Gravimetric Method for the Dynamic Measurement of Urine Flow (43636)

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Abstract. **The rate of urine formation is a primary index of renal function, but no techniques are currently available to accurately measure low rates of urine flow on a continuous basis, such as are normally found in rats. We developed a gravimetric method for the dynamic measurement of urine flow in anesthetized rats. Catheters were inserted directly into the ureters close to the renal pelves, and a siphon was created to collect all of the urine formed as rapidly as it was produced. Urine flow was determined by measuring the weight of the urine using a direct-reading analytical balance interfaced to a computer. Basal urine flow was measured at 2-sec intervals for 30 to 60 min. The dynamic response of urine flow to a rapid decrease in arterial pressure produced by a bolus intravenous injection of acetylcholine (0.5 μg) was also measured. Intrinsic drift, evaporative losses, and the responsiveness of the system to several fixed pump flows in the low physiologic range were evaluated *in vitro*. The gravimetric method described was able to continuously measure basal urine flows that averaged 37.3 ± 12.4 μl/min. Error due to drift and evaporation was negligible, totaling less than 1% of the measured urine flow. Acetylcholine-induced declines in arterial pressure were followed within 8 sec by a decline in urine flow. These data demonstrate that this new gravimetric method provides a simple, inexpensive, dynamic measurement of urine flow in the μl/min range.**

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In physiologic studies, it is often desirable to measure fluid flows that average considerably less than 1 ml/min. For example, the rate of urine formation in adult rats averages about 10–15 μl/min (1) and the rate of cerebrospinal fluid formation in dogs averages about 50 μl/min (2). Presently available flow-measuring devices based on electromagnetic or Doppler principles (3) do not have the resolution or accuracy necessary to measure such low flows continuously.

Other common methods of flow measurement include drop counters and volumetric measurement. Both of these techniques require collection of fluid at intervals and yield only time-averaged data. Drop counters usually consist of electrical devices that detect formation of drops and calculate either the number of drops per unit time or the reciprocal of the time between drops. The drop counter method is simple but cannot detect changes in flow that occur during the formation of a single drop. Timed collection of fluid via indwelling catheters, followed by volumetric measurement, is limited by the need to collect sufficient fluid for accurate and precise determination of volume. Thus with volumetric measurements, rapid changes in flow at low flows may not be detectable.

Because of the problems associated with drop counters and volumetric methods in the measurement of rapid changes of low flows, we developed a gravimetric method that uses readily available equipment to measure flow changes as low as 100 nl/unit time. We have successfully used this technique to measure the rapidity and magnitude of urine flow responses to acute changes in arterial pressure (4). This report describes our methodology and demonstrates its use in the measurement of urine formation in anesthetized rats.

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Methods

Experimental Set-up. The experimental set-up is illustrated in Figure 1. Segments of Silastic tubing (0.062 in i.d. × 0.095 in o.d.; Dow Corning Corp., Midland, MI) lead from each ureteral catheter through four-way stopcocks via a section of Silastic tubing (0.062 in i.d. × 0.125 in o.d.) and then glass tubing (0.084 in i.d. × 0.12 in o.d.) to deliver the urine to a polyethylene collection reservoir (radius 4.5 cm, height 2.0 cm) on the pan of an analytical balance (model GA110; Ohaus Corp., Florham Park, NJ). The glass tube enters the top of the balance cover through a hole (diameter, 8 mm) in the lid. A continuous column of fluid is established between the urine in each ureteral catheter and the collection reservoir on the pan of the balance by filling all tubing with saline and filling the reservoir to a level above the tip of the glass tube.

In order to measure urine flow with this method, all the urine that is produced must be collected; to accomplish this, a siphon was used. For complete collection of urine from a ureteral catheter in the dog, Ehmke *et al.* (5) applied negative pressure to the catheter at a level twice that at which urine flow became independent of the pressure. The peak urine flow in rats is 400–450 μl/min (6). Therefore, we applied sufficient negative pressure to siphon urine from the ureteral catheters at a rate twice this peak urine flow, i.e., at 1 ml/min. The siphoning pressure required to produce a flow of 1 ml/min with our set-up is −5 cm H_2O. In order to establish this pressure, the balance pan was positioned 5 cm below the level of the kidneys.

