Kidneys



Introduction:

In this experiment, you will investigate how the kidneys handle fluid loads. These include water alone, and isosmotic salt and monosaccharide solutions, as well as a hyperosmotic monosaccharide solution.



Claude Bernard (1813 - 1867) - the first to express the concept of the constancy of the internal environment.



Carl Ludwig (1816 - 1895) - a pioneer of renal physiology.

Learning Objectives

By the end of today's laboratory you will be able to:

- · Describe how the kidneys handle a water load
- Distinguish between the handling of water and isosmotic salt loads
- Explain the pattern of fluid excretion following isosmotic and hyperosmotic monosaccharide loads
- Discuss the relationship between urine osmolarity and specific gravity and how osmolarity is affected by changes in urine flow rates





- 1. Applying a drop of urine to the prism of the refractometer.
- 4. Cleaning the prism (be careful not to scratch the prism).



Procedure:

This laboratory involves the collection of urine at various time intervals and measurement of its volume and specific gravity (an indication of osmolarity). There are four different protocols, each requiring a different volunteer.

Volunteer preparation

The establishment of the diuresis depends upon fairly rapid absorption of the water so it is important not to start with a full stomach. Just eat a light meal and drink normally in the 3 to 4 hours before the laboratory starts. In addition, avoid fluids containing caffeine (coffee, tea, cola drinks) for at least 3 hours prior to the laboratory.

Be sure to note the time at which you last urinated prior to coming to the laboratory.

Refractometer measurement

1. Preparation for measurement (handle the device with care and do not touch optical lens).

Set direction of the refractometer transparent cup against the light, look into the eyepiece and sharpen the image to improve readability. You will see a circular space with a measuring scale .

2. Refractometer calibration.

Open the transparent cup, put **1 to 2 drops** of the calibration solution (distilled water) on the optical prism, close the cup and press it slightly for the solution to spread perfectly over the surface of the optical prism (without air bubbles and dry spots). Look into the eyepiece, the top of the visor should be blue, the bottom white, and the boundary should go through a calibration value of **1.3300** (left scale of the RI) If not, turn the calibration screw until the desired condition is reached.

3. Measurement.

Open the transparent cup, clean the optical prism with the **included cloth**, then put **1 to 2 drops** of the test liquid (urine) on it, close the cup and slightly press it so that the liquid can spread perfectly over the surface of the optical prism (without air bubbles and dry spots) The measured value will be represented by the intersection of the blue-white boundary on the **right measuring scale** (specific gravity).

4. Cleaning after measurement

Clean the prism and the transparent cup with a damp cloth and carefully place the device in to the case. To wet the cloth, use **Desident CaviCide** spray.

General procedures during the experiments for all volunteers

- 1. At the commencement of the experiment, note the time, collect your urine and measure its volume. Keep a small sample for measurement of specific gravity.
- Immediately after the collection of the first sample, drink the required solution (except control). Once you have drunk this solution, do not drink anything else during the laboratory.
- 3. Continue to collect urine approximately every 20 minutes, noting the time at which the bladder is emptied to the nearest minute.
- 4. It will be found most convenient for each subject to be his or her own timekeeper; there is no necessity for the subjects to keep in step with each other. The essential thing is that the intervals between urination are accurately recorded.

Cautions

△ Do not volunteer to be a subject in this laboratory class if you are suffering from kidney or circulatory problems, have any other medical problem or are on any medications.

⚠ Urine is a potentially infectious body fluid. Therefore, students are directly responsible for all measurements of the volume and specific gravity of their own urine, and are required to clean up any split urine themselves.



Experimental protocols:

There are four different protocols.

Protocol 1: Control - no fluid intake during experiment.

- 1. Drink nothing during this laboratory and collect specimens of urine each 20 minutes or so.
- 2. Measure the volume and specific gravity of your urine and then dispose of the sample down the toilet.
- Enter the volume and specific gravity of the sample into your own table

Protocol 2: To illustrate a normal water diuresis.

- 1. Drink 800 mL of Solution 2, then collect specimens of urine each 20 minutes or so.
- 2. Measure the volume and specific gravity of your urine and then dispose of the sample down the toilet.
- 3. Enter the volume and specific gravity of the sample into your own table.

Protocol 3: To illustrate effects of drinking the equivalent of an isosmotic sodium chloride solution.

- 1. Drink 800 mL of Solution 3, then collect specimens of urine each 20 minutes or so.
- 2. Measure the volume and specific gravity of your urine and then dispose of the sample down the toilet.
- 3. Enter the volume and specific gravity of the sample into your own table.

