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Development of a classroom size nephron model and urinalysis experiment

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Water, ion, and pH homeostasis is essential for all physiological processes. The renal system plays a fundamental role in maintaining this homeostasis, which is controlled by the nephron, the functional unit of the kidney. Following ultrafiltration of the blood by the glomerulus, varying amounts of tubular reabsorption and tubular secretion take place, which are adjusted based on the physiological state of the body. Current approaches for teaching students about these complicated processes involve dialysis tubing and/or construction of miniature nephrons. Mini-nephrons use beads, strainers, and spoons to model the blood components, the filtration process, and reabsorption, respectively. While effective, these miniature models are difficult to assemble, putting too much emphasis on the construction phase. To focus student learning on the physiology, we have constructed a large, reusable classroom nephron model. This model demonstrates ultrafiltration of the glomerulus, tubular reabsorption, tubular secretion, and urine excretion. The classroom model can be easily modified to demonstrate two pathologies that affect ultrafiltration and tubular reabsorption: glomerulonephritis and Type I diabetes. Modified urinalysis experiments and case studies corresponding with each disease are included in our lab lesson. Overall, we suggest that the construction of a reusable larger classroom nephron model will help students learning renal physiology. In this presentation, the model and urinalysis experiments will be demonstrated, followed by a group discussion.

Keywords: Anatomy & Physiology; Model Building; Nephron; Urinalysis

Introduction

Models are important classroom tools with proven efficacy in explaining traditionally difficult biological concepts (Gilbert 1991, 2004). One such concept is the physiology of the nephron, the functional unit of the kidney. The collective action of approximately one million nephrons per kidney is responsible for regulating water, ion, and pH homeostasis while eliminating waste through the formation of urine (Preuss 1993). These processes involve three steps. The first is ultrafiltration, in which the dissolved substances of the blood plasma are filtered through the glomerular capillaries and podocytes into Bowman's space and the tubular nephron. The second step is reabsorption, primarily of water, ions, and other materials needed to maintain homeostasis. This occurs throughout the renal tubules and returns the substance to the blood. The last step is tubular secretion, where substances move from the peritubular capillaries to the renal tubules which then secrete them into the filtrate. This process includes substances like nitrogenous waste, drugs, and excess ions.

A survey of US internal medicine subspeciality fellows reported that renal physiology was one of the most challenging topics in medical school (Jhaveri 2013). Many respondents felt they were underprepared about this topic, thus discouraging them from specializing in that field. This topic is taught to undergraduate students at most universities as well. It is taught most often as a component of a basic anatomy & physiology course as well as upper-level physiology courses. It is also taught, in a rather simplified way, through non-science major, general education courses. Similarly, it is a topic our students find difficult to understand.

Various pedagogical approaches have been used to explain the physiology of the nephron, each with their own benefits and limitations. One method challenges students to draw a diagram of the nephron (Robinson 2018), while another uses flash-based animations which emphasize how urea, water, glucose, proteins, and sodium are reabsorbed (KScience 2021).

Additionally, hands-on approaches have been implemented. In fact, laboratory supply companies sell kits focused on renal physiology. However, they emphasize the ultrafiltration step (Kidney Filtration Carolina 2021, Wards Kidney Dialysis 2021) but not the processes of reabsorption and secretion. These kits often involve using simulated kidney blood and dialysis tubing. The simulated blood remains inside the dialysis tubing while smaller molecules pass through.

One company, ScienceTake*Out, sells a buildable model that allows students to make a simplified version of a nephron (Kidney Problem, 2021). This model uses simulated blood composed of beads of various sizes, representing the different components of blood, and simulated urine. While the concepts of ultrafiltration and reabsorption are observed, the latter of which is done manually by the students using spoons, tubular secretion is not modeled. A fully functional model that is easy to assemble and reusable that highlights all three processes would allow for a more thorough introduction to the physiology of the nephron.

To address these challenges, we designed a laboratory-based lesson in which students use such a nephron model to learn about all three processes. We have designed a large classroom model for a teacher to use when discussing the kidney in lecture (Figure 1) and smaller student models for a more hands-on experience (Figure 2). The models are pre-made for the students, allowing them to focus on understanding the physiology of the nephron, rather than the construction. Our model can be reused and allows students to observe two pathologies that affect ultrafiltration and tubular reabsorption: glomerulonephritis and Type I diabetes.

In this paper, we describe the design of both the teacher and student models and how they can be used in conjunction with a urinalysis experiment. Students conduct a simulated urinalysis experiment which relates their results back to the model to explain the cause of any abnormality seen in the urine. Both the model and activity have allowed our students to have a better understanding of how the nephron works and how certain pathologies can result.

Figure 1. Classroom Nephron Model

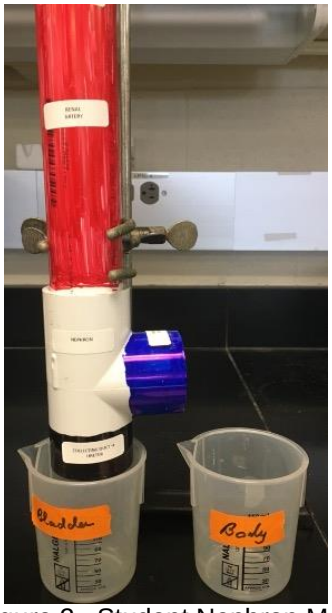

Figure 2. Student Nephron Model

Student Outline

Objectives

By the end of this lab, students will:

- Be able to describe the various functions of the nephron.
- Be able to use the student nephron model to predict urinalysis results from healthy individuals and those diagnosed with diabetes and glomerulonephritis.
- Be able to perform and interpret a urinalysis.

