**TITLE:**

Highly Sensitive Measurement of Glomerular Permeability in Mice with Fluorescein Isothiocyanate-polysucrose 70

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**KEYWORDS:**

Glomerular permeability, polysucrose, tracer, albuminuria, mouse urinary catheter, mouse central venous catheter, slit diaphragm, glomerulus

**SUMMARY:**

Here, we present a protocol to test glomerular permeability in mice using a highly sensitive, nonradioactive tracer. This method allows repetitive urine analyses with small urine volumes.

**ABSTRACT:**

The loss of albumin in urine (albuminuria) predicts cardiovascular outcome. Under physiological conditions, small amounts of albumin are filtered by the glomerulus and reabsorbed in the tubular system up until the absorption limit is reached. Early increases in pathological albumin filtration may, thus, be missed by analyzing albuminuria. Therefore, the use of tracers to test glomerular permselectivity appears advantageous. Fluorescently labeled tracer fluorescein isothiocyanate (FITC)-polysucrose (i.e., FITC-Ficoll), can be used to study glomerular permselectivity. FITC-polysucrose molecules are freely filtered by the glomerulus but not reabsorbed in the tubular system. In mice and rats, FITC-polysucrose has been investigated in models of glomerular permeability by using technically complex procedures (i.e., radioactive measurements, high-performance liquid chromatography [HPLC], gel filtration). We have modified and facilitated a FITC-polysucrose tracer-based protocol to test early and small increases in glomerular permeability to FITC-polysucrose 70 (size of albumin) in mice. This method allows repetitive urine analyses with small urine volumes (5 µL). This protocol contains information on how the tracer FITC-polysucrose 70 is applied intravenously and urine is collected via a simple urinary catheter. Urine is analyzed via a fluorescence plate reader and normalized to a urine concentration marker (creatinine), thereby avoiding technically complex procedures.

**INTRODUCTION:**

Functional or structural defects within the glomerular filtration barrier increase glomerular permeability to albumin, resulting in the detection of albumin in the urine (albuminuria). Albuminuria predicts cardiovascular outcome and is an important marker for glomerular injury1. Even low levels of albuminuria, lying within the normal range, are associated with an increased cardiovascular risk1.

Under physiological conditions, albumin is filtered through the glomerulus and is almost completely reabsorbed in the tubular system2,3. In mice, the detection of albumin in the urine is usually performed by an albumin enzyme-linked immunosorbent assay (ELISA) from 24 h of urine collection. If urine from a 24 h urine collection or spot urine is used, small differences in albumin concentrations may be missed due to assay sensitivity problems. Most researchers, therefore, use animal models in which albuminuria is induced by robust renal injury due to toxins, drugs, and renal surgery.

Therefore, the finding of a sensitive method to detect small and transient changes in glomerular permeability is very important to the field. Rippeet al. have presented a rat model to test glomerular permeability by applying a fluorescently labeled tracer, namely FITC-polysucrose 70 (i.e., FITC-Ficoll 70), at the size of albumin4. The tracer application allows the testing of short-term changes in glomerular permeability (within minutes) and is very sensitive4. Two studies have used the tracer method in mice5,6. Despite its benefits, this method, unfortunately, has disadvantages: it is technically very complex, radioactive, and invasive. Further analysis of the urine is only accomplished by using gel filtration or size-exclusion HPLC5,6.

Within this paper, we present an alternative, sensitive, nonradioactive, and fast method to measure glomerular permeability in mice using fluorescently labeled FITC-polysucrose 70. By introducing a transurethral catheter, urine collection is less invasive than bladder puncture, urethrotomy, and suprapubic catheter application, and allows urine collection at least every 30 min. Urine analysis is performed from small amounts (5 µL) using a fluorescent plate reader. Tracer concentrations in the urine are normalized to creatinine concentrations in the urine using an enzymatic creatinine assay.

Therefore, this novel method offers a sensitive tool to study early glomerular injury with increased glomerular permeability.