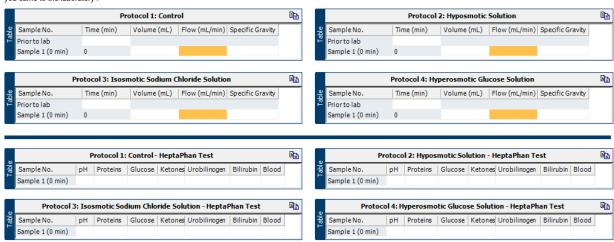
Protocol 4: To illustrate effects of drinking a hyperosmotic glucose solution.

- 1. Drink 800 mL of Solution 4, then collect specimens of urine each 20 minutes or so.
- 2. Measure the volume and specific gravity of your urine and then dispose of the sample down the toilet.
- 3. Enter the volume and specific gravity of the sample into your own table.



Sample 1: 0 min

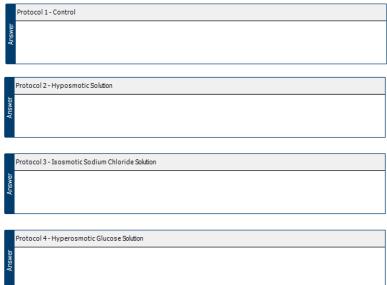
After collecting the first sample complete the fields in the table below, for each different protocol (Sample 1: 0 min). You must record the time in minutes that you last urinated before you came to the laboratory.



Study Question

While waiting to collect the next sample, answer the following question.

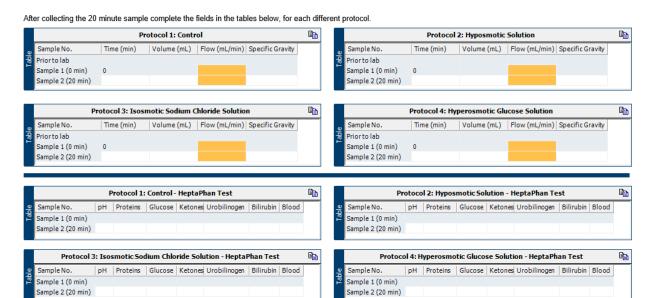
1. What changes in urine output do you expect to see in each of the protocols during this laboratory?

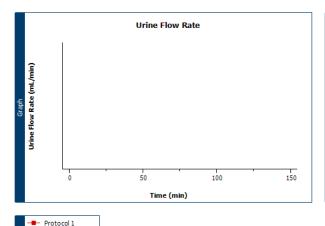


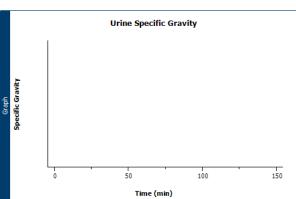


Sample 2: 20 min

Protocol 2
 Protocol 3
 Protocol 4







While waiting to collect the next sample, answer the following questions.

2. What is the osmolarity of the fluid in the interstitial space in the renal cortex? Is it the same throughout that space?



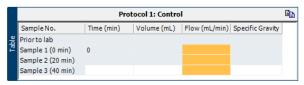
3. What is the osmolarity of the fluid in the interstitial space in the renal medulla? Is it the same throughout that space?

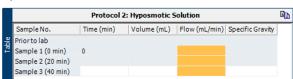


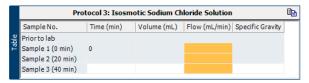


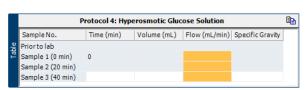
Sample 3: 40 min

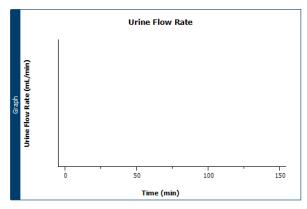
After collecting the 40 minute sample complete the fields in the table below, for each different protocol.

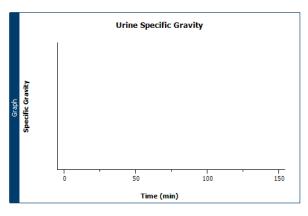














While waiting to collect the next sample, answer the following question.

4. What hormone is involved in regulating renal water excretion? What normally inhibits the release of this hormone?

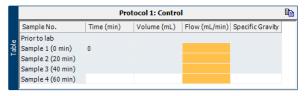


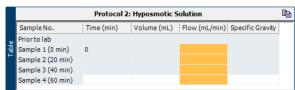


Sample 4: 60 min

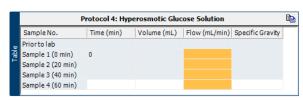
Protocol 2
 Protocol 3
 Protocol 4

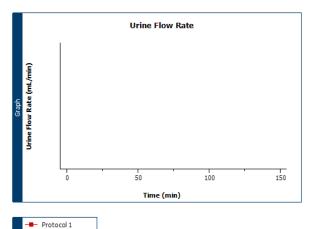
After collecting the 60 minute sample complete the fields in the table below, for each different protocol.