Data Acquisition. The weight of the urine detected by the balance was digitized each 2 sec, and the data transmitted via an RS232 output port to an AST premium 386/33 computer (model 5V; AST Research, Inc., Taiwan, ROC). The balance used in these studies had a resolution of 0.0001 g (100 μg), permitting the measure of changes in fluid volume as small as 100 nl. Data from the balance were stored in an ASCII file using Po-Ne-Mah digital acquisition software (Po-Ne-Mah digital acquisition, analysis and archive systems; Po-Ne-Mah, Inc., Storrs, CT) for subsequent off-line analysis.

***In Vitro* Testing.** *Drift.* Intrinsic drift was evaluated by placing a 10-g weight on the balance pan, and collecting data at 0.1 Hz for 60 min on three different days. Changes in weight were recorded, converted to volume equivalents, and then expressed as flows (μl/min) to evaluate the impact of drift on the measurement of flow.

Evaporation. The system was set up exactly as it would be at the start of a urine collection experiment except that no animal was used. Data were collected at 0.1 Hz for 60 min. The continuous decrease in weight that occurred was attributed to evaporation and was expressed as μl/min.

Fixed flows. A syringe-drive pump (model 600-910/920; Harvard Apparatus Co., Inc., Dover, MA) was used to deliver saline at rates comparable to the range of urine flows of an adult rat (approximately 2, 25, and 70 μl/min). Data were collected at 0.1 Hz for 30 min. These tests were conducted to determine the capability of the balance to record a range of low flows.

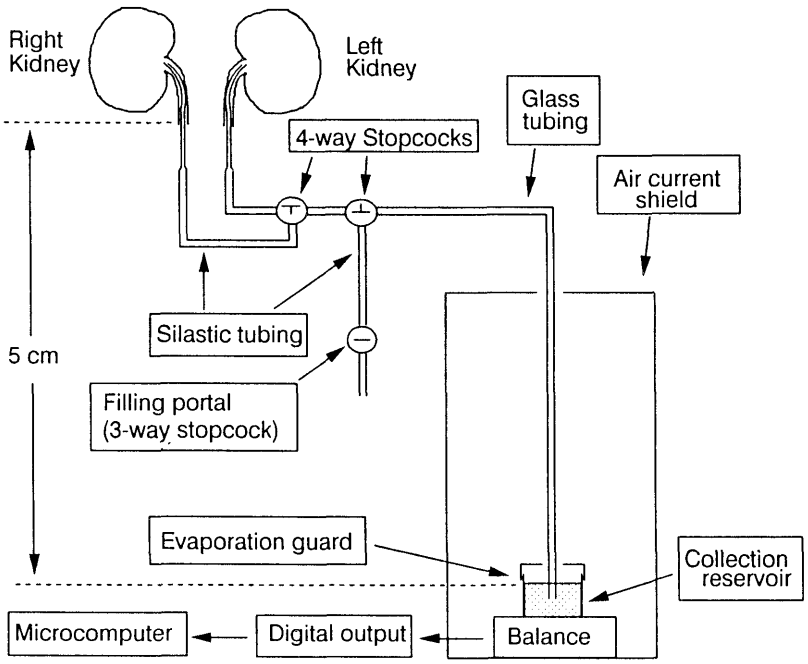

Figure 1. Schematic of method used for collection of urine on the pan of an analytical balance.

***In Vivo* Testing: Animal Preparation.** Six male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing 375–425 g were anesthetized with Inactin (thiobutabarbitol; BYK Gulden, Konstanz, Germany) (100–150 mg/kg, ip) or sodium pentobarbital (50–75 mg/kg, ip). The left femoral artery and vein were exposed. A catheter (PE-50; Clay-Adams, Inc., Parsippany, NJ) was placed in the femoral vein and saline was infused at 50–100 μl/min during the remainder of the surgery and the data collection period. The arterial catheter was constructed from a 25-cm piece of Tygon tubing (0.02 in i.d. × 0.06 in o.d.; Norton Plastics and Synthetics, Akron, OH) secured to a 5-cm piece of Teflon tubing (0.015 in i.d. × 0.027 in o.d.; Small Parts, Inc., Miami, FL) with Duro cement (Loctite Corp., Cleveland, OH). The ureteral catheters were constructed from three pieces of Silastic tubing (i.d. 0.012 in, 0.030 in, and 0.062 in, respectively) telescoped one into the other and secured with Silastic cement (General Electric Co., Waterford, NY). The length of the two smaller diameter pieces was 3 cm and the length of the largest diameter section was 10 cm. A midabdominal incision was made and the catheters were inserted into the ureters. The ureteral catheter tips were placed just distal to the renal pelvis and the catheters were allowed to fill completely with urine.

