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Understanding How the Nephron Concentrates Urea in Urine: An Experimental Approach Using Dialysis Bags

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Understanding how the nephrons of the kidney concentrate urea in urine is a particularly challenging concept for students of organismal biology/anatomy. I developed a laboratory exercise using dialysis bags to replicate the progression of filtrate through the nephron. Dialysis bags are permeable to small molecules like water and salt, but not sugar. Since the descending loop of Henle and part of the collecting duct are permeable to water, but not salt, I replaced sugar with salt for this part of the simulation. Students start with a dialysis bag of known volume and concentration of solute (10mL, 10% sugar) placed in 50% sugar water simulating the descending loop of Henle. The new volume and concentration is determined, and a new dialysis bag of the same volume and concentration of solute (salt) is placed in a beaker of 100% water simulate the ascending loop of Henle. The volume is assumed to not change and salt concentration is determined using a salinity probe. The final bag then is of the same volume and solute concentration (sugar) and placed in 50% sugar water to simulate the collecting duct. Students track the concentration of salt and urea (hypothetical) and water volume through the experiment.

Keywords: excretion, urea, kidney, nephron, inquiry-based learning

Link to Original Poster File: **https://doi.org/10.37590/able.v41.poster65**

Introduction

The nephron of the mammalian kidney is able to concentrate urea, allowing animals to excrete hyperosmotic urine, reducing water loss. The means by which the nephron accomplishes this are complex, resulting in students experiencing difficulties in understanding this process. This process is driven by the tubule of the nephron passing between the medulla and cortex, which have different concentration gradients of salt, multiple times. As a result of passive transport driven by this unique anatomy and the addition of active transport, urea is concentrated to 342-490 mM in the urine (a hundred fold difference from blood concentration). I developed an experiment which simulates the different parts of the nephron in sequential order: mathematical simulation for areas with active transport and the use of dialysis bags in beakers for areas with passive transport. Students take an initial sample of "filtrate" and pass it through each of these simulations, recording changes in the filtrate salt and (theoretical) urea concentration and volume.

Student Outline

Objectives

- Understand the structure of the kidneys, and how this structure concentrates urea in urine

Introduction

In the kidney, the salt concentration is lowest in the cortex, and raises as you go into the medulla (Urry et al. 2016). Filtrate from the blood enters the nephron through the glomerulus at the Bowman's capsule in the cortex. The filtrate flows through the proximal tubule in the cortex, then through the loop of Henle which descends into the medulla and then ascends back to the cortex. After the loop of Henle, the filtrate passes through the distal tubule, before passing into the collecting duct, which descends into the medulla before leaving the kidneys (see Table 1 for summary, including which molecules are transported at each stage). The filtrate loses water, which is driven by passing through areas of the kidney with differing salt concentrations, becoming hyperosmotic (with respect to urea) to the body. Keep in mind that very little urea leaves the filtrate; thus, loss in volume in the filtrate results in an increase in concentration of urea. Normal urea concentration in the blood is 2.5-7.1 mM, while it is 342-490 mM in the urine (a hundred-fold difference). We will use larger values in our experiment to make it easier to visualize.

Table 1. Summary of transport in regions of the nephron.

Region	Region of the Kidney Located In (Direction of movement indicated if more than one)	Type of Transport	Molecules that Can Pass Through From the Nephron Into the Main Kidney Region
Proximal Tubule	Cortex	Active	Salt, Water
Descending Loop of Henle	Cortex to Medulla	Passive	Water
Ascending Loop of Henle	Medulla to Cortex	Passive (some active transport)	Salt
Distal Tubule	Cortex	Active	Salt, Water
Collecting Duct (1)*	Cortex to Medulla	Active	Salt
Collecting Duct (2)*	Medulla	Passive	Water

* NOTE: since the collecting duct behaves differently in different regions of the kidney, it is included twice.

To see how the kidney excretes a hyperosmotic urine we will use a series of dialysis bags as the parts of the nephron in which passive transport occurs; the beaker containing the dialysis bag represents the interstitial fluid of the kidney, and mathematical simulation for the parts of the nephron in which active transport occurs. We will change out the solution in the beaker (changing the solute concentration) to represent varying interstitial fluid salt concentrations. We will change out the dialysis bag solution at times, using sugar in place of salt since salt can pass through the membrane. Sugar, in contrast, cannot pass through the dialysis bag and will be used to imitate the descending loop of Henle and the collecting duct. (The dialysis bag will be permeable to water in the ascending loop part of the experiment but should not make a difference to the results). We can thus track the change in the volume in the dialysis bag and the change in solute concentration. We will assume a starting concentration of 1% of urea.