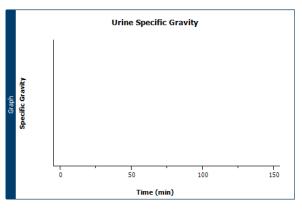




	Protocol 3: Isosmotic Sodium Chloride Solution								
	Sample No.	Time (min)	Volume (mL)	Flow (mL/min)	Specific Gravity				
a)	Prior to lab								
aple	Sample 1 (0 min)	0							
_	Sample 2 (20 min)								
	Sample 3 (40 min)								
	Sample 4 (60 min)								

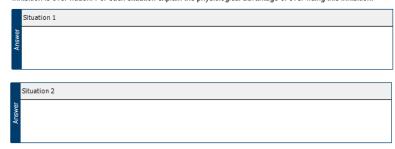






While waiting to collect the next sample, answer the following question.

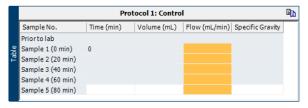
5. In question 4 you stated what normally inhibits the release of the hormone controlling water excretion. From your own experience, identify two situations where this inhibition is over-ridden. For each situation explain the physiological advantage of over-riding this inhibition.

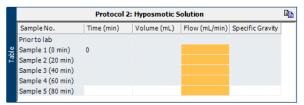


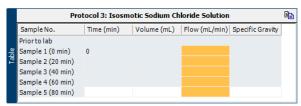


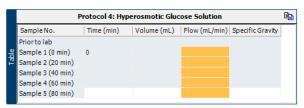
Sample 5: 80 min

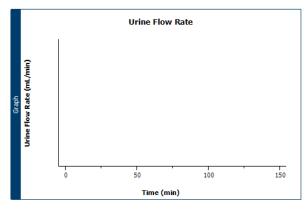
After collecting the 80 minute sample complete the fields in the table below, for each different protocol.

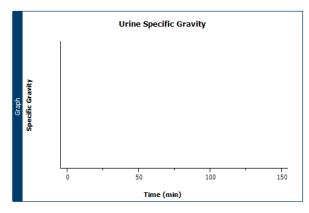








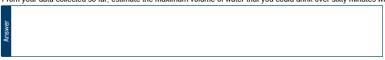






While waiting to collect the next sample, answer the following question.

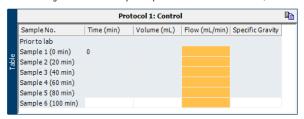
6. From your data collected so far, estimate the maximum volume of water that you could drink over sixty minutes while still remaining in water balance?

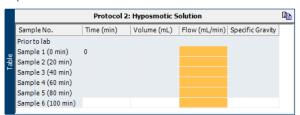




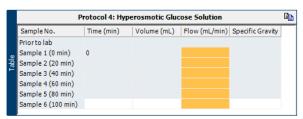
Sample 6: 100 min

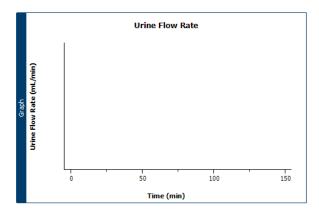
After collecting the 100 minute sample complete the fields in the table below, for each different protocol.

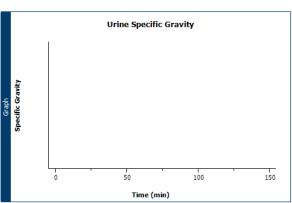




	Protocol 3: Isosmotic Sodium Chloride Solution							
	Sample No.	Time (min)	Volume (mL)	Flow (mL/min)	Specific Gravity			
	Prior to lab							
a)	Sample 1 (0 min)	0						
rable	Sample 2 (20 min)							
-	Sample 3 (40 min)							
	Sample 4 (60 min)							
	Sample 5 (80 min)							
	Sample 6 (100 min)							









While waiting to collect the next sample, answer the following question.

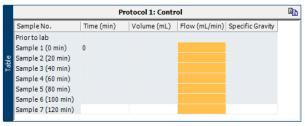
7. What would happen if your water intake over sixty minutes significantly exceeded the maximal volume you could excrete in this period? Why could this be life threatening?