Basal Urine Flow. The basal urine flow of the six anesthetized rats was measured. To collect the urine, the collection tubing was flushed to remove air bubbles and filled with saline before connection with the ureteral catheters. A siphon was established as described above, and urine flow sampled at 0.5 Hz for 30 to 60 min.

Measurement of Induced Changes in Urine Flow. In order to determine the ability of this urine collection method to detect rapid changes in urine flow, acute hypotension was induced by bolus injections of acetylcholine (0.5 μg, iv), and the urine flow response to the decrease in arterial pressure was recorded. To measure arterial pressure, the arterial catheter was connected to a Gould-Statham P23Db pressure transducer (Medical Products Division, Gould, Oxnard, CA). The signal from the transducer was amplified with a SensorMedics Dynograph recorder R611 polygraph (Anaheim, CA). Blood pressure and urine flow were sampled at 0.5 Hz.

Results

***In Vitro* Assessment.** As illustrated in Figure 2, drift in the analytical balance was minimal. During the first 60-min session, drift was continuously positive with an average increase equivalent to 0.007 μl/min. During the second session, drift was continuously negative with an average decrease equivalent to 0.015 μl/min. Drift was random during the third session with an overall average decrease equivalent to 0.003 μl/min. These values correspond to only 0.25% of the spontaneous urine flow measured *in vivo* in anesthetized rats (Fig. 4). The effect of evaporation on the gravimetric measurement of flow is also shown in Figure 2. Losses due to evaporation averaged 0.18 μl/min.

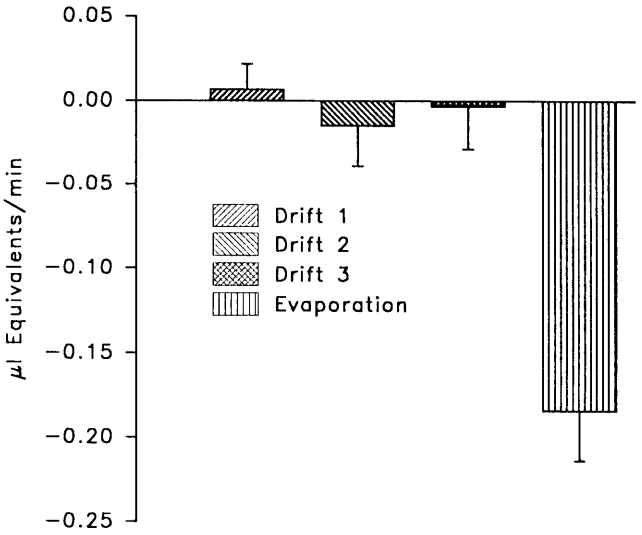

Figure 2. Mean weight changes due to drift (three trials with a 10-g weight on the balance pan) and evaporation. Weight changes are expressed in μl/min; data were collected at 0.1 Hz for 60 min. Error bars represent 95% confidence interval.

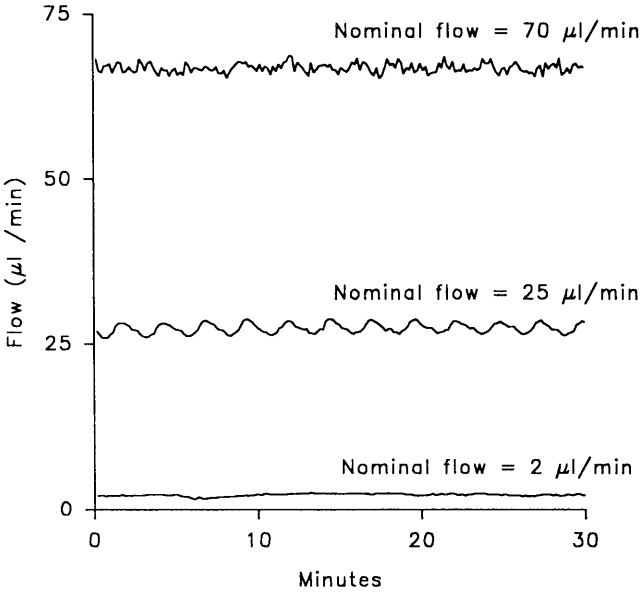

Figure 3. Flow (μl/min) from a syringe-drive infusion pump connected to the fluid collection system. Three different flows were employed; data were collected at 0.1 Hz for 30 min.