NOTE: the kidneys are more efficient than this experiment at reabsorbing water- the kidneys are able to reabsorb up to 99% of water in the filtrate!

Methods and Data Collection

Part A: Proximal Tubule

The proximal tubule uses active transport, so we will not use the dialysis bags for this part. Instead, we will assume that 5% of water volume and salt concentration has been removed from the filtrate (and returned to the bloodstream).

New volume of water is: 10mL * 0.95 = 9.5mL
New concentration of salt: 10 * 0.95 = 9.5%

We can figure out what the new concentration of urea is using the equation:

$M_1V_1 = M_2V_2$

M is the concentration (percent here instead of molarity), V is the volume. 1 indicates the starting values, 2 indicates the values after the dialysis bag was in the beaker.

$M_1V_1 = M_2V_2$
$1*10 = M_2*9.5$
$M_2 = 1.05\%$

Enter these values onto the figure in your answer sheet; you will do the same after each section, which will help you answer the questions at the end of this lab.

Part B: Descending Loop of Henle

Remember, the descending loop of Henle is permeable to water, but not salt or urea. Therefore, we will use sugar in place of salt in the dialysis bag and beaker solution to imitate the properties of this section of the nephron.

We will start with a dialysis bag filled with 9.5mL of 9.5% sugar (Bag 1); since sugar cannot pass through the dialysis bag, this will appropriately imitate the descending loop of Henle. The dialysis bag will be placed into a beaker with 50% sugar solution for fifteen minutes to imitate the higher solute concentration the filtrate encounters as it goes down the tube.

Here is a diagram of the experiment; explain which molecules you expect to move and in which direction. Answer the following before you move on. Will the volume in the dialysis bag increase or decrease? The concentration of salt (sugar)? The concentration of urea?

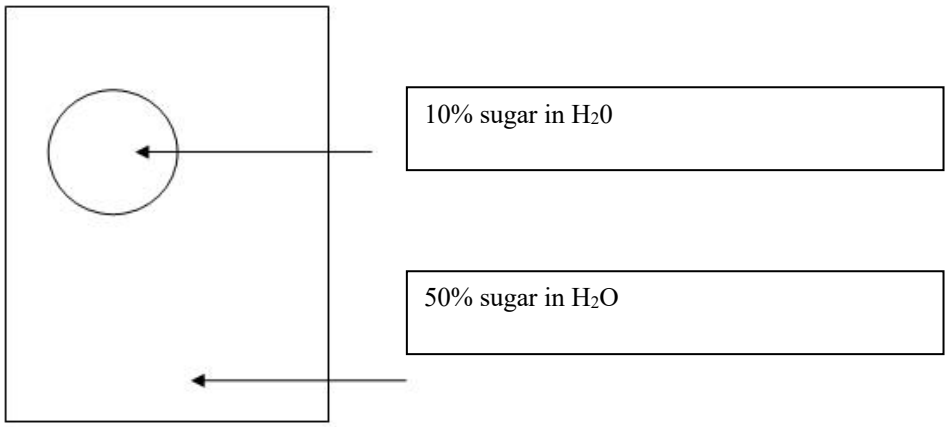

Figure 1. Simple Model for Part B.

1. Prepare the dialysis tubing. Cut a 15 cm section of dialysis bag and soak it in distilled water until flexible. Twist one end of the dialysis tubing and fold it over on itself, then seal with a yellow clip.
2. Pipette 9.5mL of 9.5% sugar solution (provided) into the dialysis bag. Seal the other end of the dialysis bag in the same way, be sure that there is no air in the dialysis bag and limited excess space (you can cut the bag shorter if needed).
3. Place the dialysis bag into the beaker with 50% sugar solution; leave for fifteen minutes.

After fifteen minutes, we need to determine the new volume (V_2 in the equation below) and concentration of our dialysis bag (both for our data and to make the new bag the same volume and concentration). Carefully open the dialysis bag and pour it into a graduated cylinder to measure; you may use the funnel to help, or the transfer pipettes provided.

