of the dog urinary bladder measured by radioactive microsphere technique was compared to the clearance of locally injected $^{99m}$Technetium pertechnate ($^{99m}$Tc) in the bladder wall. In semilogarithmic plots the $^{99m}$Tc washout curves showed a multiexponential course. From the initial slopes (median 5.7 min) the bladder blood flow was calculated to be only 30–62% of the results obtained from the radioactive microsphere technique (blood flow in the muscular layer 21.7–44.8 ml/100 g/min). These lower values imply that the rate of removal of the hydrophilic tracer $^{99m}$Tc at these flow rates is limited by the capillary diffusion capacity. The multiexponential curves reflect recirculation and excretion of $^{99m}$Tc by the kidneys with accumulation of $^{99m}$Tc in the bladder. It is concluded, that clearance studies of locally injected $^{99m}$Tc in the bladder wall are unable to evaluate bladder blood flow.

**Key words:** Urinary bladder blood flow – $^{99m}$Technetium pertechnate – Radioactive microsphere technique – Distension

From experimental in-vitro studies in different animals it is obvious that the detrusor muscle is sensitive to changes in the oxygen/blood supply and that ischemia can result in irreversible changes in the detrusor function. Bladder necrosis has been described following urinary retention and overdistension. But it is unknown whether the increased functional demand on the detrusor muscle in different diseases of the lower urinary tract causes any change in the blood supply to the bladder.

The blood flow of the urinary bladder is only sparsely investigated in animals both regarding absolute values and tissue distribution but also the effect of bladder filling. Different methods as clearance of locally injected tracers [6, 9], transillumination [18], radioisotope labelled microsphere technique [10, 21] and measurements of the venous outflow by a drop counter [1] have been used.

The use of radioisotope labelled microsphere technique is an established way to determine blood flow [2, 3, 11], but this method is not applicable in humans. Clearance of locally injected $^{99m}$Technetium pertechnate ($^{99m}$Tc) has been recommended for measuring urinary bladder blood flow [6]. In order to develop a method for measuring human bladder blood flow, we compared the results obtained from clearance of $^{99m}$Tc locally injected in the dog detrusor muscle with the results obtained from the radioactive microsphere technique in the same dogs.

## Methods

### *Procedure*

Three mongrel dogs weighing between 20–30 kg were used. Anesthesia was introduced by thiomebumal 12.5 mg/kg i.v. An endotracheal tube was introduced and artificial respiration with atmospheric air was maintained with a respirator. The anesthesia was maintained with pentobarbital as necessary. Arterial oxygen saturation, oxygen and carbon dioxide tensions and pH were repeatedly measured. Fluid replacement was performed with isotonic saline. Heart rate and arterial blood pressure (Statham pressure transducer) were continously recorded. A catheter (Fr 6.0) was inserted into the left ventricle of the heart via the left femoral artery. The correct possition of the catheter tip was secured by pressure wave recording. Another catheter (Fr 6.0) was advanced into the abdominal aorta via the right femoral artery for reference blood sampling.

A polyethylene catheter (Fr 8.0) was inserted into the bladder transurethrally. The catheter was connected to a water column to measure the intravesical pressure. The water column equipped with three-way stop-cocks at 5, 10, 15 and 20 cm above the bladder level allowing constant bladder pressures at these levels. Isotonic body-warm saline was used to distend the bladder.

A lower paramedian abdominal incision was made to expose the bladder. Blood flow measurements were performed at least 30 min after this.

### *The radioactive microsphere method*

The microsphere method with reference blood sampling [4, 11] was used to measure the regional blood flow. 15 μ microspheres

**Table 1.** The urinary bladder blood flow evaluated by clearance of locally injected $^{99m}$Tc and by radioactive microsphere technique at different intravesical pressures

| | Intravesical pressure (cm water) | Bladder blood flow (ml/100 g/min) | | |
|---|---|---|---|---|
| | | Clearance of $^{99m}$Tc | Microsphere technique | |
| | | | muscularis | mucosa |
| Dog A | 1 | 16.0 | | |
| | 5 | 14.3 | 38.4 | 21.2 |
| | 10 | 13.9 | 24.5 | 23.7 |
| Dog B | 5 | 14.1 | 38.2 | 68.8 |
| | 10 | 13.4 | 21.7 | 49.6 |
| Dog C | 5 | 11.0 | | |
| | 5 | 10.7 | 34.3 | 31.3 |
| | 10 | 13.4 | 44.8 | 59.3 |
| | 15 | 13.0 | | |