**PROTOCOL:**

The investigations were conducted according to the guidelines outlined in the Guide for Care and Use of Laboratory Animals (US National Institutes of Health Publication No. 85-23, revised 1996). All animal experiments were performed in accordance with the relevant institutional approvals (state government Landesamt für Natur, Umwelt und Verbraucherschutz [LANUV] reference number 84-02.04.2012.A397).

1. **Preparation of instruments, solutions, and equipment**
   1. Reconstitute FITC-polysucrose 70 with 0.9% sterile sodium chloride (NaCl) to a final concentration of 10 mg/mL (i.e., 100 mg in 10 mL of NaCl).
   2. Dialyze FITC-polysucrose 70 solution to remove free FITC molecules overnight at 4 °C (molecular weight cut-off [MWCO] at 10,000). Use 1 L of 0.9% sterile NaCl per 10 mL of FITC-polysucrose 70 under constant stirring. Protect from light. Aliquot the dialyzed FITC-polysucrose 70 and store it at -20 °C.
   3. For the FITC-polysucrose 70 bolus, add 4 µL of 10 mg/mL FITC-polysucrose 70 solution to 996 µL of 0.9% NaCl (the final concentration of FITC-polysucrose 70: 40 µg/mL).
   4. For the equilibration infusion solution, add 20 µL of a 10 mg/mL FITC-polysucrose 70 solution to 9.98 mL of 0.9% sterile NaCl yielding to a final concentration of 20 µg/mL.
   5. For the experimental solution, add drugs or substances to the infusion solution (e.g., for angiotensin II [Ang II] [100 ng/kg/min] for a 25 g mouse, add 3 µL of Ang II of a 1 mM solution).
   6. For the surgery, prepare one shaver, two surgical clamps, one pair of surgical scissors, two tweezers, two fine tweezers, one pair of fine scissors, and swabs. Prepare two 10 cm silk threads (4-0 to 6-0) for ligation procedures.
   7. For the placement of a central venous catheter, prepare a 10 mL syringe with a 21 G needle. Place the tip of the needle in a 30 cm-long catheter (with an inner diameter [ID] of 0.58 mm). Connect the 0.58 mm catheter to a 10 cm catheter (with an ID of 0.28 mm). Cut the tip of the smaller catheter oblique to create a sharp tip that is introduced into the jugular vein.
   8. Prepare the anesthesia (i.e., intraperitoneal anesthesia ketamine, 100 mg/kg of body weight, and xylazine, 5 mg/kg of body weight).
   9. Prepare a 22 G angiocatheter by discarding the needle and marking the catheter 1 cm from the tip. Preheat the heating pad to 37 °C.
   10. Prepare a blood pressure device and change the blood-pressure-measuring membrane if necessary.
2. **Preparation phase**
   1. **Urinary catheter**

NOTE:Section 2.1 follows the protocol as described by Reis et al.7. **Figure 1** and **Supplemental Figure 1** show the placement of a urinary catheter in female mice.

* + 1. Anesthetize the mouse with ketamine/xylazine (see step 1.8). Use the toe-pinch test to confirm proper anesthesia. To maintain anesthesia, repeat the anesthesia (e.g., with half the dosage) after 60 min.

NOTE: Female FVB mice are used in this protocol.

* + 1. Position the mouse in dorsal recumbency on a 37 °C heating pad. Tighten the lower abdomen and find the urethral ostium (e.g.,under a microscope).
    2. Use the plastic part of the catheter of a 22 G angiocatheter and lubricate it with xylocaine gel. Introduce it carefully 3 mm into the urethral ostium while paralleling the distal urethral axis (**Figure 1A**).
    3. Turn the top of the angiocatheter 180° by keeping the tip within the urethral ostium and maintaining the axis of the urethra (**Figure 1B**).
    4. Introduce the catheter 7 mm further into the mouse so that it is placed within the bladder (**Figure 1C**). Do not force the catheter beyond resistance. Correct the position of the catheter from the beginning if resistance is felt. Take note that if the position of the urinary catheter is correct, urine might already appear within the catheter.
    5. Place a 1.5 mL brown tube over the top of the angiocatheter to collect the urine. Apply 1 mL of 0.9% NaCl intraperitoneally to enhance urine production.
  1. **Central venous catheter** 
     1. Shave the neck of the mouse and place it in recumbency with the head toward the surgeon. Hyperextend the head of the mouse with a tape.
     2. Disinfect the neck with 70% isopropanol. Make a small skin incision (5 mm) below the jawline, using a tweezer and a pair of scissors. Cut the skin approximately 1 cm in the direction of the sternum until the middle of the sternum is reached.
     3. Carefully dissect the skin on the left side of the neck, using a pair of scissors. Make a rectangular incision of the skin to the right side of the mouse to expose the soft tissue of the neck. Use a pair of scissors and a tweezer. Fix the skin flaps with two clamps.