New concentration of salt:
$M_1V_1 = M_2V_2$

$10*10 = M_2V_2$

For V_2, enter the new volume you measured and solve for M_2.

New concentration of urea (same basic formula):
$M_1V_1 = M_2V_2$
$10*1 = M_2V_2$

Enter the new volume and concentration of salt and urea onto the figure in your answer sheet.

Be sure to rinse any beakers and flasks that you use between parts.

Part C: Ascending Loop of Henle

Remember, the ascending loop of Henle is permeable to salt, but not water or urea. Therefore, we will use salt in the dialysis bag and beaker solution to imitate the properties of this section of the nephron.

1. Make your Bag 2 the same volume as Bag 1 was at the end of Part B (V_2 from above equation) in the provided 25mL flask; the solution needs to be the same concentration as Bag 1 was at the end of Part A (M_2 from above). To make this solution, measure out 10mL of water, add 0.1g of salt per % salt needed (so for our example above, multiply 14.4 x 0.1 = 1.44g salt).
2. Use the pipette to measure the precise volume of solution needed; you can pipette the volume into an empty 25mL flask and use a dropper to fill the dialysis bag.

Fill in the percent of solute on the diagram below (Fig. 2) and explain which molecules you expect to move and in which direction. Will the volume in the dialysis bag increase or decrease? The concentration of salt? The concentration of urea?

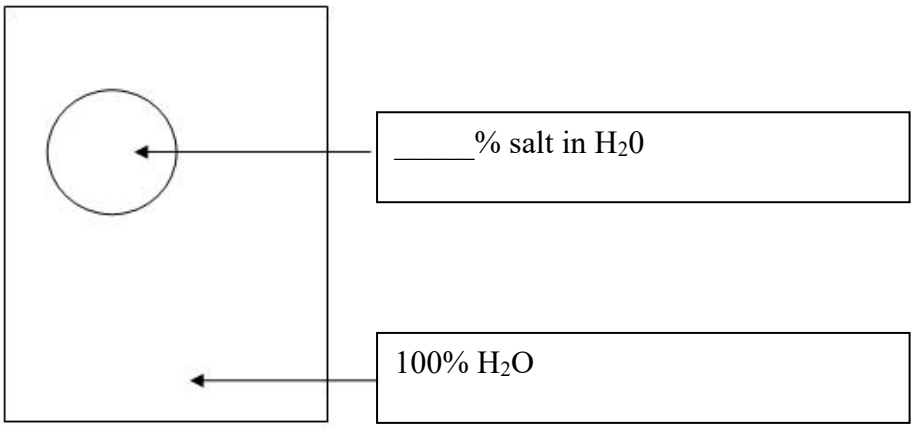

Figure 2. Simple Model for Part C.

3. Place the dialysis bag in a beaker of 100% water for 15 minutes (to imitate the reduced solute concentration of the cortex).
4. After fifteen minutes, use the conductivity probe to measure salinity as parts per million (ppm). The probe plugs in to the LabProQuest2. Be sure the switch on the connecting box is set to O-20,000uS.
5. Once turned on, go to the Sensor Menu, chose Sensor Setup. Press CH 1; select Conductivity, then Conductivity 20000MICS. Press OK, when it returns to the Sensor Setup screen, press OK again. You can change the units by pressing on the current number displayed, units is an option.
6. Pour the contents of Bag 2 from the graduated cylinder into an empty tube.
7. Pipette 9mL of water in another tube, then add 1mL of the solution from Bag 2 to this beaker and mix (use parafilm to cover tube).
8. Place the probe in the tube; take the ppm you record and divide by 1,000 to get the number as a percent (this accounts for the dilution you just did as well as converting).

While the volume may have changed slightly, we will ignore that for the purposes of the experiment and consider the volume to be unchanged. Record the "new" volume and salt concentration on the figure.

Use $M_1V_1 = M_2V_2$ to determine the concentration of urea. In this case, $V_1 = V_2$ and is the final volume in Bag 1. M_1 is the final concentration of urea in Bag 1. Solve for M_2.

$$M_1V_1 = M_2V_2$$

Part D: Distal Tubule

The distal tubule uses active transport, so we will not use the dialysis bags for this part. Instead, we will assume that 5% of water volume and salt concentration has been removed from the filtrate (and returned to the bloodstream).