Introduction

Part A: The Nephron

The renal system is responsible for maintaining our homeostasis of water, ions, and pH and is involved in numerous other physiological activities, including blood pressure maintenance, red blood cell count, and excretion of waste products. The renal system is composed of the **kidneys,** which are paired organs located in the abdominal cavity. One of their major functions is to produce urine and extract waste products and toxins from the blood, which is accomplished in combination with the other components of the urinary system. The urinary system functions to eliminate waste in the liquid form of urine which contains water-soluble waste products and exogenous compounds.

Homeostasis and urine formation is accomplished by microscopic tubules called **nephrons** (Figure 1), the functional unit of the kidney. The parts of the nephron include: 1. The renal corpuscle (composed of the glomerulus, Bowman's space and the glomerular capsule); 2. the proximal convoluted tubule; 3. The loop of the nephron (Loop of Henle); 4. the distal convoluted tubule and; 5. the collecting duct.

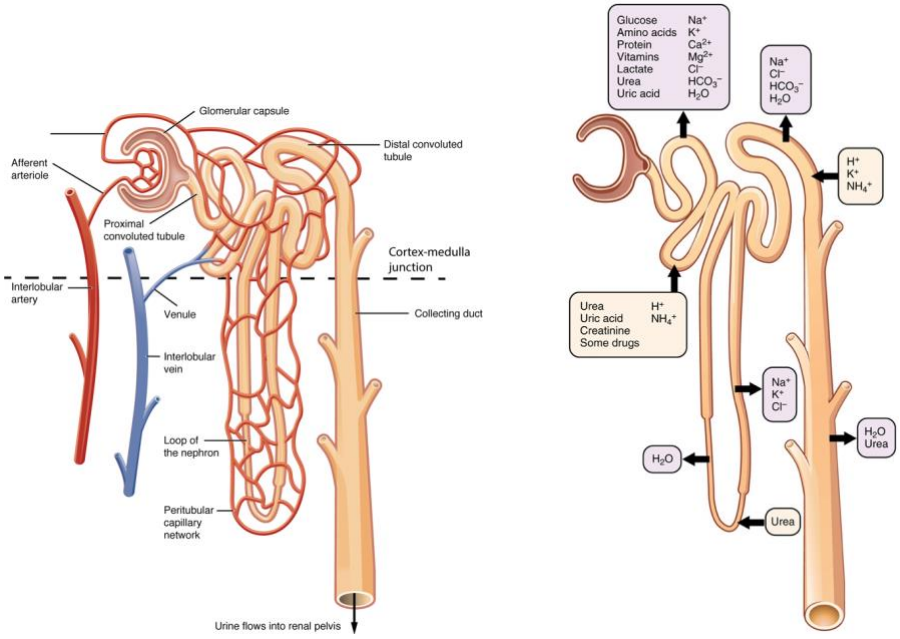

Figure 1: Anatomy of the nephron. (A). Blood to be filtered enters the nephron via the afferent arteriole, a branch of the renal artery. The renal artery is a major muscular artery of the body that divides into the segmentary arteries, followed by the interlobar arteries, and eventually leads to the afferent arterioles. Filtered blood enters the peritubular capillary network that surrounds the tubular nephron via the efferent arteriole, before returning to the systemic circulation. The peritubular capillaries connect to the interlobular veins and then to the renal vein. (B) Nephron image without blood vessels shown. Locations of reabsorption (arrow directed out of the nephron) and tubular secretion (arrow directed into the nephron) are noted. Images from OpenStax College - Anatomy & Physiology, Connexions Web site.

Within the nephron, there are three key processes involved in regulating fluid homeostasis and the formation of urine:

1. **Ultrafiltration.** Glomerular capillary blood pressure forces water and water-soluble substances such as ions, nutrients, and waste products out of the blood (through glomerular capillaries) and into Bowman's space of the nephron, forming a solution called filtrate. Initially, the filtrate is composed of the same concentration of water, ions, nutrients, and waste products as that of the blood. However, the filtrate lacks blood cells and plasma proteins, because they are too big to pass through the glomerular capillaries and are repelled by a specialized basement membrane separating the blood from the filtrate.

2. **Reabsorption.** Components of the filtrate that need to be maintained in the body move from the nephron back into the blood stream; this is called reabsorption. The most important substances that are reabsorbed are water, ions, and nutrients such as glucose. In fact, without the process of reabsorption, land animals would quickly dehydrate due to a rapid loss of water from their bodies. Reabsorption occurs when water, salts, and glucose are transported from the nephron tubules into the extracellular fluids and then into the peritubular capillaries. From here, the components will eventually move back to the renal veins and into the systemic circulation. Thus, these important substances are retained in the body and not lost in the urine.

3. **Tubular secretion.** Tubular secretion is the process of moving additional components of the blood that were not filtered in the glomerulus into the filtrate of the renal tubules. Waste and toxic substances, such as creatine or drugs, are actively transported and/or passively diffused from the peritubular capillaries to the renal tubule. These substances will eventually end up in the urine.

The processes of tubular reabsorption and secretion are modulated by the endocrine system. A central regulator of fluid homeostasis is the hormone **vasopressin** (also known as antidiuretic hormone, ADH), which is released from the posterior pituitary gland, travels in the blood to the kidneys, and signals to the principal cells of the collecting ducts. ADH signaling increases the amount of water channels (aquaporins) on the surface of the principal cells, making them more permeable to water, which allows for water retention. Consuming alcohol blocks the release of ADH, which decreases the number of aquaporins in the collecting tube. This results in a decrease in water retention and an increase in urination. The urine is diluted with more water. This results in an individual becoming dehydrated, with contributes to a feeling of a hangover.

The nephron can be negatively affected by different diseases and infections, which ultimately affects the production and composition of urine. Two common examples are **diabetes** and **glomerulonephritis**.