NOTE: The jugular vein runs along the left side of the thyroid gland or is slightly covered by the right lobe of the thyroid gland. After this step, use a microscope for surgery.

* + 1. Carefully expose the jugular vein by blunt preparation, using the tip of the fine tweezer. Avoid injury to vein branches.

NOTE: It might be necessary to remove tissue with fine scissors. Be careful when using scissors to remove tissue as it increases the risk of bleeding.

* + 1. Place and close a ligature with a silk thread (4-0 to 6-0) at the distal part of the visible jugular vein (toward the head of the mouse). Put tension on the ligature by fixing the silk thread with a tape to ensure slight tension on the jugular vein. Prepare a ligature around the proximal part of the jugular vein.
    2. Fill the catheter with the equilibration infusion solution (see step 1.4) and fix the catheter with a tape so that the catheter is aligning the jugular vein. Control for bubbles to avoid air embolism.
    3. Lift the jugular vein with fine tweezers at the site of insertion (the insertion site is 1–2 mm proximal of the ligature). Align the tubing parallel to the jugular vein. Puncture the jugular vein, aiming for the lumen, and insert for approximately 2–4 mm, paralleling the axis of the jugular vein. Avoid any brisk movements.
    4. Close the ligature to fix the catheter. Control for tightness of the ligature by carefully viewing through the microscope. Put a damp swab over the site of surgery.
  1. **Blood pressure measurement**
     1. Place the tail-cuff at the bottom of the mouse tail while the mouse lays in dorsal recumbency. Start the measurements and repeat them 10x per time point. Build a mean from the measurements.
     2. Adjust the position of the tail-cuff if blood pressure measurements do not seem correct and false data are produced.

1. **Equilibration phase**
   1. Change the urinary collection tube before the equilibration phase starts and place it on ice (urine 0 min).
   2. Introduce a 10 mL syringe filled with FITC equilibration phase solution (see step 1.4) with a 21 G needle and the larger catheter (with an ID of 0.58 mm) and place it into the syringe pump.
   3. Fold a swab and put it around the small-vessel catheter (with an ID of 0.28 mm) that is introduced into the jugular vein. Lay the catheter inside the swab. Place a clamp over the swab to abolish retrograde blood flow or air embolism. Connect a 27 G needle with a 1 mL syringe filled with the FITC bolus to the end of the small-vessel catheter.
   4. Open the clamp and apply the FITC bolus (100 µL). Close the clamp again and connect the larger catheter with the smaller catheter. Start the syringe pump with 0.008 mL/min (0.480 mL/h). Continue the infusion for 60 min.
2. **Experimental phase**

NOTE: In this phase, the effect of drugs on glomerular permselectivity can be investigated.

* 1. Change the urinary tube (time point 0 min) to another 1.5 mL brown tube. Put a swab around the small-vessel catheter and close a clamp as indicated in step 3.3.
  2. Disconnect the large catheter from the small-vessel catheter. Change the syringes to the experimental phase solutions (see step 1.5). Let the infusion pump run so that the large catheter is filled with the equilibration solution.
  3. Reconnect the catheters while avoiding air embolism and reopen the clamp. Start the syringe pump at 0.008 mL/min.
  4. Continue running the syringe pump for 60 min and collect the urine within the urinary tube thereafter (urine 60 min). Place the urine tube on ice.
  5. Sacrifice the mouse by decapitation during anesthesia.