New volume of water is: former concentration * 0.95 =
New concentration of salt: former concentration * 0.95 =
New concentration of urea:
$M_1V_1 = M_2V_2$

Part E: Collecting Duct Part 1

The first part of the collecting duct uses active transport, so we will not use the dialysis bags for this part. Only salt is removed, however. We will assume that 5% of salt concentration has been removed from the filtrate (and returned to the bloodstream). The volume and concentration of urea will not change.

New salt concentration: former concentration * 0.95 =

Part F: Collecting Duct Part 2

Remember, the second part of the collecting duct is permeable to water, but not salt or urea. Therefore, we will use sugar in place of salt in the dialysis bag and beaker solution to imitate the properties of this section of the nephron.

1. To make the solution for the dialysis bag, add 0.1g of sugar per % sugar needed; multiple the percent you need by 0.1 to determine this number. Weigh your sugar and add to 10mL of water.
2. Use the pipette to measure the precise volume of solution needed; you can pipette the volume into an empty 25mL flask and use a dropper to fill the dialysis bag.

Fill in the percent of solute on the diagram below (Fig. 3) and explain which molecules you expect to move and in which direction. Will the volume in the dialysis bag increase or decrease? The concentration of solute?

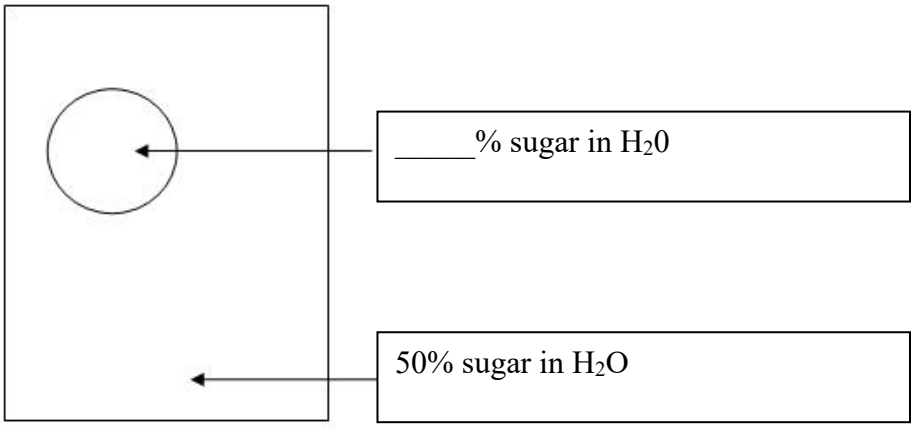

Figure 3. Simple Model for Part F.

3. Place the dialysis bag in a beaker of 50% sugar for 15 minutes.
4. Determine the final volume of the dialysis bag as you did previously after Bag 1.

New concentration of salt:

$M_1V_1 = M_2V_2$

New concentration of urea:
$M_1V_1 = M_2V_2$

Cited References

Urry LA, Cain ML, Wasserman SA, Minorsky PV, Jackson RB, Reece JB. 2016. Campbell Biology. 11th edition. New York, NY: Pearson.

Materials

Materials per group:

- 45cm of dialysis tubing - 15.9mm x 25mm (Ward's Science,#470163-402)
- 2 clips to seal dialysis tubing (Ward's Science, #470206-374); if clips are not available, dental floss may be used, although tubing is more likely to leak.
- Scissors
- Ruler
- Transfer pipettes
- 10mL pipettor and tips
- 1 Small beaker or flask
- Sugar (from grocery store is fine)
- Salt (Fisher Scientific, #7647-14-5)
- 2 weigh boats
- Balance measuring in grams to at least 2 decimal places
- 3 - 400mL beakers
- Small beaker or flask to mix solutions in
- Graduated cylinder (10-25mL) to measure final volume in dialysis bags
- Conductivity Probe (Vernier, CON-BTA)
- LabProQuest (Vernier, LABQ2) -older systems available from the vendor work as well
- 50% sugar water (500g sugar per liter): 600mL per group
- Distilled water

Notes for the Instructor

The substitution of sugar for salt initially can be confusing for students; it is recommended that this be explained by the instructor before starting the experiment. This substitution is necessary because dialysis tubing that will allow water, but not salt, to diffuse is not available.