(NEN-TRAC, New England Nuclear) labelled with $^{103}$Ru and $^{46}$Sc were used. A volume of 4 ml microsphere solution containing $5.0 \times 10^6$ spheres was used at each flow determination. The spheres were vigorously shaken on a Vortex mixer for 5 min immediately before injection. The spheres were injected into the left ventricle over a period of 30 s. Starting about 15 s before the injection and continuing for about 2 min after the end of the injection a reference blood sample was drawn from the aortic catheter with a constant flow of 2.0 ml/min.

The dogs were sacrified with a dose of saturated potassium chloride. The bladder was totally resected and very carefully separated into mucosa and muscularis. The tissue was divided into approximately 2 g pieces and placed in preweighed plastic vials to a maximum height of 2 cm for gamma radiation counting. The reference blood samples and tissue samples were counted in a 2-channel scintillation system with the channels set for the 2 principal energies for the two isotopes. The counts in each channel were corrected for background and cross talk from the other isotope. The total counts for the single reference blood sample and the single tissue sample were calculated. The regional blood flow rate was calculated using the equation [11]:

$$\text{Regional blood flow rate (ml/100 g/min)} = \frac{\text{counts/100 g tissue} * V}{\text{counts of reference sampling}}$$

where V is the reference sample rate (ml/min).

### *The local isotope clearance method*

$^{99m}$Technetium-pertechnate ($^{99m}$Tc) was used. $^{99m}$Tc is a diffusible hydrophilic tracer with a molecular weight of 163 and a half-life of 6 hours. Initially is about 70% of intravenous injected pertechnate ions bound to plasma proteins. This binding is weak and reversible. The pertechnate binding plasma proteins have generally a molecular weight greater than 70.000 and therefore are confined to circulate in the vascular compartment. The free pertechnate ions diffuse rapidly to the interstitial fluid through pores in the capillary membrane. When the concentration of the free pertechnate ions in the vascular compartment decrease, it causes a rapid release of weakly protein-bound pertechnate. Furthermore, pertechnate is removed from the vascular compartment by the stomach, thyroid, bowel, salivary glands, choroid plexus and by the kidneys [14, 19].

By injection of $^{99m}$Tc locally in the bladder wall the same weak and reversible binding to proteins in the tissue is assumed. The free pertechnate ions diffuse rapidly to the vascular compartment and diffusion equilibrium of pertechnate ions across the capillary wall is rapidly achieved.

Assuming a single homogenous tissue with steady flow and diffusion equilibrium between the tissue in the counting field and the blood leaving it, capillary blood flow can be expressed per 100 g of tissue by:

$$f = (\ln 2/t_{1/2}) * \lambda * 100 \text{ (ml/100 g/min)}$$

where $t_{1/2}$ is the half-time measured from the washout curve and $\lambda$ is the partition coefficient between the tissue and blood [15]. The $\lambda$-value for $^{99m}$Tc was assumed to be 1.0. The isotope solution of 0.05 ml was with great care slowly injected in the detrusor muscle through a 26 G needle. The needle was left in situ for 10–15 s after the injection before withdrawal. About 15 min before the injection the bladders were distended to the wanted intravesical pressure.

The clearance of $^{99m}$Tc was measured by a collimated sodium iodide detector placed 10 cm above bladder level. The collimation was wide to obtain count rates from the whole bladder and to secure stable geometry during the washout. Only gamma emission around the 142 KeV peak of $^{99m}$Tc was recorded. The crystal was connected to a digital ratemeter. The counting of radioactivity began immediately after the injection. The counts were accumulated over 20 s, corrected for background and plotted in semilogarithmic diagrams. When the injection was repeated in the same dog, the counts were corrected for residual activity in the bladder wall. The biological half-time was determined by fitting a straight line by eye to the recorded points.