- **Type I Diabetes** (also known as juvenile diabetes) is a disease that leads to the destruction of cells in the pancreas called beta-cells, which results in little to no insulin production. Insulin is an important hormone because it regulates the amount of glucose in our blood by allowing our cells to use it or store it for energy. **Type II Diabetes** is the much more common form. This occurs when the body becomes resistant to insulin, or fails to make enough insulin. If a person does not have enough insulin, then glucose levels in the blood will rise. In diabetic individuals, blood glucose can be so high that not all of the glucose is reabsorbed by the proximal convoluted tubule. Hence, much of the glucose ends up in the urine.

- **Glomerulonephritis** is a disease which is caused by a number of different factors, one of which includes an inflammatory response following a Streptococcal infection. This results in inflammation of the glomeruli and other small blood vessels entering the nephron. This alters the processes of filtration, reabsorption, and secretion, resulting in very abnormal urine.

Part B: Urinalysis

The visual and chemical examination of urine is known as **urinalysis**. Urinalysis can be used to indicate if the kidneys are functioning properly or to determine if an individual has a particular disease. There are numerous properties of urine that can be examined during a urinalysis including:

- **Color.** Many things affect urine color, including fluid balance, diet, medicines, and diseases. How dark or light the color is tells you how much water is in it. Some medicines and foods, like blackberries, beets, and rhubarb, can turn urine red-brown. **Gross hematuria** is when blood is visible in the urine.

- **Clarity.** Urine is normally clear and sterile (free of all microbes). Microscopic analysis can be performed to check for sterility. Bacteria, blood, sperm, mineral crystals, or mucus can make urine look cloudy.

- **Odor.** Urine does not smell very strong, but it has a slightly "nutty" odor. Some diseases cause a change in the odor of urine. Diabetic urine has a sweet smell due to the presence of glucose in the urine.

- **Specific gravity.** This checks the amount of substances and water in the urine. The higher the specific gravity, the more solid material is in the urine. When you drink a lot of fluid, your kidneys make urine with a high amount of water in it, which has a low specific gravity. When you do not drink fluids, your kidneys make urine with a small amount of water in it, which has a high specific gravity. For reference, the specific gravity of distilled water is 1.000 while in uncontrolled diabetes it may reach as high as 1.045.

- **pH.** The pH is a measure of how acidic or alkaline (basic) the urine is.

- **Protein.** The presence of protein in urine is known as **proteinuria**.

- **Glucose.** The presence of glucose in urine is known as **glucosuria.**

Methods and Data Collection

Part A: Modeling the Nephron

1. Your instructor has a classroom model of a functioning nephron. The simulated “blood” contains water, red balls (representing red blood cells/erythrocytes), yellow and green balls (representing proteins), and lead sinkers (representing glucose). Watch the instructor pour the blood into the classroom model. For each scenario below, pour the simulated “blood” at your lab bench into your small nephron model and note how each blood component travels through the nephron and where it is found at the end.

NORMAL NEPHRON	Did this enter the renal vein? Was this due to filtration/reabsorption or secretion?	Did this enter the bladder to be excreted as part of urine? Was this due to filtration/reabsorption or secretion?
Water		
Red Blood Cells		
Proteins		
Glucose		

TYPE I DIABETES	Did this enter the renal vein? Was this due to filtration/reabsorption or secretion?	Did this enter the bladder to be excreted as part of urine? Was this due to filtration/reabsorption or secretion?
Water		
Red Blood Cells		
Proteins		
Glucose		

GLOMERULO-NEPHRITIS	Did this enter the renal vein? Was this due to filtration/reabsorption or secretion?	Did this enter the bladder to be excreted as part of urine? Was this due to filtration/reabsorption or secretion?
Water		
Red Blood Cells		
Proteins		
Glucose		

Part B: Urinalysis

2. At your bench is a synthetic urine sample from an individual. Perform urinalysis on this sample and use this information to diagnose your patient. Describe the properties of the urine that have allowed you to draw your conclusion. Record the sample letter of your assigned urine sample here: _____

Test	How to perform test	Result
Color	Visual observation.	
Clarity	Visual observation	
Odor	Waft sample	
Specific gravity	Pour sample into glass cylinder. Drop in hydrometer. Read the # at the bottom of the meniscus. Read as 1.0____ (ex: 1.0<u>10</u>, 1.0<u>20</u>, etc..)	
Gross hematuria	Visual observation.	
pH	Use pH paper (or pH probe)	

Take your sample and divide it equally into 2 test tubes and test for glucosuria and proteinuria.

Glucosuria	Add 400 µl of Benedict's Solution to a test tube. Place the tube in a hot water bath for 5 minutes. Glucose is present if the color of the solution changes from <u>blue to any other color</u>. If it remains blue, then glucose is not present.	
Proteinuria	Add 400 µl of Biuret Solution to a test tube. Protein is present if the color changes from <u>blue to purple</u>.	

The table below shows the common properties of various types of urinalyses:

	Color	pH	Gross hematuria	Glucosuria	Proteinuria	Specific Gravity	Microbes	Clarity	Smell
Normal Urine	Yellow-Amber	4.6 - 8	no	no	no	1.010-1.025	no	clear	mild
Diabetic	light yellow	< 4.6	no	yes	could be present	> 1.025	no	clear	sweet
Urinary Tract Infection (UTI)	light yellow (or darker if gross hematuria)	> 8	could be present	could be present*	could be present	1.008-1.020	yes	cloudy	mild
Hydrated Individual (Following alcohol consumption)	colorless	< 4.6	no	no	no	< 1.010	no	clear	mild
Glomerulo-nephritis	red (from hematuria)	< 4.6	yes	yes	yes	< 1.025	No	clear	mild

* glucosuria can lead to the development of a UTI.

(Data collected from www.webmd.com, https://pubmed.ncbi.nlm.nih.gov/17026722/ and Carolina Biological Supply Company)

Conclusion/Discussion

3. Based on the results of your urinalysis, what conclusion can you draw? Be sure to explain how you arrived at your answer.

