Measuring dynamic kidney function in an undergraduate physiology laboratory

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Medler S, Harrington F. Measuring dynamic kidney function in an undergraduate physiology laboratory. *Adv Physiol Educ* 37: 384–391, 2013; doi:10.1152/advan.00057.2013.—Most undergraduate physiology laboratories are very limited in how they treat renal physiology. It is common to find teaching laboratories equipped with the capability for high-resolution digital recordings of physiological functions (muscle twitches, ECG, action potentials, respiratory responses, etc.), but most urinary laboratories still rely on a "dipstick" approach of urinalysis. Although this technique can provide some basic insights into the functioning of the kidneys, it overlooks the dynamic processes of filtration, reabsorption, and secretion. In the present article, we provide a straightforward approach of using renal clearance measurements to estimate glomerular filtration rate, fractional water reabsorption, glucose clearance, and other physiologically relevant parameters. The estimated values from our measurements in laboratory are in close agreement with those anticipated based on textbook parameters. For example, we found glomerular filtration rate to average 124 ± 45 ml/min, serum creatinine to be 1.23 ± 0.4 mg/dl, and fractional water reabsorption to be ~96.8%. Furthermore, analyses for the class data revealed significant correlations between parameters like fractional water reabsorption and urine concentration, providing opportunities to discuss urine concentrating mechanisms and other physiological processes. The procedures outlined here are general enough that most undergraduate physiology laboratory courses should be able to implement them without difficulty.

glomerular filtration rate; kidney; renal clearance; undergraduate laboratory

RENAL FUNCTION is a highly complex area of physiology that is often challenging for students to comprehend (7, 8). Most students come into the laboratory recognizing that the kidneys play an important role in the elimination of wastes, but many fail to appreciate the vital role of the kidneys in maintaining the precise composition of the extracellular fluids. It is an oversimplification to view the primary physiological role of the kidneys as simply to remove wastes from the body. Although excretion is certainly one important function of the kidneys, viewing waste elimination as their only physiological role ignores the vital importance of the urinary system for other processes of osmoregulation. These include ion and water balance, acid-base regulation, and the selective retention of vital solutes like glucose and amino acids (8). In addition, students frequently have difficulty understanding the concepts of renal clearance and the fact that clearance differs among specific molecules (7). The standard approach to renal physiology in undergraduate teaching laboratories likely reinforces an overly simplistic view of vertebrate kidney function.

Currently, the most common approach to investigating renal function in physiology laboratories relies on a "dipstick" approach of urinalysis (4, 11). In these exercises, students collect a sample of urine sample and then use a urinalysis strip to measure several parameters including urine concentration, pH, nitrogenous wastes, and other physiological indicators. In some cases, students may be instructed to drink different liquids and then measure urine output and composition (11). While these exercises can be beneficial, they are limited in a number of ways. First, they focus on urine simply as a "finished product" and generally assess whether solutes are inside of "normal" ranges. In doing so, they fail to examine the dynamic physiological processes of filtration, reabsorption, and secretion. Yet, these are the fundamental processes that affect the final composition and volume of the urine. Nor does this approach provide an opportunity to calculate renal clearances or directly examine urinary concentrating mechanisms. In the Biology Department at the State University of New York (SUNY, Fredonia, NY), we frequently observe that our students need practice in performing quantitative analyses of data. The need for additional practice encompasses problems with fairly simple calculations of solute concentrations as well as more sophisticated statistical comparisons and analyses. Collecting and working with data require students to sharpen their quantitative and analytic skills, hopefully encouraging higher levels of critical thinking.

We are aware of only a single published protocol for the direct measurement of renal physiological parameters in a student-based laboratory (12). In 1995, Walker and Olson (12) presented a student exercise for the measurement of renal function in the rat. Their laboratory exercise has the benefit of requiring students to make calculations of renal clearances, including the estimation of glomerular filtration rate (GFR). A potential drawback for that exercise, however, is that it involves experimentation with vertebrate animals. Part of the authors' rationale for using the rat was due to growing concerns, at that time, about the hazards related to the collection of human fluids in a laboratory setting (12). Currently, the hurdle of obtaining Institutional Animal Care and Use Committee approval for animal experiments in a student-based laboratory may outweigh the potential problems with handling human fluids. In addition, that protocol requires specialized laboratory equipment (i.e., flame photometer, chloride titrator, and osmometer) as well as animal handling procedures (cardiac puncture) that may not be suitable for some teaching laboratories. Finally, many students with an ambition of pursuing a career in a healthcare profession are very interested in the clinical implications of renal function in humans.

In the present article, we present straightforward methods for the determination of GFR, fractional water reabsorption (FWR), glucose clearance, urine concentration, and other physiological parameters. These measurements are based on the analysis of a small volume of blood (~100–200 µl) from a single finger puncture and on collection of a single urine sample. These samples are used to determine serum and urine creatinine, glucose,

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and other solute concentrations. The measured values are then used in conjunction with the urine flow rate and urine concentration to determine renal clearances and other physiological parameters. This procedure is simple and can easily be completed within a single laboratory period.

The procedures in this exercise encourage students to use their understanding of renal clearance and test their ability to apply that knowledge. After completing calculations from their own measurements, students are provided with renal parameters from the entire class and are instructed to look for significant correlations among the data. Our goal is to help students understand the functional relationships among parameters like FWR and urine concentration and to provide an exercise that encourages them to develop their quantitative and analytic skills.