1. **Urine analysis**
   1. **Fluorescence measurement**
      1. Thaw the urinary pool and dilute it 1:10 with phosphate-buffered saline (PBS).

NOTE: The urinary pool is a pool of urine that has been collected from healthy mice in a 12–24 h urine collection in a metabolic cage.

* + 1. Prepare standards for the fluorescence measurement. Take 10 brown 1.5 mL tubes and label them for blank (1:10 diluted urine pool) and the following FITC-polysucrose 70 concentrations (0.625, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 µg/mL).
    2. Pipet 246 µL of diluted urine pool into a 1.5 mL brown tube and add 4 µL of the FITC-polysucrose 70 stock concentration (see step 1.1) to get a final concentration of 160 µg/mL FITC-polysucrose 70. Pipet 100 µL of the diluted urine pool into all other tubes.
    3. Dilute 1:2 by pipetting 100 µL of the 160 µg/mL FITC-polysucrose 70 tube into the 80 µg/mL tube and continue with the other concentrations. Assure proper mixture of the diluted concentrations (e.g., by vortexing the tube before continuing).
    4. Dilute the mouse urine samples (0 min, 60 min) 1:10 with diluted urinary pool.
    5. Pipette 5 µL of each standard FITC-polysucrose 70 concentration and of the mouse urine samples as triplicates in a black 384-well plate. Centrifuge the plate at 1,000 x *g* for 15 s to avoid bubbles.
    6. Analyze the 384-well plate in a plate reader. Perform excitation at 496 nm and measure the fluorescence at 525 nm.
  1. **Creatinine measurement**
     1. Follow the manufacturer’s instructions. In brief, prepare creatinine standards by diluting 10 µL of the 100 mM creatinine standard solution with 990 µL of creatinine assay buffer to prepare a 1 mM standard solution.
     2. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM creatinine standard solution into a 96-well plate to generate 0, 2, 4, 6, 8, and 10 nmol/well standards, respectively. Add creatinine assay buffer to each well to bring the volume to 50 µL.
     3. Prepare the reaction mixes by adding 42–44 µL of creatinine assay buffer, 2 µL of creatinase, 2 µL of creatininase, 2 µL of creatinine enzyme mix, and 2 µL of creatinine probe. For the blank, use 44 µL of creatinine assay buffer, 2 µL of creatinase, 2 µL of creatinine enzyme mix, and 1–2 µL of creatinine probe.
     4. Add 50 µL of the appropriate reaction mix to each well in the 96-well plate and incubate it for 60 min on a horizontal shaker at 37 °C. Protect the plate from light during the incubation. Measure the absorbance at 570 nm on a plate reader.
     5. Calculate the creatinine concentrations by subtracting the blank value from all readings. The result will have the unit nanomoles/microliter. Multiply the concentration of creatinine by the molecular weight of creatinine (113.12 ng/nmol) to receive the unit nanograms/microliter.

1. **Data analysis**
   1. Calculate the FITC-polysucrose 70 urine concentration from the standard curve.
   2. Reference the FITC-polysucrose 70 urine concentrations to creatinine concentrations in the representative urine sample.
   3. Reference the urine 60 min samples to urine 0 min samples of controls and treated mice.

**REPRESENTATIVE RESULTS:**

As depicted in **Figure 2**, the method to test glomerular permeability in mice is built up in three phases. The first phase is called the preparation phase, in which a urinary catheter and a central venous catheter are placed. The second phase is called the equilibration phase, starting with an intravenous bolus injection of FITC-polysucrose 70 and followed by the continuous infusion of FITC-polysucrose 70 for 60 min. The last phase is called the experimental phase. In this phase, the infusion of FITC-polysucrose 70 is continued and drugs or other substances can be tested for influencing glomerular permeability. Urine is collected at the end of each phase.