## Results

Table 1 shows the results obtained from both methods. The microsphere results showed that the muscularis blood flow in all 3 dogs at an intravesical pressure of 5 cm water ranged from 34.3–38.4 ml/100 g/min. When the pressure was raised to 10 cm water, the muscularis blood flow decreased by about 30% in two dogs but increased about 30% in one dog. The mucosa blood flow showed greater variance: from 21.2–68.8 ml/100 g/min at a pressure of 5 cm water, and with the pressure raised to 10 cm water, the mucosa blood flow was either unchanged, decreased about 30% or increased about 100%. None of the microsphere injections caused any changes in blood pressure or heart rate.

None of the semilogarithmic plots of $^{99m}$Tc washout curves showed a monoexponential course (Fig. 1). Only the first 4.2–9.2 min (median 5.7 min) of the curves could be fitted to straight lines. Blood flow calculated from these initial slopes varied from 10.7–16.0 ml/100 g/min. In the first two dogs there was a little decrease in blood flow, when the intravesical pressure was raised to 10 cm water. But in the last dog the blood flow increased a little with increasing pressure. The bladder pressures were kept constant during the investigations. One detector placed over the leg muscle showed no significant increase in radioactivity with time. The absorbed $^{99m}$Tc recirculated and was excreted through the kidneys. It could be measured by emptying the bladder. Thus, the multiexponential course of the washout curves is mainly due to the accumulation of $^{99m}$Tc in the bladder.

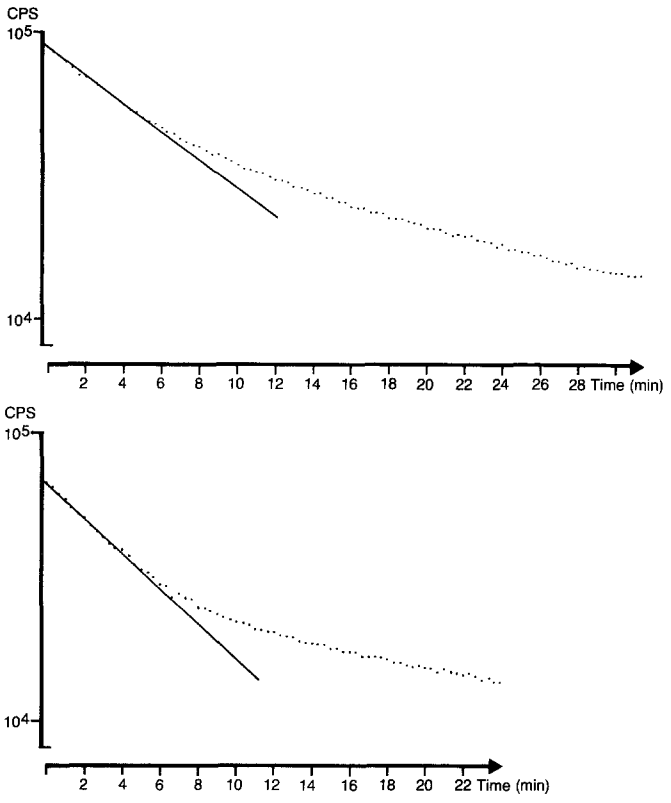


**Fig. 1.** Two representative $^{99m}$Technetium washout curves in semilogarithmic plots

## Discussion

The radioactive microsphere technique is an established method of measurement of regional organ blood flow [2–4, 10, 11]. Only about 1% of microspheres sized 15 μ are found to bypass the systemic circulation [2, 8, 11, 24]. Therefore, the use of 15 μ microspheres results in a measure of the capillary blood flow. Our results for the detrusor muscle in undistended bladders are higher than others reported in the literature (Table 2). It is not known whether the urinary bladder contains arterio-venous (AV) shunts, so maybe our microsphere results underestimate the true blood flow. A reliable estimate of the total organ blood flow including the blood flow in possible AV shunts should use microspheres greater than 150 μ, but because of effects on the circulation it is not practicable [11]. In the present study the stomach and renal blood flow determined by the microsphere technique gave results in agreement with microsphere results reported elsewhere.