The results from the fixed flow (pump) studies are summarized in Figure 3. The capability of the balance to record not only a range of flows but also to detect small changes in flow on a moment-to-moment basis was demonstrated. The small, cyclic changes in flow, particularly noticeable at the middle flow (25 μl/min), were attributed to the mechanical nature of the infusion

pump because similar cyclic changes in flow were not observed in the drift or evaporation tests.

***In Vivo* Assessment.** Urine flow varied among the rats as well as within the recording session for each rat. A typical 30-min recording from one animal is illustrated in Figure 4. Urine flow averaged 21.7 ± 4.93 (mean ± SD) μl/min and varied from 12.6 to 29.8 μl/min in this particular example. Spontaneous urine flow from the six rats averaged 37.3 ± 12.4 μl/min with a range of 14.2–49.4 μl/min.

After bolus intravenous administration of acetylcholine, mean blood pressure for the six rats decreased from 97.1 ± 3.1 mm Hg to 71.6 ± 4.2 mm Hg in 5.1 ± 1.0 sec and urine flow decreased from 40.8 ± 6.6 μl/min to 22.6 ± 4.4 μl/min in 11.8 ± 1.3 sec. Figure 5 shows a representative tracing of the blood pressure and urine flow responses to acetylcholine administration in one rat.

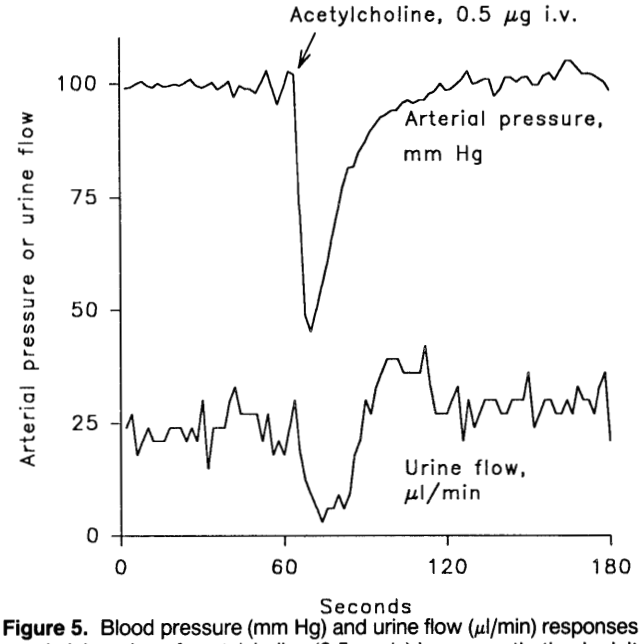

Figure 5. Blood pressure (mm Hg) and urine flow (μl/min) responses to administration of acetylcholine (0.5 μg, iv) in an anesthetized adult rat. Data were collected at 0.5 Hz.

Discussion

The gravimetric method reported here permits the accurate and continuous measurement of urine flow at rates in the microliter range and the detection of flow changes as low as 100 nl/unit time. Unlike the time-averaging volumetric or drop counter techniques, this new method has the advantage of being able to detect changes in flow during relatively brief intervals. The equipment required is common to many laboratories and the method is simple, making this technique readily available to most investigators.

By creating a continuous fluid column between the ureters and the collection reservoir, this system eliminates the problems associated with the formation and counting of fluid drops or with other time-averaging techniques. The accuracy, precision, and reproducibility provided by using an analytical balance to weigh the fluid that is collected contributes to the utility of the system. Error due to drift combined with evaporative losses amounted to less than 1% of the measured value of urine flow in rats. The resolution of the measurement is limited only by the resolution of the analytical balance used. We used a balance with a resolution of ±100 μg, and thus were able to detect a change in flow of 100 nl/unit time.