METHODS

Participants

This laboratory procedure was developed in 1998–1999 for a senior-level vertebrate physiology laboratory at Louisiana State University and has now been successfully implemented at the SUNY (Fredonia, NY) from 2011 to the present. We have used this exercise as part of a junior-level physiology laboratory and in conjunction with a sophomore-level human anatomy and physiology laboratory. All students participating in the exercise volunteer to do so, and students are allowed to "opt out" of the exercise at their discretion. Students are instructed to handle only their own body fluids, to reduce the possibility of accidental contact with another student's bodily fluids. All students provided written agreement allowing the use of their anonymous data for publication in *Advances in Physiology Education*, and the activity was approved by an Institutional Review Board of SUNY (Fredonia, NY). In addition to students participating in the laboratory exercises, some of data were collected by one of the authors (S. Medler) over several different years.

Student Prelaboratory Activities

Students are encouraged to study the interactive physiology tutorials (Interactive Physiology, Pearson-Benjamin Cummings) covering the kidneys before attending the laboratory. These interactive exercises provide dynamic animations of the renal system and are available as accessory learning materials with their class textbook (8). Once in the laboratory, students are given a short prelaboratory lecture focusing on the basic physiological roles of the kidneys. We emphasize the role of the kidneys not only in excretion but also in the homeostasis of ions, water, and other physiological processes (osmoregulation). We then focus on the three major processes that govern the final volume and composition of urine: filtration, reabsorption, and secretion. Within this discussion, we introduce the concept of renal clearance and talk about the idea that clearance depends on how specific molecules are handled by the kidneys. Next, we introduce the idea that if a molecule is freely filtered, but neither reabsorbed nor secreted, its clearance provides a direct measure of GFR. We then talk about why creatinine clearance provides a convenient measure of GFR, even though some minor levels of secretion take place (2). At this point in the laboratory, it might be useful to group students into pairs and have them calculate a clearance problem based on urine and serum parameters provided by the instructor.

Laboratory Procedures

Ensuring responsible handling of bodily fluids. Handling human bodily fluids always carries a risk of the transmission of infectious diseases. We use several basic tactics to minimize these risks. First, we provide a handout to students before the laboratory describing the potential risks and outlining the rules for handling fluids in the laboratory. One of these is that students have the right to opt out of the laboratory exercise if they choose to. Another is that students are directed to handle their own blood and urine, thus minimizing the potential for cross-contamination. Furthermore, students wear gloves during the exercise, clearly marked biohazard bags and sharps containers are available for waste disposal, and the benches are covered with absorbent paper. Finally, if any accidental spills do occur, the laboratory instructors disinfect the area immediately. We feel that safely handling these fluids presents a learning experience in itself, since many of these students are preparing to enter into health professions and it is important that they learn to handle potentially hazardous fluids responsibly.

Renal clearance measurements. GFR is measured by comparing the excretion rate of a filtration marker with the marker's serum concentration (6, 8, 12). GFR estimation is simply a special case of the more general estimation of renal clearance but with the criteria that the solute is freely filtered but not reabsorbed or secreted. This means that it is straightforward to estimate GFR or the clearance of any solute, as long as that solute can be measured in the blood and in the urine. The solute excretion rate is then calculated as the urine concentration multiplied by the urine flow rate, which can be measured directly.

The general formula for the clearance (6, 8, 12) of a solute (X) is as follows:

$$\text{Urine flow rate (in ml/h)} \times [X]_{\text{urine}}(\text{in mg/ml})/[X]_{\text{serum}}(\text{in mg/ml})$$

For the estimation of creatinine and glucose clearance, urine and serum concentrations of these molecules are substituted for the concentration of solute X in the urine and serum ($[X]_{\text{urine}}$ and $[X]_{\text{serum}}$, respectively) (6, 8, 12).

FWR can be calculated, once creatinine clearance (~GFR) has been calculated, as follows:

$$\text{FWR} = 1 - (\text{urinary flow rate/GFR})$$

Serum and urine collection. Serum and urinary creatinine concentrations are used to determine creatinine clearance, which is used as an estimate of GFR. Approximately 100–200 μl of blood are collected into heparinized capillary tubes after finger puncture with a sterile lancet. Samples are immediately spun down with a capillary centrifuge, and the percent hematocrit is measured and recorded. The capillary tube is then scored with a file and broken between the serum-cell interface, and the serum is collected in a microcentrifuge tube and held on ice until it is used for the determination of creatinine.

Students are instructed to record their last time of urination before coming to class. After ~1 h or more, students collect urine in a 1-liter beaker and measure its volume. They then dispose of most of the volume, retaining only ~100 ml for subsequent analyses. Students then determine the urine flow rate as the volume of urine produced over the time since their last time of urination.

Determination of creatinine. Creatinine is measured using a modified version of the alkaline picrate method (5), adjusted for use with small volumes. At one time, a commercially available creatinine assay kit was available from Sigma (12), but we are unable to identify any commercially available kits at the present time. In our assay, a small volume of serum, urine, or creatinine standard (~50 μl for serum and 10 μl for urine) is added to 1 ml of 0.8% picric acid solution. Next, 100 μl of 10% NaOH are added to the picric acid solution, and the tube is inverted several times to mix the solution. After 5–10 min of incubation at room temperature, the entire mixture is transferred to a cuvette, and the absorbance at 520 nm is recorded.

We constructed a standard curve by serially diluting a known concentration of creatinine (C4255, Sigma) and measuring absorbances at 520 nm. We have found that the absorbance is a linear function of creatinine concentrations between 1 and 20 μg. Concentrations of unknowns from serum and urine are determined from the

standard curve. Urine creatinine concentrations are typically fairly high, and absorbance readings fall within the midregions of the standard curve. If the urine samples exceed the range of the standard curve, smaller volumes are used. Serum creatinine concentrations are typically near the lower ranges of the standard curve, and estimated concentrations are very sensitive to measurement errors in the standard curve. Proteins present in the serum lead to elevated estimates of serum creatinine. To remove these interfering substances, a small volume of saturated trichloroacetic acid (2 μl/50 μl serum) is added to the sample, and the sample is placed on ice for 5 min. The resulting pellet is then spun down in a microcentrifuge for 5 min, and the fluid phase is used in the creatinine assay.