_____

_____

_____

_____

Materials

Construction of the Nephron Model

All materials necessary to construct the classroom and student models can be found at local hardware and/or pet stores. Detailed explanations of how to build the nephron models can be found in Appendix A.

Preparation of Simulated Blood

Beads of various sizes are needed to prepare the simulated blood samples for the models. Erythrocytes were modeled by 24 mm red beads, proteins by 12 mm beads of various colors, and glucose molecules by soft lead split shot (size 1). All of these materials can be bought at local crafting stores or on Amazon (Appendix B).

Blood samples were prepared by adding all beads to beakers containing 500 mL of water (classroom size) or 150 mL (student size). Twice as many glucose molecules should be added to the diabetic and glomerulonephritis samples.

Urinalysis

All materials needed to prepare the simulated urine samples and urinalysis reagents can be found in Appendix C. Simulated urine samples were derived from Carolina Biological Supply Company urine samples. Our procedure uses Benedict's and Biuret Reagents to test for the presence of glucose and protein, respectively. However, instructors can substitute test strips for these reagents.

Notes for the Instructor

Nephron Models

Both the classroom and student nephron models should show that erythrocytes and proteins are not filtered, while glucose is fully reabsorbed and returned to the body. Most of the water enters into the bladder in these models, so students should be made aware that most water is normally reabsorbed.

Urea is a nitrogenous compound and is an end product of protein metabolism. Our model does not show urea since it is reabsorbed in the proximal tubule, secreted in the loop of Henle, and partially reabsorbed back into the body at the collecting duct. Our model focuses primarily on complete filtration, reabsorption, and excretion. Although the model and simulated blood does not include urea and other soluble ions and compounds, students should be made aware that these molecules would be present in the urine that collects in the bladder.

The diabetic nephron lacks the screen mesh (Appendix A). Therefore, both water and glucose will enter into the urine, thus simulating glycosuria. This allows the teacher to explain that urinalysis can be used to monitor a patient's blood glucose levels. In other words, when blood glucose levels are higher than the resorptive capacity of the nephron, then it will be excreted in the urine. The glomerulonephritis nephron lacks a filter, simulating the fact that components of blood that are usually filtered at the glomerulus pass through and are excreted in the urine (i.e., hematuria (blood in urine) and proteinuria (protein in urine)).

While the student model only shows the end result of what blood components end up in the urine and what is returned to the body, the classroom model demonstrates the filtration, reabsorption and tubular secretion steps, while identifying the proximal and distal tubules. The yellow tubing in the classroom model allows for students to see the components of blood that were not filtered in the glomerulus pass into the filtrate of the renal tubules. This allows teachers to explain how certain drugs end up in an individual's urine.

Follow-Up Activity (for advanced classes):

Our model can be altered to mimic nephron behavior in diabetics and glomerulonephritis patients. Continuing with that same idea, students could be assigned a follow-up assignment where they are asked to conceive of ways of changing the model to mimic different physiological conditions such as differing levels of ADH or the use of diuretics, which would alter water permeability in the model.

Response to the Activity:

These models have been used in our university's non-science major lab course, anatomy & physiology, and upper-level systemic physiology courses. In all courses, both instructors and students have commented that the models have helped with their understanding of the function of the nephron. In a survey given to non-science majors, two questions were asked in a pre- and post-lab survey ("What is the function of the nephron?" and "What molecules are present in the urine of an individual with glomerulonephritis?"). There was an increase in an understanding of what the nephron does after watching the model demonstration, despite the students having learned about it in lecture. Only 7% of students answered the survey correctly before the lab. Following the lab, approximately 40% of the students answered the survey correctly. The model produced an increased understanding of what passes through the glomerular capillaries in an individual with glomerulonephritis.

An instructor teaching anatomy and physiology felt that using the models seemed to help students understand nephron filtration. A student taking anatomy and physiology commented that the models were "a great way to demonstrate how different conditions can affect the urinary system and the nephron."

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About the Authors

Aelin G. Shea received her Bachelor of Science from Saint Joseph's University in 2018 and recently obtained a master in public health degree (MPH) in Spring 2021 from Florida State University. She is currently a clinical research/ coordinator/ psychometrician working in Alzheimer's research and treatment. While a junior and senior at Saint Joseph's University, Aelin was as summer scholar, where her love for research began.

Matthew D. Nelson earned his Ph.D. in Biology at New York University and completed his postdoctoral training in Dr. David Raizen's lab at the University of Pennsylvania. He is currently an associate professor in the biology department at Saint Joseph's University. His research is focused on understanding the cellular and molecular nature of complex behaviors, such as sleep. Also, he is an active mentor for pre-health students and teaches human physiology and histology.

Louis D'Angelo received his B.S. from St. John's University, his M.S. from Long Island University and was a Ph.D. candidate at New York University for four years. He spent 10 years in the pharmaceutical industry as a protein purification chemist. In 1998 he joined the faculty of St. Joseph's University where he taught Cells, Genetics, Human Biology, Heredity and Evolution and Anatomy and Physiology for biology majors and non-majors who were wishing to enter nursing or allied health science programs.

Gabrielle Mikalonis, PT, DPT received her Bachelor of Science from Saint Joseph's University in 2018. While there, she worked closely with Dr. Brian

M. Forster assisting in research and as a Teaching Assistant for his general biology labs. She later went on to earn her Doctorate of Physical Therapy from Thomas Jefferson University in 2020. Ms. Mikalonis currently works as a physical therapist in Philadelphia, PA.

Brian M. Forster received his Ph.D. from Cornell University. In 2011, he joined the faculty of Saint Joseph's University. Dr. Forster is the laboratory coordinator for laboratory-based classes designed for students who are not science majors. He teaches courses in general biology, heredity, environmental science, and a course in microbiology designed for students wishing to enter nursing or allied health programs.