To investigate glomerular permeability within this tracer model, it is essential to place a urinary catheter properly and without injuring the mucosa. The placement of a urinary catheter into a female mouse bladder is demonstrated in **Figure 1**. The catheter is placed 3 mm into the urethral ostium, parallel to the craniocaudal urethral axis (**Figure 1A**). The catheter is turned 180° toward the tail of the mouse (**Figure 1B**) and introduced 7 mm further into the bladder, parallel to the murine spine (**Figure 1C,D**).

As described above, previous methods to test glomerular permeability in mice used HPLC to purify FITC-polysucrose 70 signals from urine samples5,6. As this method uses fluorescence measurement without a previous purification of the tracer, PBS, mouse urine, and FITC-polysucrose 70 in mouse urine were analyzed. **Figure 3A** shows the fluorescence scan of PBS with a fluorescence peak at 325 nm, which seems to be an effect of the excitation flash at 290 nm. The fluorescence scan of native mouse urine shows a fluorescence maximum at 395 nm (**Figure 3B**). FITC-polysucrose 70 dissolved in mouse urine displays a fluorescence maximum at 525 nm (**Figure 3C,D**). **Figure 3C** shows that mouse urine does not disturb the fluorescence measurement of FITC-polysucrose 70 in mouse urine. Increasing concentrations of FITC-polysucrose 70 show an increased fluorescence intensity (**Figure 3E**).

To prove that this method is capable of detecting differences in glomerular permeability, Ang II was applied in the experimental phase of the model. Ang II increased the glomerular permeability in mice 60 min after a continuous administration in nonblood-pressure-relevant dosages (**Figure 4A,B**). The increase in glomerular permeability due to Ang II could be blocked by an Ang II-receptor blocker (ARB), namely candesartan (**Figure 4A**). Ang II washout decreased glomerular permeability (**Figure 4A**). Blood pressure was measured via the tail-cuff method and did not show significant differences between the groups (**Figure 4B**). Polysucrose 70- and FITC-polysucrose 70-infused animals serve as controls for FITC-polysucrose 70- and Ang II-treated animals (**Figure 4C**).

**FIGURE LEGENDS:**

**Figure 1: Placement of a urinary catheter in female mice.** Lateral view of the mouse abdomen. (**A**) The marked and lubricated catheter is introduced 3 mm into the external urethral ostium of a female mouse. The craniocaudal axis of the urethra is paralleled with the catheter. Careful tension on the lower abdomen with the left index finger facilitates the introduction of the catheter into the external urethral ostium. (**B**) The catheter is turned 180° toward the tail of the mouse. The tip of the catheter remains 3 mm within the external urethral ostium. (**C**) The catheter is now introduced 7 mm further into the bladder. The direction of the catheter is aligned with the murine spine. (**D**) Ventral view of the mouse abdomen. The catheter is placed 10 mm into the mouse. (**E**) Urine appears with a correct insertion into the bladder.

**Figure 2: Schematic illustration of the experimental procedures on a timeline.** After narcosis of the mouse, a urinary catheter is placed. The central venous catheter is implanted and baseline urine is obtained (0 min). Afterward, the FITC-polysucrose 70 bolus is applied via the central venous catheter and a continuous infusion of FITC-polysucrose 70 is started. The equilibration phase for FITC-polysucrose 70 lasts 60 min. Urine is collected after the equilibration phase (0 min) and before the experimental phase starts. Within this phase, drugs or other substances (like angiotensin II) can be applied. At the end of the experimental phase, urine is obtained for analysis (60 min).

**Figure 3: Fluorescence of PBS and mouse urine with and without FITC-polysucrose 70.** (**A**) Fluorescence frequency scan (excitation of 290 nm, emission of 325–700 nm) of PBS shows a signal at 325 nm, which seems to be an effect of the excitation flash at 290 nm. (**B**) In the frequency scan of mouse native urine (urine pool diluted at 1:10 and 1:20), there is a signal with a maximum at 395 nm, probably caused by urinary protein autofluorescence. (**C**) Mouse urine containing FITC-polysucrose 70 (40 µg/mL) shows a signal peak at 525 nm that does not interfere with the measurement of the urine autofluorescence. (**D**) Magnification of the FITC-polysucrose 70 fluorescence peak depending on the emission wavelength. Different concentrations of FITC-polysucrose 70 (1.25, 2.5, 5, 10, 20, and 40 µg/mL) are displayed. (**E**) Increasing FITC-polysucrose 70 concentrations show an increase of fluorescence intensity at the emission of 525 nm.