Glucose measurements. Serum glucose levels are measured using an automated system for blood glucose monitoring (OneTouch Ultra-Mini blood glucose monitoring system, LifeScan). This system includes a small automated lancet device, and the automated measurement system only requires ~10 μl of blood. Glucose in the urine is measured with a test strip (Multistix 10 SG, Siemens).

Cl^- measurements. In the reported experiments, we used an automated chloride titrator to measure serum and urine Cl^- concentrations (Buchler-Cotlove Chloridometer, Buchler Instruments). However, Cl^- can also be estimated through alternative titration methods relying on colorimetric determination (5, 11).

Urine osmolarity. Urine concentration is estimated from specific gravity measurements taken from a handheld refractometer (Uricon-PN, Atago). Specific gravity is converted to mosM by multiplying the decimal units of specific gravity by ~31–33 (1, 3), and we used a factor of 32 for our estimates. For example, a specific gravity reading of 1.002 corresponds to an estimated urine concentration of 64 mosM.

Other urinary parameters. The same commercially available test strip used to measure urine glucose (Multistix 10 SG, Siemens) is also used to measure bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrite, and leukocytes.

Student Postlaboratory Activities

During the laboratory session, students obtain and record values for serum and urine creatinine concentrations as well as urinary flow rate. Before returning to laboratory the following week, students are instructed to calculate creatinine clearance as an estimate of GFR and FWR. Students are advised to consult the section of their textbook covering renal clearance (8). This assignment requires that students go through the process of using their own data to calculate these physiological parameters and, in doing so, makes them confront difficulties in their understanding of basic concepts. In addition, students can compare their calculated GFR and FWR numbers with textbook values to get a sense whether they are on the right track or not.

Each student is asked to turn in a data sheet containing his or her raw data at the end of the first week's laboratory. The instructor copies these data sheets and hands back the originals, so that students can make their own calculations. The instructor then compiles the data for each student into a spreadsheet that can then be used to perform statistical analyses for the class as a whole. Once completed, a copy of this spreadsheet is provided to each of the students to look for trends on their own. These exercises provide a different level of analysis, where students can use correlative analyses for the whole class to look for meaningful trends. At a minimum, students should be instructed to plot urine concentration versus FWR, urine concentration versus urine flow rate, and urine flow rate versus GFR. They may also be assigned specific problems relating to kidney function (see the APPENDIX). These analyses must be completed before the following laboratory period.

Data analysis laboratory. We use the laboratory period after the data collection laboratory to discuss individual parameter estimates and to examine and discuss broad patterns in the data. We have adopted this approach for two reasons. First, basic renal physiology is a complex enough topic to warrant a second period of discussion. We

begin the laboratory with a short lecture that revisits some of the main ideas covered in the first laboratory as well as a more indepth discussion of fluid and ion handling in different regions of the nephron and of urinary concentrating mechanisms. Second, students need some time to perform their own calculations using their own data to see if they understand how to use their numbers. In earlier versions of the laboratory, we tried to leave time for discussion at the end of the data collection laboratory but found that there was too little time to discuss the data in detail. It also became clear that students needed more time to work with their own numbers before they became comfortable with the calculations. At the end of the short lecture on renal physiology, we go through the class data and discuss our interpretations of what it tells us.

Before the laboratory, the instructor prepares graphs showing correlations between different parameters from the previous laboratory. We then review the data for the class as a whole and examine correlations among different parameters, e.g., examine how urine concentration changes as a function of FWR (Fig. 1A). We also study the correlation (or lack thereof) between GFR and urine flow rate (Fig. 1C). Students will have completed their own analyses before coming to class, so they should already be generally familiar with these trends. As we examine these correlations, we ask students to interpret whether these patterns make sense, or not, based on the discussions we have had in the laboratory. We are able to discuss many different aspects of dynamic renal function through these discussions. For example, the relationship between FWR and urine concentration provides an opportunity to discuss the effects of antidiuretic hormone on the permeability of the collecting ducts and the overall regulation of urine concentration. The lack of correlation between GFR and urine output allows us to discuss the general strategy of filtering large volumes of plasma and then reclaiming what is needed.

Overall, we find that students are required to perform quantitative analyses at three levels of increasing sophistication. At the most basic level, students must obtain absorbance readings and convert them into creatinine concentrations. They are also required to convert specific gravity readings into estimates of urine osmolarity. The next level of complexity is in using urine and serum creatine concentrations, together with urine flow rates, to calculate creatinine clearance and FWR. Finally, students are challenged to analyze the interrelationships among the physiological parameters that they have obtained together as a class. Students typically differ in their preparedness to tackle these analyses, and we find that the data analysis laboratory provides an opportunity to assist students with whichever level they need help.

RESULTS AND DISCUSSION

We were consistently able to collect physiological parameters defining basic kidney function, including GFR, FWR, urinary flow rate, and urine concentration (see Table 1). GFR averaged 124 ± 44.5 ml/min, glucose clearance was uniformly 0 ml/min, and Cl^- clearance averaged 2.24 ± 0.49 ml/min. The average serum creatinine concentration was 1.23 ± 0.4 mg/dl. FWR was consistently high (average: 0.968 ± 0.025) but varied from 0.91 to 0.995. Urine concentration varied over almost an order of magnitude (96–832 mosM, average: 405.3 ± 226.75 mosM). The urine flow rate averaged 3.47 ± 2.01 ml/min but also ranged over about an order of magnitude (0.784–7.35 ml/min). The average pH of the urine was 6.16 ± 0.55.