Appendix A:
Construction of the Classroom and Student Nephron Models

Materials for Classroom Model:

- Two 2-Liter Soda Bottles (empty).
- PVC Piping (2 inch/5.08 cm diameter, two pieces with a length of 30 cm, one piece with a length of 25 cm, two pieces with a length of 12 cm). Materials can be bought at a local hardware store (Lowes/ Home Depot). If piping cannot be obtained, hamster tubing from a pet store or a cut wiffle ball bat may be used.
- Two 90-degree 2 inch/5.08 cm diameter Schedule 40 Tee PVC. Materials can be bought at a local hardware store.
- 1 plastic rectangle filter (see below for directions).
- 2 plastic funnels (must allow beads of 24 mm and smaller to pass through).
- Screen mesh from local hardware store.
- 2 Rubbermaid 1.2 L plastic food storage containers.
- 2 Glad 24 oz plastic food storage containers.
- Glue.
- Red paint.

Construction Details:

(1) Begin by preparing plastic rectangle filters (Figure A1):
   - Cut a piece of plastic from a soda bottle (5 cm x 10 cm).
   - Drill holes into the plastic using a Dremel tip. Holes should be no bigger than 12 mm (big enough for glucose/lead shot to pass through, but too small for protein beads to pass).

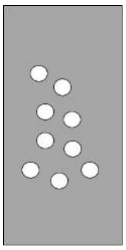

Figure A1: Plastic rectangular filter

(2) Prepare Bowman's capsule:
   - Insert plastic rectangle filters into one of the tees (Figure A2). This filter will allow water and glucose shot to pass down. All other beads (erythrocytes and proteins) will be diverted into the side. The second tee will not have a plastic rectangle filter inserted. This tee will be used to make the glomerulonephritis model.

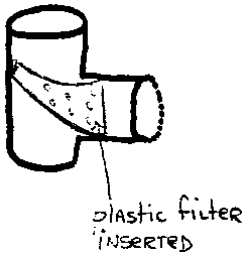

Figure A2: Insertion of plastic rectangular filter into tee

(3) Prepare efferent arteriole (Figure A3):
   - Take a Rubbermaid container and drill a hole in bottom of the container and attach PVC pipe of 12 cm. Pipe should sit flush to bottom of the container. Glue pipe into place.
   - Paint pipe red to indicate that this is a blood vessel.

- The efferent arteriole container should be clamped and placed on a ring stand.

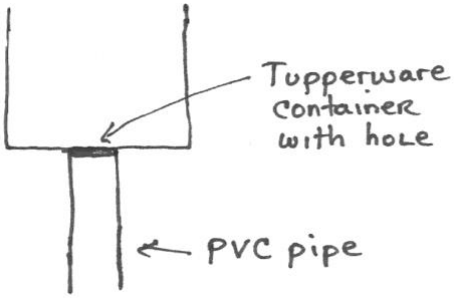

Figure A3. Efferent arteriole design.

(4) Prepare proximal tubule and loop of Henle (Figure A4):
- Take a plastic Rubbermaid container and cut holes in bottom that remove almost all of the whole bottom.
- In right corner of that same container, drill a hole and attach PVC pipe of 12 cm. Pipe should sit flush to bottom of the container. Glue pipe into place.
- Paint pipe red to indicate that this is a blood vessel.
- The proximal tubule/loop of Henle container should be clamped and placed on a ring stand.
- Cut a piece of screen mesh to insert into the Rubbermaid container. When the screen mesh is in place, the model can be used to demonstrate normal nephron activity. When the screen mesh is removed, the model can be used to demonstrate nephron filtration in a diabetic patient.

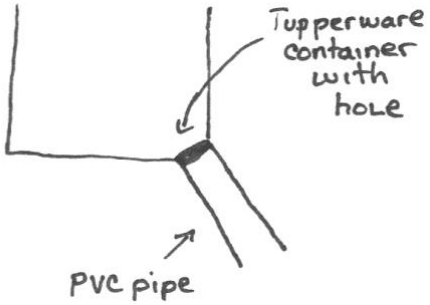

Figure A4. Proximal tubule and loop of Henle design.

(5) Prepare afferent arteriole (Figure A5):
- Take the two 2-liter soda bottles and cut off the tops.
- Insert a funnel with a diameter that can allow erythrocyte beads to pass through (24 mm) into each bottle. Cut stem off funnel if needed.
- Cut bottoms of soda bottle and connect the 30 cm PVC piping with glue.
- Paint bottle and pipe red to indicate that this is a blood vessel.

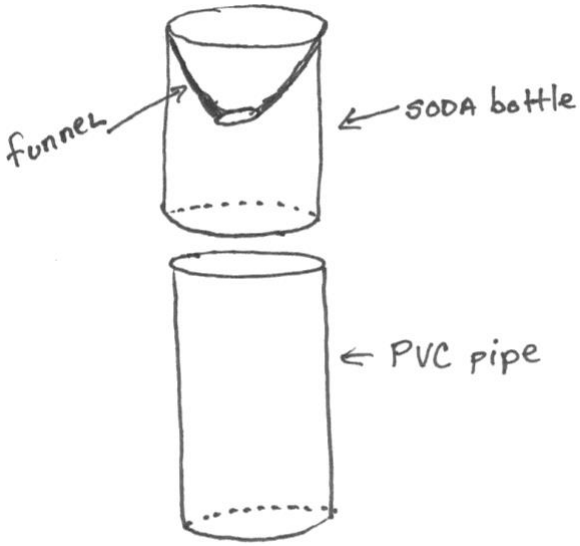

Figure A5. Afferent arteriole design.