**Figure 4: Angiotensin II (Ang II) increases glomerular permeability.** (**A**) Ang II significantly increases the glomerular permeability in mice, measured by FITC-polysucrose 70 detection in the mice’s urine (mean + SEM; *n* = 5; *p* < 0.004, tested by Kruskal–Wallis test). FITC-polysucrose 70 concentrations in the urine were referenced to urine creatinine concentrations. The white columns (0 min) represent FITC-polysucrose 70 levels before the start of Ang II stimulation, the black columns (60 min) represent FITC-polysucrose 70 levels 60 min after the start of Ang II stimulation, and the grey columns (120 min or +60 min) after an additional 60 min of Ang II stimulation or 60 min of Ang II washout. (**B**) Systolic blood pressure was monitored by the tail-cuff method (mean + SEM). No significant blood pressure differences between the control and Ang II-treated groups were noted. (**C**) Polysucrose 70 and FITC-polysucrose 70 do not alter glomerular permeability in mice significantly. Ang II increases glomerular permeability in mice significantly (mean + SEM; *n* = 7, *p* < 0.005). This figure has been modified from Konigshausen et al.8.

**Supplemental Figure 1: Schematic diagram of the placement of a urinary catheter in female mice.** Lateral view of the mouse abdomen. (**A**) The marked and lubricated catheter is introduced 3 mm into the external urethral ostium of a female mouse. The craniocaudal axis of the urethra is paralleled with the catheter. Careful tension on the lower abdomen with the left index finger facilitates the introduction of the catheter into the external urethral ostium. (**B**) The catheter is turned 180° toward the tail of the mouse. The tip of the catheter remains 3 mm within the external urethral ostium. (**C**) The catheter is now introduced 7 mm further into the bladder. The direction of the catheter is aligned with the murine spine. (**D**) Ventral view of the mouse abdomen. The catheter is introduced 3 mm into the external urethral ostium of a female mouse. (**E**) After the catheter is turned 180°, it is introduced 7 mm further into the mouse bladder. The direction of the catheter is aligned with the murine spine. This figure has been modified from Reis et al.7.

**DISCUSSION:**

The presented method enables the investigator to test glomerular permeability in mice in a very sensitive manner using a tracer. With this method, short-term increases in glomerular permeability can be diagnosed using only small amounts of urine. The most critical steps for successfully mastering this technique are 1) developing manual expertise in mouse surgery, especially in the cannulation of a central vein, 2) placing the urinary catheter without harming the mucosa, and 3) manual expertise in handling 384-well plates with small volumes of samples.

When placing the central venous catheter, it is essential that the catheter does not penetrate the jugular vein. To avoid penetration, we recommend putting tension on the jugular vein with a distal ligature (see step 2.2.7) and to lift the jugular vein with fine tweezers to extend the lumen of the jugular vein. To keep the insertion site small, try to avoid gross movements while inserting the catheter and always ensure the best view of the surgical field by removing extra tissue. The most critical step in the placement of the urinary catheter is the final insertion into the bladder. If resistance is felt, we recommend starting over from the beginning in order not to cause false tracks and injury to the mucosa. Catheter rotation, lubrication, and gentle movements, as well as training, help in difficult cases7.