We also detected meaningful correlations among some of our measured parameters (Fig. 1). Urine concentration increased significantly as a function of FWR ($P < 0.0033$, $r^2 = 0.64$ in linear regression; Fig. 1A). Urine concentration was inversely correlated with the urine flow rate ($P < 0.0244$, $r^2 = 0.45$; Fig. 1B). However, GFR was independent of the urine
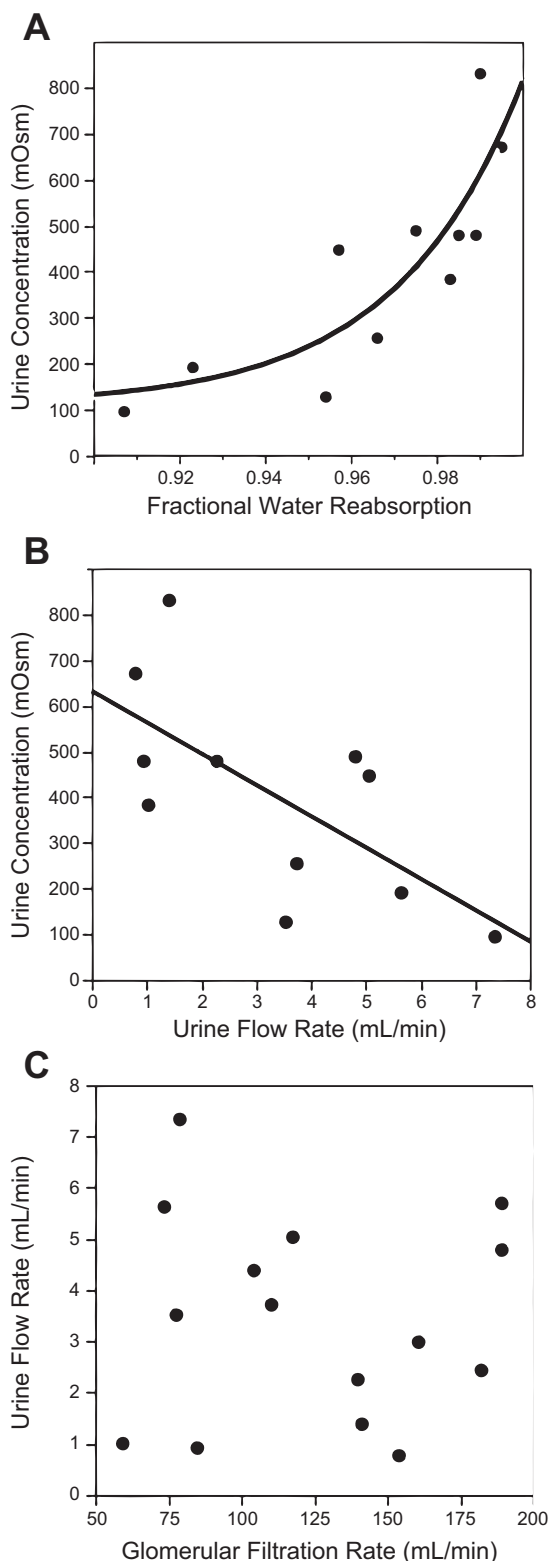
A

B

C

Fig. 1. Representative data collected from the physiology laboratory. *A*: urine concentration (in mosM) was positively correlated with fractional water reabsorption ($P < 0.0033$, $r^2 = 0.64$). In the graph, a curvilinear power function was fit to the data ($r^2 = 0.72$). *B*: urine concentration (in mosM) declined significantly as a function of urine flow rate ($P < 0.0244$, $r^2 = 0.45$). *C*: urine flow rate was not significantly correlated with glomerular filtration rate ($P > 0.90$, $r^2 = 0.001$).

flow rate (Fig. 1*C*) and was not correlated with any other measured parameters.

These measured values and the trends among them provide an opportunity to discuss several important trends in renal physiology. A common misconception among students is that the excretion of wastes is the only physiological role of the kidneys. This assumption overlooks the central osmoregulatory role of the kidneys in broadly maintaining the precise constituents of the extracellular fluid environment (volume, osmolarity, glucose, ions, pH, etc.) (2, 8). Simply measuring GFR provides a chance to emphasize the magnitude of filtration by the mammalian kidneys. The kidneys are only 0.4% of total body mass in mammals, but 20–25% of cardiac output is delivered to these metabolically active organs (2, 8). At a rate of 125 ml/min, GFR in humans results in ~180 liters of blood plasma being filtered each day. This amounts the entire volume of plasma being filtered ~50–60 times every day, which provides continuous control over plasma composition (2).

GFR is not a primary determinant of urine output, as demonstrated by the lack of correlation between these two parameters (Fig. 1*C*). Instead, GFR is remarkably constant in mammals, because most of the adjustments to fluid volume and composition take place within the renal tubules, downstream of filtration. The high GFR in mammals and birds reflects the tight coupling between the filtration capacity of the kidney and active metabolic processes (9, 13). This trend becomes apparent from comparative physiology, since mammals and birds have a GFR that is almost two orders of magnitude higher than that of comparably sized ectothermic vertebrates (13). In addition, GFR within both mammals and birds scales as mass$^{0.75}$, in parallel with metabolic rate (9, 13). Absolute GFR is not only higher in magnitude, but the relative filtration rate (filtered load relative to plasma volume) is also higher in animals with higher metabolic rates, enabling them to make rapid adjustments to plasma parameters (6). A small mammal like a rat or a mouse filters its entire plasma volume in only 5–10 min, whereas a similarly sized reptile requires several hours to filter a comparable amount of plasma (6). Since the plasma must be filtered before any adjustments can be made to the fluids, GFR represents a rate-limiting step in the osmoregulatory processes of the kidneys. Humans and other mammals enjoy the capacity to rapidly eliminate wastes and to make quick adjustments to their extracellular fluid composition because they filter and process their entire plasma volume several times each day (2).