(6) The PVC pipe that is 25 cm long will act as the distal tubule.

(7) Connect afferent arteriole to Bowman's capsule tee:
- For first nephron model, glue an afferent arteriole with the Bowman's capsule tee that contains the plastic filter. This model will serve as the normal nephron and the diabetic nephron.
- For second nephron model, glue an afferent arteriole with the Bowman's capsule tee that lacks the plastic filter. This model will serve as the glomerulonephritis nephron.

(8) Assemble the model (Figure A6):
- For the normal nephron,
  - Clamp the first afferent arteriole/Bowman's capsule to a ring stand. Position the proximal tubule/Loop of Henle and Efferent Arteriole containers as shown below.
  - Take two Glad containers. Label one "bladder" and the other "renal vein."
  - Insert a piece of screen mesh into the proximal tubule container.
  - Connect the distal tubule by a clamp and ring stand such that the distal tubule leads directly into the bladder.
- For the diabetic nephron,
  - Follow the same procedure as the normal nephron.
  - Do not insert the screen mesh into the proximal tubule/Loop of Henle container.
- For the glomerulonephritis nephron,
  - Clamp the second afferent arteriole/Bowman's capsule to a ring stand. Position the proximal tubule/Loop of Henle and Efferent Arteriole containers as shown below.
  - Take Glad containers. Label one "bladder" and the other "renal vein."
  - Insert a piece of screen mesh into the proximal tubule container.
  - Connect the distal tubule by a clamp and ring stand such that the distal tubule leads directly into the bladder.

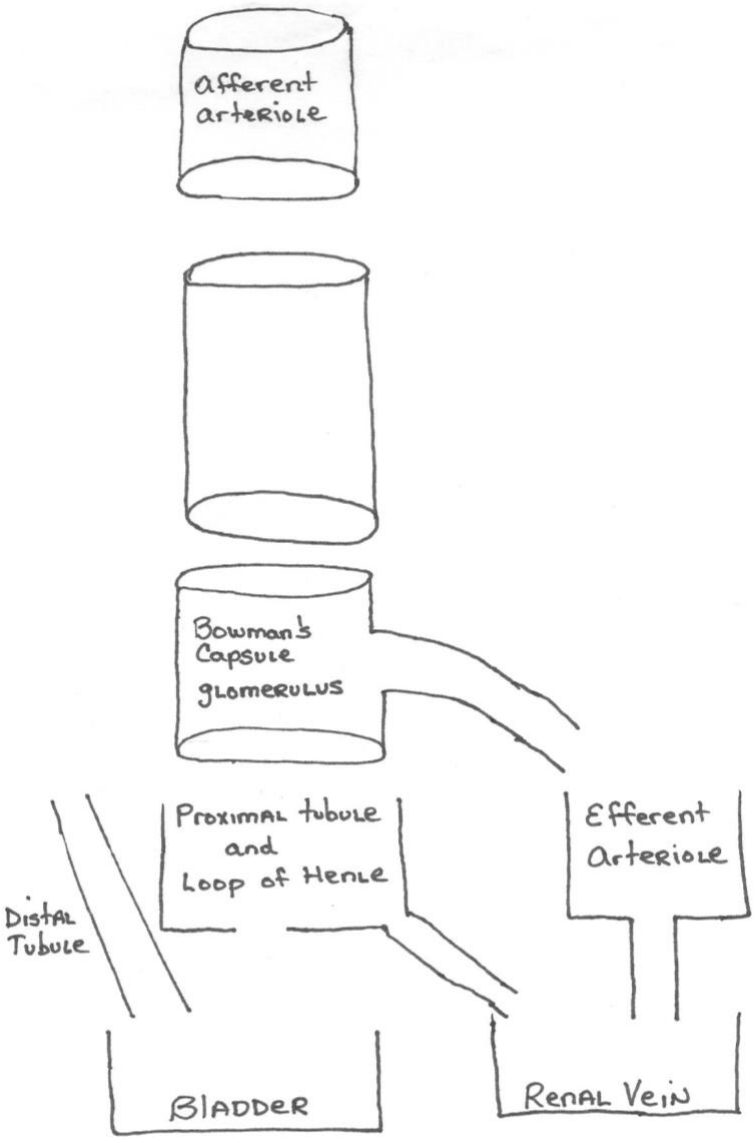

Figure A6. Final assembly of classroom nephron model.

Model Images:

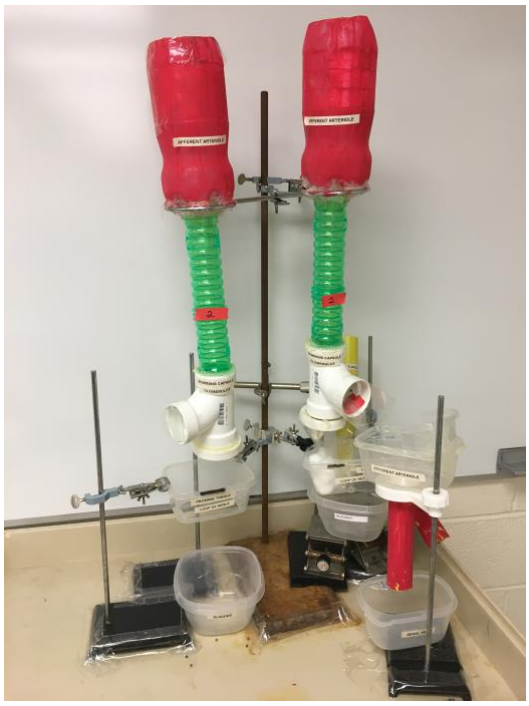

Figure A7. Complete set-up of classroom model. Model is held by clamps on ring stands. *(Note: This is Figure 1 from the main text)*