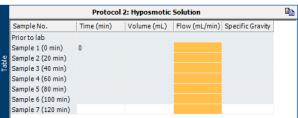


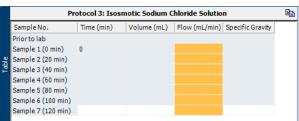


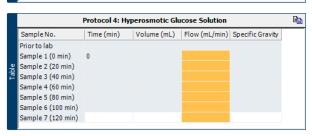
Sample 7: 120 min

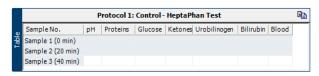
After collecting the 120 minute sample complete the fields in the table below, for each different protocol.



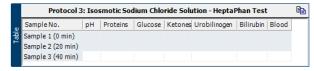




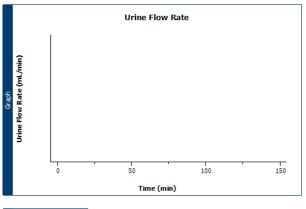


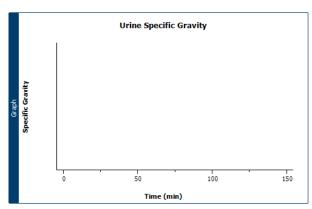


a)	Protocol 2: Hyposmotic Solution - HeptaPhan Test								
	Sample No.	pН	Proteins	Glucose	Ketones	Urobilinogen	Bilirubin	Blood	
Table	Sample 1 (0 min)								
-	Sample 2 (20 min)								
- 1	Sample 3 (40 min)								
- 1									



	Protoco	ol 4: H	yperosmo	tic Gluco	se Soluti	on - HeptaPh	an Test		
	Sample No.	pН	Proteins	Glucose	Ketones	Urobilinogen	Bilirubin	Blood	
a ap	Sample 1 (0 min)								
٦	Sample 2 (20 min)								
	Sample 3 (40 min)								





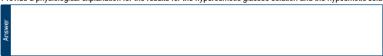


Before continuing to the analysis pages, answer the following questions.

8. Provide a physiological explanation for the results for the isosmotic sodium chloride solution and the hyposmotic solution (Protocol 3 - green vs Protocol 2 - blue)?



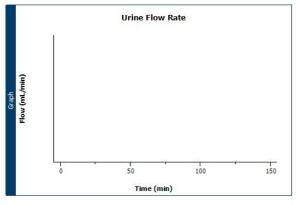
9. Provide a physiological explanation for the results for the hyperosmotic glucose solution and the hyposmotic solution (Protocol 4 - pink vs Protocol 2 - blue)?

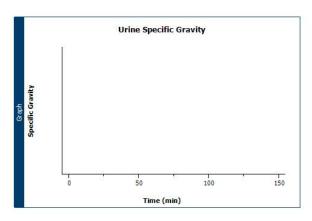




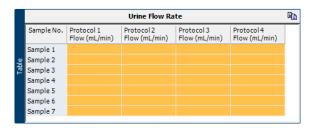
Analysis 1: Flow and Osmolarity

Composite final results for flow and osmolarity, for all four protocols, are shown below.









		ı	Urine Specific Gr	avity		
	Sample No.	Protocol 1 Specific Gravity	Protocol 2 Specific Gravity	Protocol 3 Specific Gravity	Protocol 4 Specific Gravity	
	Sample 1					
a	Sample 2					
Table	Sample 3					
	Sample 4					
	Sample 5					
	Sample 6					
	Sample 7					

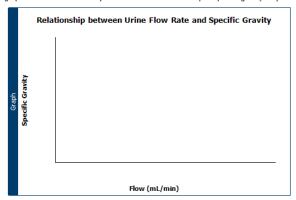
10	Reflect on your predictions for the trends	of each protocol in the spaces	below. Note that your original	l answers are shown but should not be changed

Answer	Protocol 1- Control (Prediction)
	Protocol 1 - Control (Result)
Answer	
Answer	Protocol 2 - Hyposmotic Solution (Prediction)
Answer	Protocol 2 - Hyposmotic Solution (Result)
₹	
Answer	Protocol 3 - Isosmotic Sodium Chloride Solution (Prediction)
Answer	Protocol 3 - Isosmotic Sodium Chloride Solution (Result)
	Protocol 4 - Hyperosmotic Glucose Solution (Prediction)
Answer	
	Protocol 4 - Hyperosmotic Glucose Solution (Result)
Answer	



Urine Flow and Specific Gravity Relationship

The graph below shows a scatterplot of urine flow in relationship to specific gravity for your results.