Our measurements of FWR ranged from just over 90% to 99.5%, which is consistent with the capability of the human kidneys to produce between 0.5 and 20 liters of urine per day (2). The relatively high values from FWR underscore important patterns. First, because GFR is such a large fraction of the total plasma volume, it is imperative that most of the water is reabsorbed, or a person would quickly become fatally dehydrated. Second, an analysis of the entire data set shows that FWR and urine concentration are significantly correlated with one another. More precisely, higher values of FWR lead to more concentrated urine (Fig. 1*A*). Students should understand that FWR and urine output are variable among individuals, likely because of differences in hydration state. This trend also provides an opportunity to discuss the effects of antidiuretic hormone on collecting duct permeability.

The primary control point in the mammalian kidney is not in the process of filtration but from the renal tubule, where water

Table 1. *Physiological parameters defining basic kidney function*

	Glomerular Filtration Rate, ml/min	Glucose Clearance, ml/min	Cl^{-1} Clearance, ml/min	Serum Creatinine Concentration, mg/dl	Fractional Water Reabsorption	Urine Concentration, mosM	Urine Flow Rate, ml/min	pH
Mean	124	0	2.24	1.23	0.968	405.3	3.47	6.16
SD	44.5	0	0.49	0.4	0.025	226.75	2.01	0.55
Range	59.2–189	0	1.68–2.7	0.7–2.1	0.907–0.995	96–832	0.784–7.35	5.0–7.0
n	15	15	4	15	15	11	15	11

The glomerular filtration rate was determined from creatinine clearance. Fractional water reabsorption was the proportion of the filtered load reabsorbed.

and solute reabsorption is regulated. The results presented here demonstrate that not all solutes are handled in the same manner by the kidneys. Some solutes, like creatinine, are continuously excreted in the urine. Others, like glucose, are completely reabsorbed so that under normal circumstances none of this solute is lost. Cl^- and other solutes are typically intermediate to these extremes, and it is expected that variability will be observed among students in the laboratory, depending on their particular physiological state (Table 1). It should be a fairly straightforward process to adapt these laboratory procedures to include other solutes, such as Na^+, urea, or para-aminohippuric acid, depending on the facilities available at other universities. We have also included a short problem set in the APPENDIX to illustrate the kinds of problems that might be used in conjunction with the calculations in the exercise. These problems encourage students to apply their quantitative skills and to reinforce their understanding of renal clearance, filtration loads, and other important concepts.

Accuracy of Measurements

Overall, our estimated renal parameters are remarkably close to the ranges anticipated for normal individuals (see Table 1). Textbook values of GFR for an "average" person are close to a value of 125 ml/min (2, 8), which is well within the range of our average of 124 ± 44.5 ml/min. Clinically, serum creatinine concentrations are used as a basic indicator of renal function (10). Normal serum creatinine values in healthy individuals are typically very close to 1 mg/dl but can vary significantly in relation to diet and the subject's muscle mass (10).

The primary challenge to obtaining accurate measurements is that serum creatinine concentrations are near the limits of detection, when using the small volumes described here. Because of these small concentrations, it is important to obtain accurate absorbance readings for the standard curve, particularly at the lower end of the curve. Another potential problem with the serum samples is that the protein in the serum is an interfering solute and can lead to elevated readings of creatinine (10). In our measurements, we have observed that using whole blood plasma-containing proteins results in artificially elevated absorbance readings. We have subsequently found that precipitating serum proteins with trichloroacetic acid on ice for 5 min and then centrifuging the samples efficiently removes the protein fraction and leads to more accurate values from serum creatinine (Fig. 2). Other sources of error include very small serum volumes (<10 µl) and hemolysis of red blood cells before the sample is spun down in the centrifuge.

Even when measured GFR values are outside the range of expected values, these values do not detract from the learning experience. On the contrary, they often provide a useful learning experience when students struggle to understand where

measurement errors might have impacted their final values. In the majority of these cases, GFR estimates are lower than expected, because serum creatinine estimates are elevated if protein has not been precipitated from the sample. Indeed, the alkaline picrate method of creatinine determination generally suffers from problems with interfering agents (10). As students go back through their clearance estimates, they realize that their estimates are impacted by three values: serum creatinine concentration, urine creatinine concentration, and urine flow rate. They then typically recognize that the small volumes and low concentrations of the serum creatinine provide the most likely source of error. We also have them compare their serum creatinine concentrations with the normal levels of ~1 mg/dl, and students can then see that their estimates are high. These analyses also provide segue into a discussion of how kidney function is assessed clinically. For general screening purposes, basic kidney function is determined from serum creatinine concentrations, and anything approaching 2 mg/dl is considered to indicate potentially impaired function (10). We discuss the fact that serum creatinine levels alone are only one component of GFR determination and that, without creatinine excretion rates, it is not possible to accurately estimate GFR. We also discuss the fact that even in clinical settings, obtaining accurate serum creatinine concentrations is notoriously difficult (10).