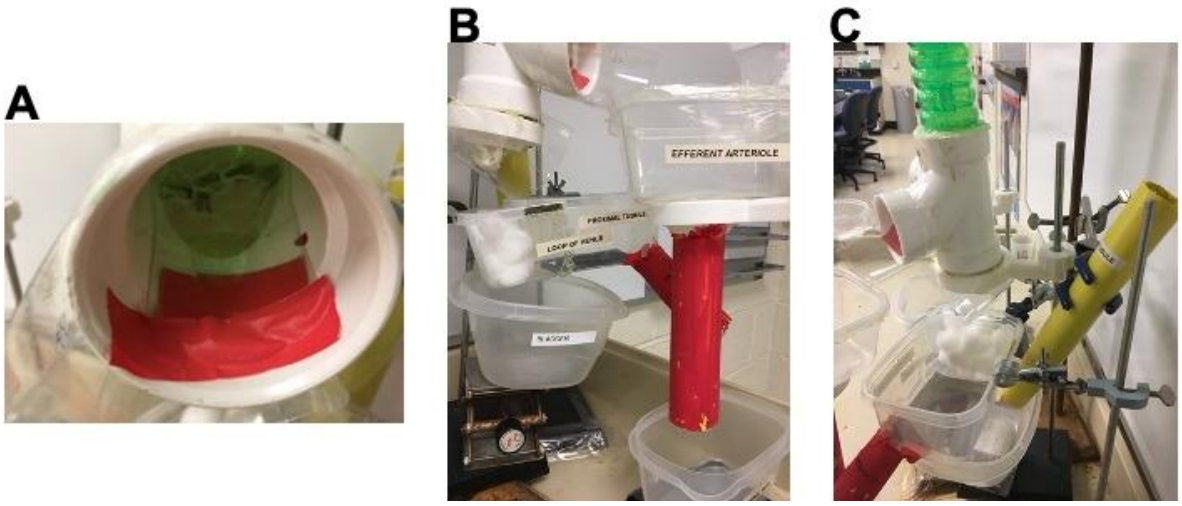

Figure A8. (A) Close up of plastic filter in tee (Bowman's Capsule). (B) Assembly of nephron with proximal tubule/loop of Henle, bladder, efferent arteriole and renal vein containers. (C) Image of the distal tubule behind tee (Bowman's Capsule) leading directly to the bladder to simulate tubular secretion.

Materials for Student Models:

- 3 pieces of PVC piping (1.25 inch/ 3.2 cm diameter, cut to a length of 16.5 cm)
- 3 90-degree Tee PVC fittings (1.25 inch/ 3.2 cm diameter)
- 2 plastic rectangle filters (see below for directions)
- Screen mesh
- Glue
- Sharpie markers (red, blue, black)
- 2 150 ml beakers

Construction Details:

(1) Begin by preparing plastic rectangle filters (Figure A9):
   - Cut 2 pieces of plastic from a soda bottle (4 cm x 9.5 cm).
   - Drill holes into the plastic using a Dremel tip. Holes should be no bigger than 12 mm (big enough for glucose/lead shot to pass through, but too small for protein beads to pass).
   - To one piece of plastic, cut and paste a piece of screen mesh over the plastic.

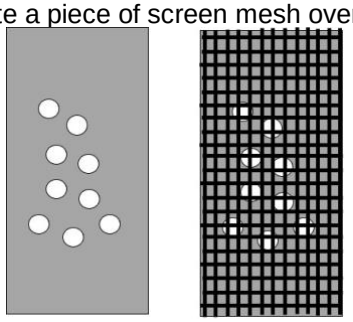

Figure A9: Plastic rectangular filters without and with screen mesh added.

(2) Insert plastic rectangle filters into tees (Figure A2):
   - To the first tee, insert the plastic filter with the screen mesh.
     - This tee will be used to make the normal nephron model.
     - Insert the plastic filter as shown below.
     - This filter will allow only water to pass down. All beads will be diverted into the side.
   - To the second tee, insert the plastic filter without the screen mesh.
     - This tee will be used to make the diabetic nephron model.
     - Insert the plastic filter as shown below.
     - This filter will allow water and glucose shot to pass down. All other beads (erythrocytes and proteins) will be diverted into the side.
   - The third tee will not have a plastic rectangle filter inserted.
     - This tee will be used to make the glomerulonephritis model.

(3) Build nephron model:

- Connect PVC pipe to the tee as shown below (Figure A10).

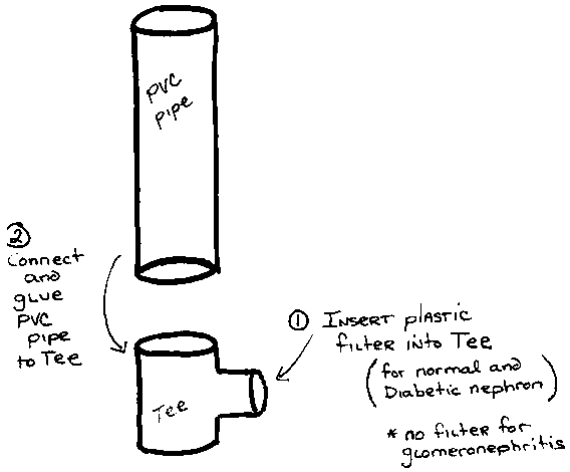

Figure A10: Connection of PVC pipe to tee

- Color the PVC and label each part:
  - Color PVC pipe red (label renal artery).
  - Middle of tee is kept white (label nephron).
  - Color bottom portion of tee black (label collecting duct→ureter).
  - Color side portion of tee blue (label renal vein).