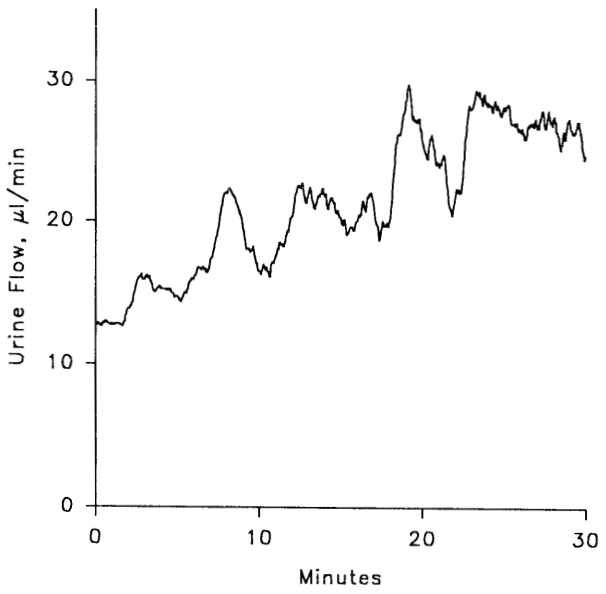

Figure 4. Spontaneous urine flow of an anesthetized adult rat. Urine flow was sampled at 0.5 Hz for 30 min; data were smoothed with 30-sec moving average filter. Mean urine flow was 21.7 ± 4.93 μl/min.

An example of the utility of this dynamic method of measuring urine flow is our observation that the delay time between changes in arterial pressure and urine flow is 6 sec (4). Determination of the delay time provided a test of a hypothesis regarding the long-term nature of the pressure diuresis mechanism. It has been hypothesized that pressure diuresis is a long-term mechanism that is essential for regulation of extracellular fluid volume and arterial blood pressure. However, no one has previously explicitly defined or investigated the character of the long-term aspect of this mechanism. The observation that the delay time between changes in arterial pressure and urine flow is 6 sec supports the hypothesis that the long-term character of pressure diuresis is a result of the cumulative effect of many small changes in urine flow, occurring in response to the spontaneous changes in arterial pressure that occur at a frequency of 6/min (7) in the conscious animal. Thus the ability to measure dynamic changes in urine flow may provide important insights into physiologic mechanisms that cannot be obtained using time-averaged, steady state measurements (e.g., 8). Another pos-

sible use of this dynamic measure of urine flow would be in association with monitoring of regional blood flow in the kidney with laser Doppler methodology. This combination of methods would allow the simultaneous determination of arterial pressure, renal hemodynamics, and urine flow.

Previous measurements of urine flow have all involved time averaging, and thus are incapable of detecting dynamic changes of short duration. For example, the urine collection period in clearance studies to evaluate renal function generally varies from 15 min to 24 hr. New methods utilizing suction or flushing techniques have recently been developed that can reduce collection periods to 5 min (9, 10) or even 1 min (1). Despite the importance of these newer collection techniques, changes in flow that occur within each collection period still go undetected. The capabilities of drop counters to detect and record drops have improved in recent years. Hester and Barber (11) developed a drop interval flowmeter employing a photodetector that also allows the reciprocal of time between drops to be converted into an analog voltage for display on a computer. Their system, however, still fails to correct the basic problem of drop counters: variation in flow during the formation of a single drop cannot be detected. In this study, for example, there was a rapid decrease in urine flow from 41 μl/min that occurred within 12 sec after administration of acetylcholine (Fig. 5). This decrease could not have been detected by the drop counter method. Also, evaporation will have a greater impact on the rate of drop formation at lower flows than at higher flows because drop formation is slower, allowing more time for evaporation. Therefore, low flows may be underestimated due to the effects of evaporation on the rate of formation of a single drop.

Changes in urine flow within the ureter have been measured in dogs by the Doppler technique (12) and with a thermistor flowmeter (13). While these techniques accurately measure changes in urine velocity related to urinary tract peristalsis and urine flow, they cannot measure the absolute rate of urine flow. In our preparation, the ureteral catheter tip was positioned just below the renal pelvis. This positioning of the catheter tip enables the measure of urine flow independent of ureteral peristalsis, so that changes in flow primarily reflect changes in urine formation. Our method could be adapted to measure the effects of ureteral peristalsis on urine flow, however, by positioning the tip of the catheter at the distal end of the ureter.

In summary, the gravimetric technique described in this paper to measure urine flow has advantages over time-averaging techniques currently in use. Some of these advantages include: (i) the ability to measure flows over relatively short intervals (2–10 sec), which allows for study of the dynamic aspects of urine formation; (ii) the ability to detect a change in flow as small as 100 nl/unit time; (iii) the availability of the required equipment; and (iv) the simplicity of the method.

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