One way to check the accuracy of the GFR measurements is to examine the correlation between creatinine excretion rate and serum creatinine concentration (Fig. 3). These two param-
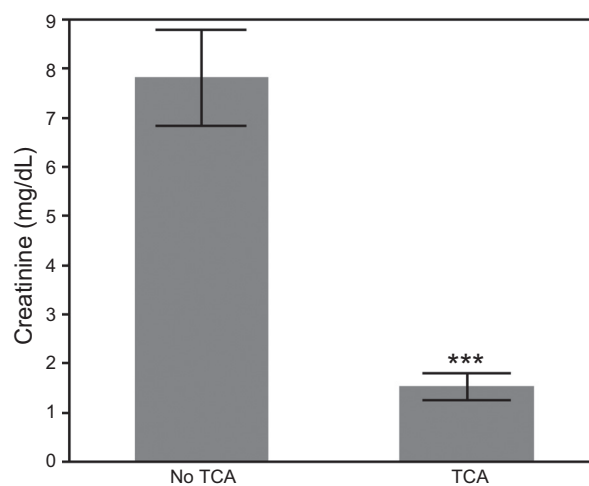

Fig. 2. Serum creatinine concentrations determined without and with trichloroacetic acid (TCA). Creatinine concentrations were significantly higher (***$P <$ 0.0001) when determined from whole serum. When serum protein was precipitated using TCA, the subsequent creatinine concentrations were within a normal range.
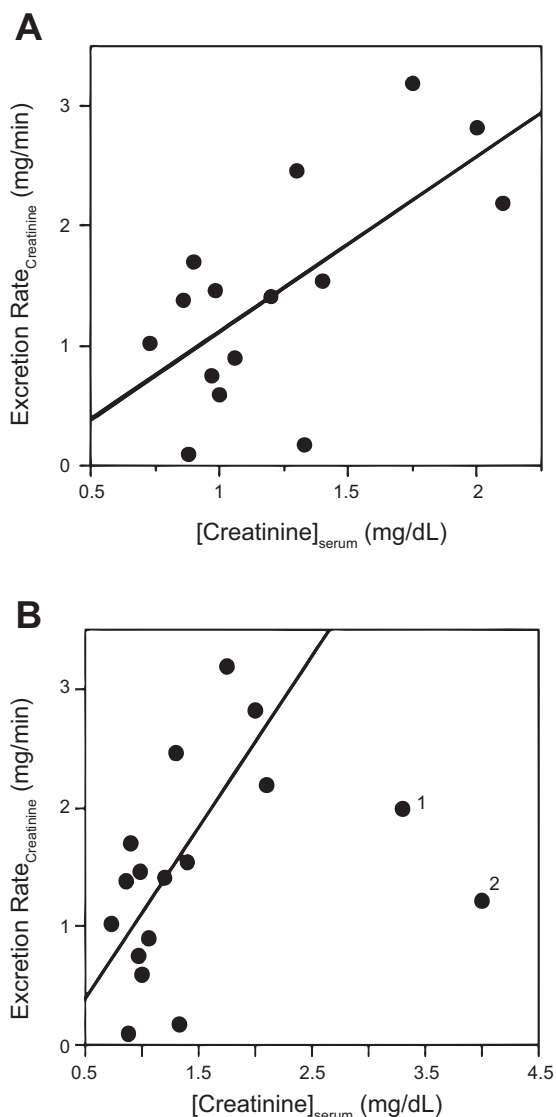
Fig. 3. Correlation between creatinine excretion rate (Excretion rate$_{Creatinine}$) and serum creatinine concentration ([Creatinine]$_{serum}$). *A*: a significant correlation was observed between these two parameters for the data shown in Table 1 ($P < 0.006$, $r^2 = 0.46$). This correlation supports the accuracy of the measurements, as excretion rate and serum concentrations are expected to be correlated with one another. *B*: two outliers were detected using this analysis. *Subject 1* had a very small serum sample available (<10 μl), whereas *subject 2* had a serum sample with hemolysis of red blood cells. These two data points were omitted from subsequent analyses because of the detected errors. (The data points and line of best fit in *B* are the same as those shown in *A* and are included here as a reference.)

eters are measured independently, but if the plasma concentrations are accurate and the GFRs within the group are within a normal range, the two should be positively correlated with one another (Fig. 3*A*). If, on the other hand, most of the variability in GFR estimates is from measurement error (particularly likely for the plasma measurements), then there may not be a significant correlation. If there are problems with specific measurements, they may be detected as outliers on this kind of plot (Fig. 3*B*). Particular attention should be given to serum creatinine concentrations that are >2–3 mg/dl since these are well outside of the expected average of ~1 mg/dl.

APPENDIX

Outline of the Kidney Physiology Laboratory

Prelaboratory activities. The following are prelaboratory activites:

- Provide the students with renal physiology material and a prelaboratory handout to review before class.
- Ask the students to note the time of their last bladder voiding, up to several hours before the start of the laboratory.

Introduction. The following topics should be introduced:

- Discuss how filtration, reabsorption, and secretion lead to the formation of urine.
- Go over safe bodily fluid handling.
- Outline the basic steps of the laboratory procedure.

Laboratory activity (blood and urine sampling can be done in any order). Laboratory activities comprise the following:

- Generate a standard curve for creatinine versus absorbance at 520 nm. This can be done by the students in class or by the instructor beforehand if time is an issue.
- Note the time and urine volume of a second voiding of their bladder. Bring ~100 ml back to the laboratory for the analysis of creatinine concentration, specific gravity (osmolarity), glucose concentration, and any other parameters available.
- Collect and process blood samples for the creatinine assay.
- Perform a blood glucose assay with an automated glucose monitor.
- Have the students fill out a data sheet with their values measured in the laboratory. Make a copy for the instructor.

Student postlaboratory activities. Postlaboratory activites comprise the following:

- Have students calculate GFR, FWR, urine concentration, glucose clearance, and other parameters and compare their results with ranges reported in their textbook.
- Instructor compiles class data and provides the complete set to students.
- Instruct students look for trends among class data.

Data analysis laboratory. The following activities are done during the data analysis laboratory:

- Review concepts of renal clearance.
- Go over the individual student results and the class summary.
- Perform statistical analysis of the data and discuss interpretation of the data.