It is recommended that students review diffusion and osmosis before this laboratory exercise, potentially by doing homework problems as a pre-lab exercise. This is especially helpful if students have not recently covered these topics.

If desired, a dye that cannot pass through the dialysis tubing can be used in place of theoretical urea, but this requires creating a concentration curve and complicates making calculations and making new solutions. Another solution would be to add the same number of drops of a food dye to example dialysis bags of different volumes so students can visualize how urea concentration increases with decreasing volume in the dialysis bag.

A potential follow up student-directed laboratory exercise could involve students researching factors that affect the re-absorption of water and salt by the nephron. For each, students should determine which parts of the nephron are targeted and how/in what direction, considering the influence of both vasopressin and RAAS/ANP. Students could then alter the experiment to reflect these changes.

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About the Authors

Lisa M. Danish has been an Instructor at the Nazareth College since 2016 and Laboratory Coordinator since 2018, where she teaches and does curriculum development for introductory biology laboratories.

Appendix: Student Answer Sheet

Answer the following questions:

1. What is the percent salt reabsorbed? (calculate: 10-final salt concentration/10% * 100).

2. What is the percent change of urea concentration? (calculate final urea concentration/1% * 100).

3. How many mL of water were reabsorbed into the “kidneys” in your experiment?

4. What percent of the original 10mL is this? (divide the previous answer by 10, multiply by 100)

5. The human body passes approximately 67L/hour of filtrate. How much of these 67L would have been reclaimed by your model kidney? (multiply your percent * (1/100) * 67)

6. What happened to the volume of water in Bag 1 and Bag 3? What caused the change?

7. What happened to the salt concentration in Bag 2? What caused the change?

8. When salt leaves the ascending loop of Henle (Bag 2), where does it go? How does this help maintain the different salt concentrations of the medulla and cortex?

9. Explain how changing volume of water and salt results in an increasing concentration of urea as the experiment proceeds.