FITC-polysucrose 70 is a fluorescently labeled, branched, and cross-linked polymer of sucrose and epichlorohydrin9. It behaves in solution as a globular molecule with a spherical shape and with a low shape asymmetry but with a molecular deformability9. Like other sucrose molecules, FITC-polysucrose 70 is filtered by the glomerulus and not reabsorbed in the tubular system4. To avoid free FITC molecules in the infusion, we dialyzed the FITC-polysucrose 70 solution before application to the mice. It is possible to store dialyzed FITC-polysucrose 70 molecules at -20 °C for months. As FITC-polysucrose 70 is applied intravenously in this and other protocols, it is essential that no blood contaminates the urine samples. Therefore, the urinary catheter needs to be placed with caution and anticoagulant substances within the experiment should be avoided. The standard curve for FITC-polysucrose 70 is dissolved in mouse urine pool to get the most accurate results. Due to concentration differences in the urine at different experimental time points and between groups, FITC-polysucrose 70 concentrations need to be referenced to a marker in the urine that reflects urine concentration (e.g.,creatinine). Interference of FITC-polysucrose 70 fluorescence with the measurement of creatinine in an enzymatic assay is unlikely.

The analysis of FITC-polysucrose 70 fluorescence should be performed in black 384-well plates as small urine volumes of 5 µL can be measured in these plates. We do not recommend reusing the same wells within the 384-well plates, even after washing the plates, as fluorescence is still measurable. As bubbles interfere with the fluorescence reading, the plates should be centrifuged before analysis. Alternatively, bubbles can be destroyed manually with the tip of a pipette.

The use of polysucroses to test glomerular permselectivity was described almost 40 years ago10. Fluorescently labeled polysucrose has been investigated in glomerular injury studies almost exclusively in rats in the past years4,11–20. This may have anatomical reasons due to easier surgical procedures in rats than in mice. In rats, urethrotomy is used to obtain urine samples4,5,9,12–14,21. To analyze plasma and urine samples, probes are subjected to high-performance size exclusion chromatography4,5,9,12–14,21. In addition, the glomerular filtration rate (GFR) is measured by radioactive 51Cr-ethylenediamine tetraacetic acid (EDTA)4,5,9,12–14,21. Two previous studies have applied FITC-polysucrose 70 in mice5,6. In mice, a suprapubic urinary catheter is introduced into the bladder6,20. Urinary and plasma probes are subjected to gel filtration before the analysis of FITC-polysucrose 70 fluorescence. Similar as in rats, GFR was analyzed via radioactivity6,20. The second publication concerning the application of FITC-polysucrose 70 in mice uses a model of peritoneal permeability and, therefore, did not analyze mouse urine for FITC-polysucrose 70 fluorescence22. The presented method was, therefore, modified to facilitate urine sampling and analysis. Urine samples can be easily obtained through a noninvasive transurethral urinary catheter. Urine analysis for FITC-polysucrose 70 fluorescence is performed without any previous high-performance size exclusion chromatography or gel filtration. By referencing FITC-polysucrose 70 fluorescence to mouse urine creatinine, the measurement of GFR with a radioactive assay is not needed.

Increases in blood pressure enhance glomerular permeability. Therefore, blood pressure monitoring is essential whilst investigating glomerular permeability. The most accurate blood pressure measurements in mice are performed via a central arterial catheter23 needing heparinization of the catheter to prevent clotting. We have experienced problems with hematuria after low-dose heparinization and urinary catheter placement. Therefore, we decided to perform the tail-cuff technique to measure blood pressure, which is noninvasive and does not need heparinization. Depending on the depth of anesthesia, blood pressure monitoring with the tail-cuff method is sometimes challenging. Blood pressure membranes need to be checked in advance and the position of the mouse should be optimized before blood pressure recording.

In addition to challenges in blood pressure measurement, this technique is also limited by producing arbitrary units of FITC-polysucrose 70. So far, this technique has only been investigated in animals and, therefore, its relevance to the human glomerular filter is still unknown. Time intervals to investigate increases in glomerular permeability depend on mouse urine production. Therefore, very short-term increases in glomerular permeability (in minutes) may be missed due to the lack of mouse urine production. In this protocol, FITC-polysucrose 70 is normalized to urine creatinine, which is removed from the blood mainly via glomerular filtration, but also by proximal tubular secretion. This will introduce an error when estimating glomerular permeability using this method, reducing the measured fractional clearance of polysucrose24.

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**DISCLOSURES:**

The authors have nothing to disclose.

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