Problem Set

Problem 1. Glucose clearance is normally zero, but glucose may appear in the urine of diabetics. Why might a diabetic person have glucose in their urine? Where in the kidney does glucose reabsorption normally take place? What transporters in the renal tubules normally ensure the efficient reabsorption of glucose?

Problem 2. The kidney has a tremendous capacity to reabsorb glucose and other solutes. Nevertheless, when solute transporters become saturated with solute in the tubular fluid,

these molecules can "spill over" into the urine. In humans, the maximum transport capacity for glucose is ~325 mg/min. If the normal blood glucose concentration is 100 mg/dl, what is the normal filtration load for glucose? How much of a safety factor exists for glucose reabsorption? At what critical blood glucose concentration will this sugar begin to spill over into the urine?

Problem 3. Routine blood panels typically include a test for serum creatinine as a baseline measure of kidney function. Serum values of >2 mg/dl are frequently considered to be an indicator of possible renal pathology. Based on what you know about creatinine clearance, is this parameter an accurate measure of kidney function? What limitations are inherent in this approach?

Problem 4. During the collection of blood samples in this laboratory exercise, a student waited too long before spinning down their sample, and the red blood cells ruptured (hemolyzed). The resultant hemoglobin spilled into the serum and interfered with the absorbance readings in the creatinine assay. How would this interference affect the determination of serum creatinine concentration? What effect would this mistake have on the subsequent estimation of GFR? FWR?

Problem 5. A 24-h urine collection from a patient results in total urine volume of 2.2 liters, and laboratory tests show the Na^+ concentration in the urine to be 100 mM. Given that the extracellular fluid typically has a Na^+ concentration of ~140 mM, what is this patient's Na^+ clearance? What is the ratio of Na^+ to creatinine clearance? What does this ratio tell you about how the kidney handles Na^+?

Problem 6. Clearance of a molecule that is freely filtered, but neither reabsorbed nor secreted, provides an estimate of GFR. The polysaccharide inulin is a molecule that is often used experimentally for GFR measurements because it fits these criteria. A different molecule, para-aminohippuric acid, is commonly used to measure a different physiological parameter: renal plasma flow. How would you predict the kidney handles para-aminohippuric acid differently than it processes inulin? Would you expect para-aminohippuric acid or inulin clearance to be greater in magnitude? What renal parameter does the ratio of inulin to para-aminohippuric acid clearance approximate?

Problem 7. Antidiuretic hormone (or vasopressin) actively regulates water reabsorption in the kidney. Antidiuretic hormone is released by the posterior pituitary, and it directs the renal tubules and collecting ducts cells to insert aquaporin channels into their apical membranes. What is the physiological role of aquaporin channels in this process? How do changes in the number of aquaporin channels affect urine volume and concentration? Alcohol inhibits antidiuretic hormone secretion. What affect does alcohol consumption have on urine flow rate and osmolarity?

Problem 8. Although many blood components are freely filtered by the glomerulus, some are trapped in the blood. Which components of blood can versus cannot be freely filtered into the forming urine? In some conditions like glomerulonephritis or diabetic nephropathy, the normal filtration barrier is altered. What tests might be done to detect this type of kidney damage?

Answers

Answer to problem 1. Glucose is freely filtered at the glomerulus but is normally almost immediately reabsorbed so that this energy-rich molecule is not wasted. Glucose transport occurs in the proximal convoluted tubule, where it is coupled to the active transport of Na^+. Glucose transporters are embedded in the apical membrane of the tubular epithelial cells. In diabetes, poorly regulated blood glucose concentrations may rise to much greater than normal levels. When this happens, the filtered load of glucose (blood glucose concentration × GFR) may saturate the glucose transporters in the renal tubules, and the leftover glucose can spill over into the urine. Because glucose is an osmotically active solute, water stays associated with the glucose and induces a diuresis (the term "diabetes" is derived from a Latin term meaning excessive urination).

Answer to problem 2. The filtered load of a solute is the amount of that solute delivered per time to the renal tubules for processing. Filtered load is calculated simply as the product of the blood solute concentration and GFR. For normal blood glucose concentrations, the filtered load is 100 mg/dl × 125 ml/min = 125 mg/min (note that there are 100 ml per 1 dl). The safety factor for reabsorption is the maximum reabsorptive capacity in relation to the normal filtration load, or 325 mg/min ÷ 125 mg/min = 2.6. This means that the normal reabsorption capacity for glucose is ~2.6 times greater than what is normally needed. This safety factor is essentially insurance that glucose does not get wasted in the urine. When the filtered load exceeds the reabsorption capacity of the kidney, then glucose will begin to spill over into the urine. This occurs when the blood glucose level reaches 260 mg/dl (260 mg/dl × 125 ml/min = 325). (In reality, glucose first appears in the urine at a blood glucose level of ~220 mg/dl. This glucose threshold is lower than maximum transport capacity because of variability in the maximum transport among nephrons. Those nephrons with the lowest transport capacity will allow some glucose to leak through before filtration load exceeds transport capacity.)

Answer to problem 3. Serum creatinine levels provide some measure of basic kidney function, because creatinine clearance provides an estimate of GFR. As GFR declines, as occurs in glomerulonephritis and other conditions, filtration capacity drops and blood levels of creatinine become elevated. However, to accurately measure creatinine clearance, we must measure creatinine excretion rates in the urine in conjunction with blood concentrations. Since creatinine is the excretory form of creatine phosphate from the skeletal muscles, it is continuously released from these tissues. While normal creatinine concentration are ~1 mg/dl, individuals with higher muscle mass and those who eat large amounts of meat may have higher than average circulating creatinine concentrations. So, while serum creatinine may provide a first indicator of kidney function, a measure of excretion rate is also required to get an accurate estimate of creatinine clearance.