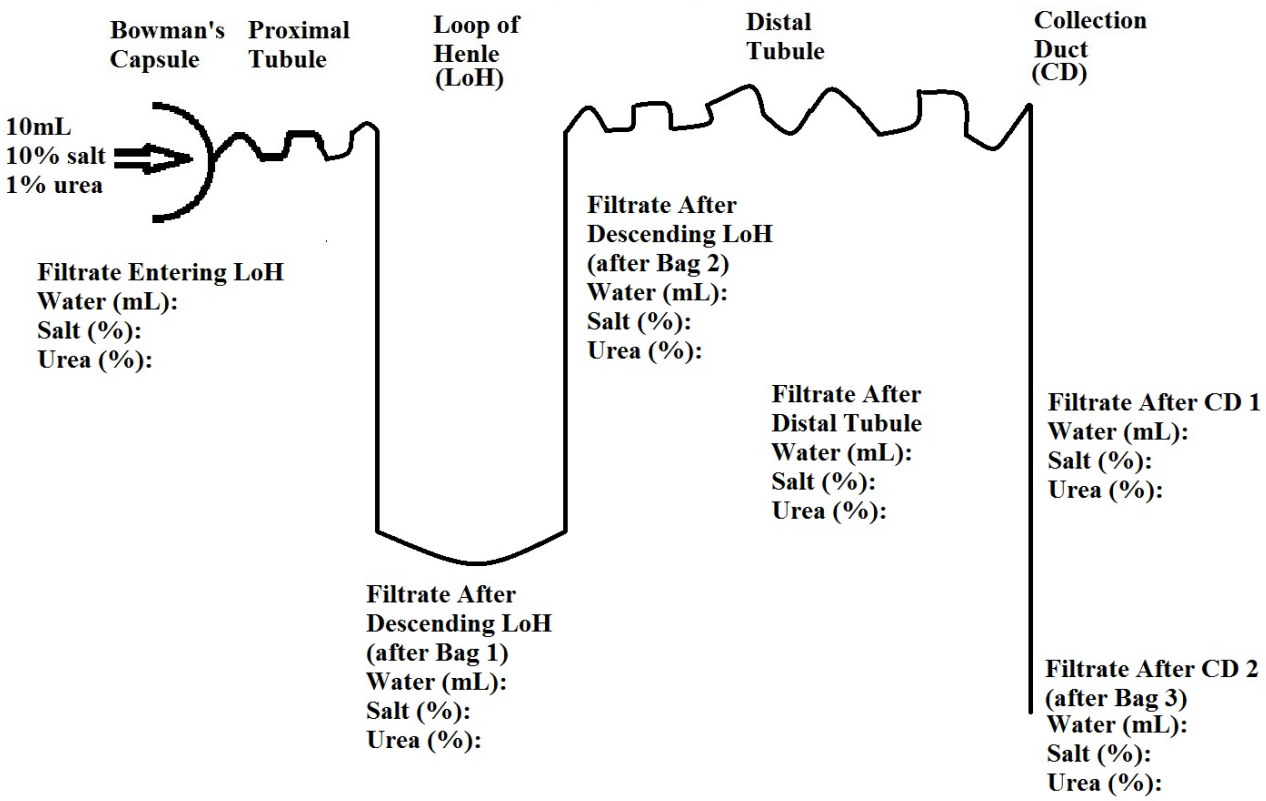
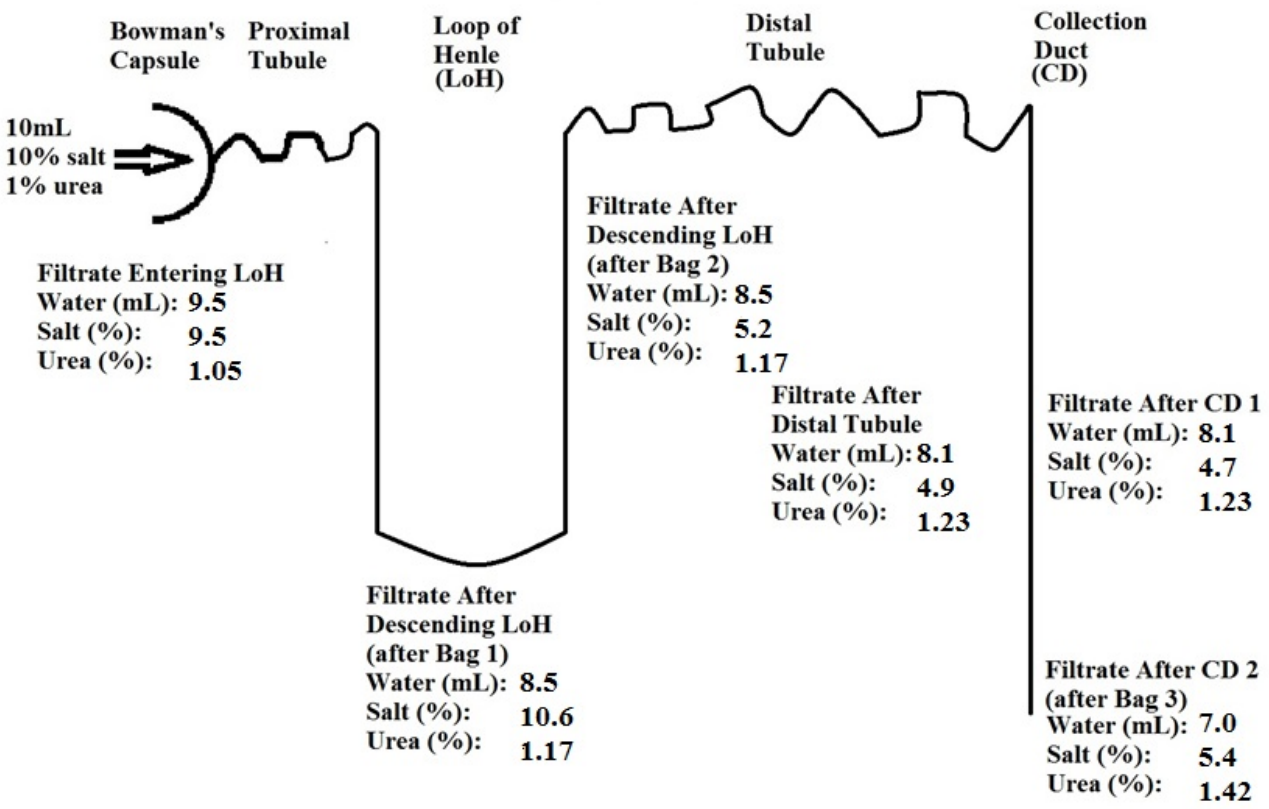


Figure 4. Diagram of the nephron to enter results.

Appendix: Sample Student Data



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