The spheres were injected into the left ventricle and this should be sufficient to ensure a thorough mixing when measuring flow in peripheral tissues [3]. No difference between heart rate and blood pressure measured immediately before and after the injection of spheres and before and after distension of the bladder could be demonstrated. According to Buckberg et al. [3] the statistical variation in flow determinations is 10% when 400 spheres are found in the tissue biopsy as well as in the reference blood sample. The number of spheres used in the present study was sufficient to measure tissue blood flow with an accuracy of at least 10%.

With microsphere technique Nemeth et al. [21] found a mucosa to muscularis blood flow ratio of 13:1 which decreased to 2.3:1 at an intravesical pressure of 20 cm water. Total bladder flow decreased 20%. By measuring the venous outflow in cats, Andersson et al. [1] found a sustained increase in blood flow with distension. We found that the blood flow of the undistended detrusor muscle was the same in all dogs. The mucosa muscularis blood flow ratio varied from 1:2 to 2:1. When the bladder was distended to a pressure of 10 cm water, the muscularis blood flow decreased 30% in two dogs and increased 30% in the last. The distension caused a very varying change in mucosal blood flow. Possibly, this could be attributed to real differences in the single animals.

Microangiographic studies of the bladder blood flow have given different results. Sarma [22] found a very sparse perfusion of the mucosa, whereas Hohlbrugger [12] showed a considerably better perfusion of the mucosa than of the muscularis. Thus, the mucosa muscularis blood flow ratio in undistended and distended bladders is not adequately clarified.

Clearance of locally injected radioactive tracers for measurements of blood flow have been applied to many organs as the skin, the subcutaneous tissue, the skeletal muscle, the prostate, the uterus and the myocardium [17]. Clearance of locally injected $^{99m}$Technetium-Pertechnate has in particular been used in the evaluation of the skin blood flow and of the skin perfusion pressure [5, 13, 20].

Dunn [6] studied the bladder blood flow in rabbits by measuring the clearance of locally injected $^{99m}$Tc. In semilogarithmic plots he found monoexponential clearance curves over two decades. The bladder blood flow was reduced to the half at an intravesical pressure of 20 mmHg, to a quarter at a pressure of 40 mmHg and to 1/20th at a pressure of 80 mmHg. However, in our study

**Table 2.** Urinary bladder blood flow

| Authors | Methods | Results (ml/100 g/min) |
|---|---|---|
| Andersson et al. [1] (cats) | Venous outflow – dropcounter | 7.0 ± 0.8 (mean, SEM) |
| Dunn [6] (rabbits) | Local clearance of $^{99m}$Tc | 18.2 |
| Nemeth et al. [21] (dogs) | Microsphere technique | 19.0 (mean) |
| Gatenbeck et al. [10] (rats) | Microsphere technique | 14.0 ± 9 (mean, 1 SD) |
| Finkbeiner et al. [9] (dogs) | Local clearance of $^{85}$Kr | 21.2 ± 7.4 (mean, 1 SD) |

only the first median 5.7 min were fitted to straight lines, because $^{99m}$Tc was rapidly excreted through the kidneys and accumulated in the bladder. The bladder blood flow calculated from these initial slopes was only 30–62% of the values obtained from the simultaneously performed microsphere technique. Even if correction for final slope was attempted flow values were lower than the microsphere figures. These low figures can be explained by the fact that $^{99m}$Tc, although a small hydrophilic tracer, is restricted to diffuse through waterfilled pores that occupy a very small fraction of the capillary wall. Especially at high blood flow (greater than 20 ml/100 g/min), the clearance rate of the hydrophilic tracers is a measure of the capillary permeability and not of the blood flow [16, 23].

$^{133}$xenon is a freely diffusible lipophilic inert gas, which is eliminated from the body via the lungs and recirculation is therefore negligeable. Further studies should be conducted to compare the results from clearance of locally injected $^{133}$Xenon with the results obtained from simultaneously performed microsphere technique. The Xenon clearance technique might be used in humans although trapping in perivesical fat might be a source of error [7].

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