Answer to problem 4. Hemoglobin released into the serum represents an interfering agent in the creatinine assay and will result in an artificially elevated estimate of serum creatinine. This may result in an estimate of somewhere in the range of 3–5 mg/dl for serum creatinine rather than the normal ~1 mg/dl. Since creatinine clearance is determined as the ratio of excretion rate to plasma concentration, the erroneously high estimate of serum creatinine will lead to an underestimate of

GFR. This should make sense intuitively to students, since elevated creatinine levels are interpreted clinically as a sign of impaired kidney function. The error will also carry through in the determination of FWR, leading to overestimation of this parameter.

Answer to problem 5. Clearance is calculated as a solute's excretion rate divided by its serum concentration. We already have the plasma Na^+ concentration, but we need to calculate the excretion rate. Here, Na^+ excretion rate is urine flow rate (2.2 liters/24 h = 0.0916 l/h) × urine concentration (100 mmol/l) = 9.16 mmol/h or 0.153 mmol/min. Clearance is then calculated as 0.153 mmol/min ÷ 140 mmol/l = 0.00109 l/min or 1.09 ml/min. Relative to creatinine clearance, this value is 1.09/(125 ml/min), or ~0.087%. This indicates that Na^+ clearance is a small proportion of creatinine clearance. Using creatinine clearance as an estimate of GFR, this indicates that >99% of the Na^+ filtered by the kidney is reabsorbed. Na^+ clearance will vary depending on the osmotic conditions in the body but will always be significantly lower than creatinine clearance.

Answer to problem 6. In addition to being freely filtered and not reabsorbed, para-aminohippuric acid is actively secreted by the renal tubules. This means that nearly all (~90%) of the para-aminohippuric acid that enters the kidney through the renal arteries is eliminated in the urine. Therefore, para-aminohippuric acid clearance can be used to estimate renal plasma flow. Since para-aminohippuric acid is secreted into the renal tubules in addition to begin freely filtered, para-aminohippuric acid clearance should always be greater than inulin clearance. The ratio of inulin to para-aminohippuric acid clearance provides an approximation of GFR to renal plasma flow, also known as the filtration fraction, which is typically ~20% of renal plasma flow. The remaining 80% of plasma enters into the peritubular capillaries, where exchange (reabsorption and secretion) takes place with the renal tubules.

Answer to problem 7. Aquaporins are channel proteins located in cell membranes that facilitate water movement along concentration gradients. The establishment of the concentration gradient in the interstitial fluids of the kidney provides the driving force for water reabsorption, while aquaporins provide a route of water flow out of the collecting ducts and back into the blood. Antidiuretic hormone binding to receptors on the basolateral membranes of cells of the collecting ducts stimulates cytoplasmic vesicles containing aquaporins to fuse with the apical cell membrane. Insertion of these aquaporins results in the reabsorption of up to 99% of the water filtered by the kidney, thereby decreasing urine volume and increasing urine osmolarity. With alcohol consumption, antidiuretic hormone secretion is blunted, and large volumes of dilute urine are produced. This diuresis may help to flush some of the water-soluble, but still slightly toxic, breakdown products of alcohol from the body. The downside is that too much alcohol consumption can result in dehydration.

Answer to problem 8. Most small molecules, including water, ions, amino acids, and sugars, can be freely filtered out of the blood and into the forming urine. The major components in the blood that cannot be filtered are larger proteins and blood cells. Damage to the glomerular membranes may allow these proteins and blood cells to escape into the urine. The presence of either protein or blood cells in the urine is an indicator of damage to the renal corpuscle and may be a sign of kidney disease.

REFERENCES

1. **Dorizzi R, Pradella M, Bertoldo S, Rigolin F.** Refractometry, test strip, and osmometry compared as measures of relative density of urine. *Clin Chem* 33: 190, 1987.
2. **Guyton AC, Hall JE.** *Textbook of Medical Physiology.* Philadelphia, PA: W. B. Saunders, 1996.
3. **Leech S, Penney MD.** Correlation of specific gravity and osmolality of urine in neonates and adults. *Arch Dis Child* 62: 671–673, 1987.
4. **Marieb E.** *Human Anatomy and Physiology Laboratory Manual.* New York: Benjamin Cummings, 2003.
5. **Mark D, Zimmer A.** *Atlas of Clinical Laboratory Practices. Volume I Clinical Chemistry.* New York: McGraw Hill, 1967, p. 185.
6. **Medler S.** Physiological time and the measurement of glomerular filtration rate. *J Theor Biol* 176: 59–65, 1995.
7. **Richardson D, Speck D.** Addressing students' misconceptions of renal clearance. *Adv Physiol Educ* 28: 210–212, 2004.
8. **Silverthorn D.** *Human Physiology: an Integrated Approach.* New York: Pearson/Benjamin Cummings, 2010.
9. **Singer MA.** Of mice and men and elephants: metabolic rate sets glomerular filtration rate. *Am J Kidney Dis* 37: 164–178, 2002.
10. **Stevens LA, Coresh J, Greene T, Levey AS.** Assessing kidney function - measured and estimated glomerular filtration rate. *N Engl J Med* 354: 2473–2483, 2006.
11. **Tharp G.** *Experiments in Physiology.* New Jersey: Prentice Hall, 1997.
12. **Walker R, Olson M.** Renal function in the laboratory rat: a student exercise. *Adv Physiol Educ* 13: 49–55, 1995.
13. **Yokota SD, Benyajati S, Dantzler WH.** Comparative aspects of glomerular filtration in vertebrates. *Renal Physiol* 8: 193–221, 1985.