Model Images:

Figure A11: Complete set-up of student model. Model is held by a clamp on a ring stand. Two beakers are set up beneath the nephron. One beaker directly below the tee represents the bladder. The other beaker to the side represents what was filtered and returned to the body. *(Note: This is Figure 2 from the main text)*

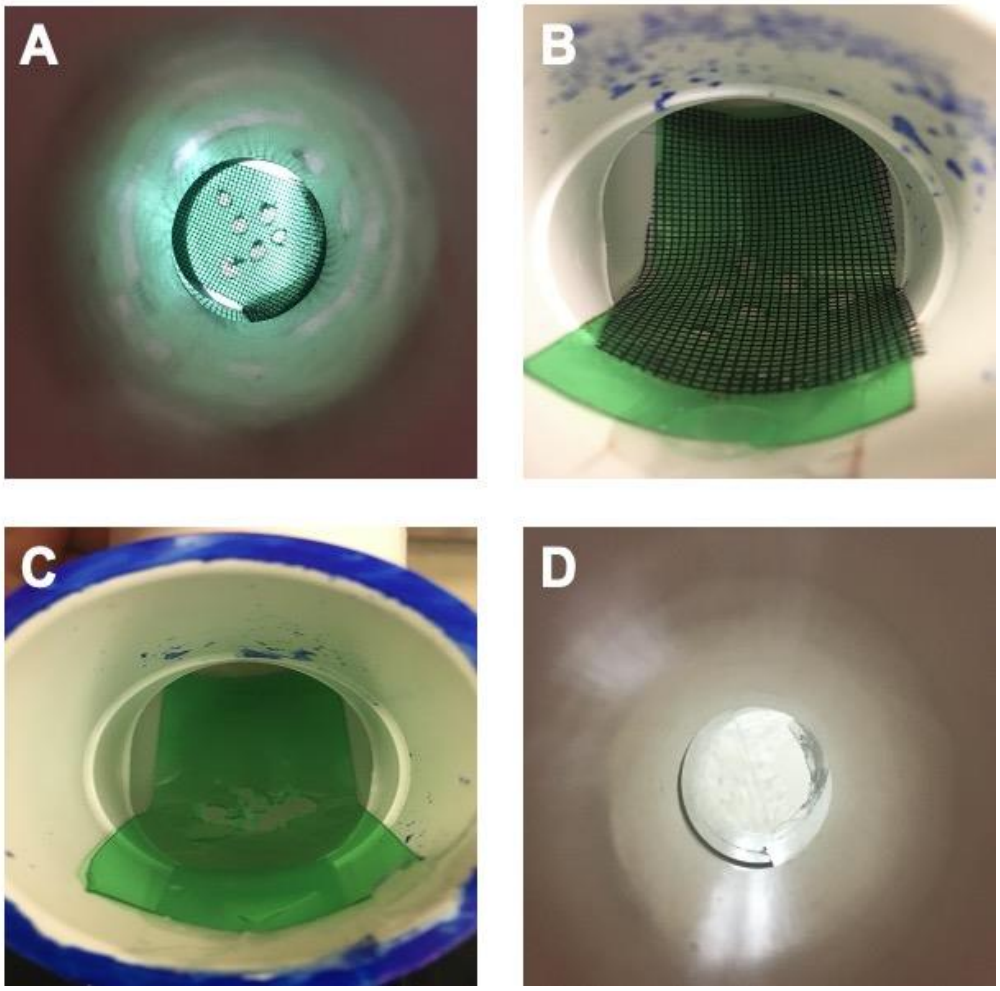

Figure A12: (A) View of plastic filter looking down renal artery in normal nephron. (B) Side view of filter in normal nephron. (C) Side view of filter in diabetic nephron. Note the lack of screen mesh. (D) View of glomerulonephritis model looking down renal artery. Note the lack of a plastic filter.

Appendix B:
Construction of Simulated Blood

Beads of various sizes are needed to prepare the simulated blood samples for the models.

- Erythrocytes were modeled by 24mm, red beads: AMAZON LINK
- Proteins were modeled by 12mm beads of various colors: AMAZON LINK
- Glucose molecules were modeled by soft lead split shot (size 1): AMAZON LINK

Appendix C:
Preparation of Urinalysis Samples

Urinalysis materials

- Simulated Urine Samples from Carolina Biological (Normal (#695955), Urine with Glucose (#695951), Urine Low (#695954)).
- Salted caramel syrup (e.g. Torani, to emphasize sweet odor from diabetic urine)
- 2 M Sodium Hydroxide Solution
- Bovine Serum Albumin (BSA) Solution (20 mg/mL).
- Red Food Coloring Solution (1 drop food coloring in 50 mL of deionized water).
- Overnight culture of *Escherichia coli* (any non-pathogenic strain will work, i.e., K12)

Urinalysis materials needed at student bench

- Simulated Urine Sample (see below)
- Benedict's Reagent (either Carolina Biological, Wards Science or Flinn Scientific)
- Biuret Reagent (either Carolina Biological, Wards Science or Flinn Scientific)
- Hydrometer (for specific gravity). (Carolina Biological #722660)
- Water bath on and set to 60°C (for Benedict's test for glucose).
- Two test tubes (18 x 150 mm).
- 1 mL Pipets (or micropipettors P200 and P1000)

Urinalysis Sample Preparations:

Urine Sample	Diagnosis	Preparation
A	Diabetic (without proteinuria)	• 10 mL Urine with Glucose • 200 μl of salted caramel syrup
B	Glomerulonephritis	• 10 mL Urine with Glucose • 500 μl of red food coloring solution • 1 mL BSA Solution
C	Normal Urine	• 10 mL Normal Urine
D	Alcohol Consumption	• 10 mL Urine Low
E	Urinary Tract Infection	• 10 mL Normal Urine • 1 mL BSA Solution • 100 μl of 2 M sodium hydroxide • 500 μl of overnight *E. coli* culture
F	Diabetic (with proteinuria)	• 10 mL Urine with Glucose • 200 μl of salted caramel syrup • 1 mL BSA Solution

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