Study Question

11. Explain the relationship, shown in the graph above, between the urine flow rate and specific gravity.



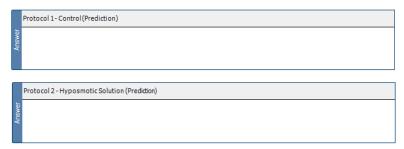


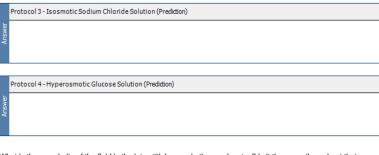


Study Questions

Check your answers to the questions from each of the Sample pages, as shown below.

1. What changes in urine output do you expect to see in each of the protocols?





2. What is the osmolarity of the fluid in the interstitial space in the renal cortex? Is it the same throughout that space?



3. What is the osmolarity of the fluid in the interstitial space in the renal medulla? Is it the same throughout that space?



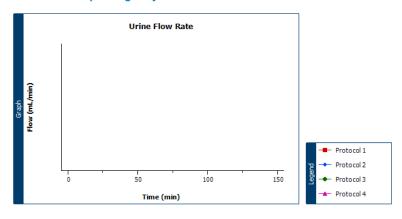
4. What hormone is involved in regulating renal water excretion? What normally inhibits the release of this hormone?

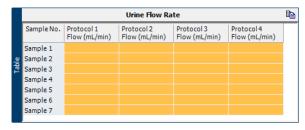


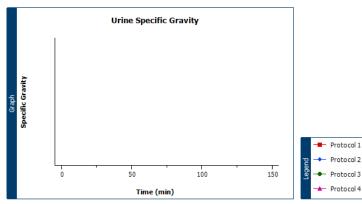
In question 4 you stated what normally inhibits the release of the hormone controlling water excretion. From your
own experience, identify two situations where this inhibition is over-ridden. For each situation explain the
physiological advantage of over-riding this inhibition.



Results: Flow and Specific gravity







		ı	Urine Specific Gr	avity		B
	Sample No.	Protocol 1 Specific Gravity	Protocol 2 Specific Gravity	Protocol 3 Specific Gravity	Protocol 4 Specific Gravity	
	Sample 1					
<u>•</u>	Sample 2					
Table	Sample 3					
	Sample 4					
	Sample 5					
	Sample 6					
	Sample 7					

6. From your data, estimate the maximum volume of water that you could drink over sixty minutes while still remaining in water balance?



7. What would happen if your water intake over sixty minutes significantly exceeded the maximal volume you could excrete in this period? Why could this be life threatening?

Answer	

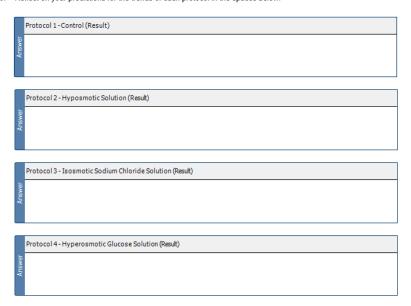
8. Provide a physiological explanation for the results for the isosmotic sodium chloride solution and the hyposmotic solution (Protocol 3 - green vs Protocol 2 - blue)?

Answer	

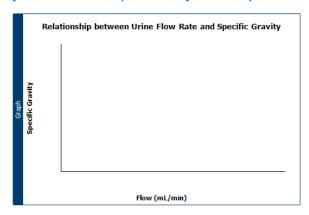
9. Provide a physiological explanation for the results for the hyperosmotic glucose solution and the hyposmotic solution (Protocol 4 - pink vs Protocol 2 - blue)?

Answer

10. Reflect on your predictions for the trends of each protocol in the spaces below.



Analysis: Urine Flow and Specific Gravity Relationship



11. Explain the relationship, shown in the graph above, between the urine flow rate and specific gravity.



Results: HeptaPhan Test



	Protocol 2: Hyposmotic Solution - HeptaPhan Test								
	Sample No.	pН	Proteins	Glucose	Ketones	Urobilinogen	Bilirubin	Blood	
able	Sample 1 (0 min)								
-	Sample 2 (20 min)								
	Sample 3 (40 min)								

Protocol:	Phan Test	an Test						
Sample No.	pН	Proteins	Glucose	Ketones	Urobilinogen	Bilirubin	Blood	
Sample 1 (0 min)								
Sample 2 (20 min)								
Sample 3 (40 min)								

	Protocol 4: Hyperosmotic Glucose Solution - HeptaPhan Test								
Table	Sample No.	pН	Proteins	Glucose	Ketones	Urobilinogen	Bilirubin	Blood	
	Sample 1 (0 min)								
	Sample 2 (20 min)								
	Sample 3 (40 min)								
	l								