Virtual experiment    Experiment 13

Regulation of urine formation

[Objectives]
1. To investigate the effects of increased plasma volume, hyperglycemia, norepinephrine, furosemide, pituitrin and vagus nerve activation on volume of urine, and to understand the underlying mechanisms.
2. To examine the secretion function of renal tubules by the phenol red time test.

[Principles]
The formation of urine includes three renal processes: (1) glomerular filtration, (2) tubular reabsorption of filtrate and (3) tubular secretion. Any factors that affect these three processes of urine formation will influence the urine volume and components in the urine (Figure 1).

Because of the high permeability of the glomerular membrane, all the components in the plasma except plasma protein are filtered into Bowman’s capsule. The volume of filtrate is determined not only by hydrostatic pressure and colloid osmotic forces across the glomerular membrane, but also by the permeability and surface area of filtration membrane. Norepinephrine (NE), which is a neurotransmitter released from sympathetic nerve endings, constricts renal arteries to decrease glomerular hydrostatic pressure, and decrease the volume of urine. Activation of the vagus nerve depresses arterial blood pressure (including renal arterial pressure) to decrease glomerular hydrostatic pressure, and decrease the volume of urine.

When filtrate flows through the renal tubule, some substances such as glucose are almost completely reabsorbed, and others such as sodium ions are partly reabsorbed. With solute reabsorption, more than 99% of filtered water is absorbed too. But in patients with diabetes mellitus, when there is more than 180 mg/dl glucose in blood as well as in filtrate, all the glucose in the filtrate cannot be reabsorbed due to saturation of the glucose transport capacity of the proximal tubule (concentration of blood glucose exceeds the threshold level for reabsorption by the kidneys). So some glucose stays in the urine, increasing its osmolarity, and causing increased urine volume.

Furosemide, an inhibitor of the Na⁺-K⁺-2Cl⁻ co-transporter located in the thick segment of the ascending limb, can greatly decrease the reabsorption of these ions, to cause large quantities of water to be excreted (polyuria) (Figure 1).

Atrial natriuretic peptide (ANP) is synthesized and secreted by special atrial muscle cells. It acts on the tubules to inhibit sodium and water reabsorption, causing
the volume of urine to increase (Figure 1). After injection of 20 ml saline solution into a rabbit vein, the expansion of plasma volume extends the walls of the atria to stimulate ANP release from atrial cells, so more sodium and water is excreted.

Pituitrin is composed of antidiuretic hormone (ADH) and oxytocin, which are both stored in the posterior pituitary. ADH activates V$_2$ receptors, which are distributed in the epithelia of the distal tubule and collecting ducts, to increase the permeability of tubules to water (Figure 1). So increased ADH in the circulation induces more water reabsorption and excretion of less concentrated urine.

Renal tubule epithelia can transport some substances (e.g. phenol red) from circulation to filtrate. Phenol red cannot be filtered in the glomerulus, but can be uptaken by tubule cells and be secreted into the filtrate (Figure 1). If the function of the transport system in the tubule is lost or weakened, phenol red does not appear in the urine or its appearance is delayed. So the phenol red time (time from phenol red injection to its excretion in urine) is usually applied in the clinic for evaluating the secretion function of tubules.

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**Figure 1.** The processes of urine formation
[Experimental animal]
Rabbit

[Experimental equipment]
Balance, syringe, scalpel, scissors, forceps, hemostatic forceps, operating table, electrode, stimulator, bladder catheter, drop counter, 20% ethyl carbamate, 0.01% norepinephrine, 10 mg/ml furosemide, 1 U/ml pituitrin, saline solution, 20% glucose solution, 0.6% phenol red, 10% NaOH.

[Virtual Experiment Procedure]
On the desktop of WinXP, select the “rabbit” icon and double-click it to run the program.
In this program, select Experiment 17 in the catalog list (Figure 2).

![CATALOGUE]

- EXP 9 Effects of several drugs and extracellular ions on isolated toad heart
- EXP 10 Aortic nerve firing
- EXP 11 Human electrocardiogram
- EXP 12 Nervous and humoral regulation of arterial blood pressure in rabbit
- EXP 13 Carotid baroreceptor reflex
- EXP 14 Electrical activity of diaphragm muscle and respiration
- EXP 15 Regulation of respiration in rabbit
- EXP 16 Effects of several drugs and extracellular pH on isolated small intestinal muscle
- EXP 17 Factors affecting urine formation

**Figure 2. Catalog list of virtual experiments**

Select “Methods” in Figure 3 to learn the procedure of surgical operation and bladder intubation step by step. Firstly, the rabbit is anesthetized and fixed on the operating table. Then, cervical surgery is started and the common carotid artery is intubated by
catheter for measuring the SP/DP. Finally, ventral surgery is started and the bladder is intubated for measuring the volume of urine.

**Methods of experiment**

Select the simulation experiment to start work (Figure 4). You will find an anesthetized rabbit lying on its back on the operating table. Move the mouse pointer to the scalpel icon. Click the left mouse button and drag the scalpel on the neck of the rabbit to activate a video window for carotid intubation. After finishing that, ventral surgery is done and the bladder is intubated. The urine flows through the bladder catheter and is measured by a drop counter. The data on urine volume and SP/DP are shown on the right panel of the recorder (circled in Figure 4).
Experimental items

1. Saline solution: Inject 20 ml saline solution into the ear vein quickly by moving the syringe on the ear. Record the maximum value of urine volume as well as SP/DP from the right panel of the recorder.

2. Glucose solution: Inject 5 ml 20% glucose into the ear vein by moving the syringe on the ear. Record the maximum value of urine volume as well as SP/DP from the right panel of the recorder.

3. NE: Inject 0.3 ml NE into the ear vein by moving the syringe on the ear. Record the minimum value of urine volume as well as SP/DP.

4. Furosemide: Inject 15 mg furosemide into the ear vein by moving the syringe on the ear. Record the maximum value of urine volume as well as SP/DP.

5. Pituitrin: Inject 2 unit pituitrin into the ear vein by moving the syringe on the ear. Record the minimum value of urine volume as well as SP/DP.

6. Stimulation of vagus nerve: To start the experiment, move the scalpel using the mouse pointer on the vagus nerve to cut it. Turn on the stimulator and move the electrode onto the efferent end of vagus nerve to start stimulation. Record the minimum value of urine volume as well as SP/DP.

7. Phenol red: Inject 0.5 ml phenol red into the ear vein by moving the syringe on the ear. Record the time from phenol red application to excretion in urine.
[Results]
1. Record the volume of urine and SP/DP, and complete Table 1.
2. Record the time from phenol red injection into the vein to phenol red excretion in the urine.

Table 1. Effects of 20 ml saline, 20% glucose, NE, furosemide, pituitrin and vagus nerve stimulation on volume of urine and SP/DP

<table>
<thead>
<tr>
<th></th>
<th>Volume of Urine (drops per min)</th>
<th>SP/DP(mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline solution</td>
<td></td>
<td></td>
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<tr>
<td>20% Glucose</td>
<td></td>
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<tr>
<td>NE</td>
<td></td>
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<tr>
<td>Furosemide</td>
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<tr>
<td>Pituitrin</td>
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<tr>
<td>Stimulate the efferent end of vagus nerve</td>
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</table>

Time from phenol red injection to excretion in urine was:

[Conclusion and Discussion]
1. How did the volume of urine change when 20 ml saline solution was injected into the rabbit vein? Please describe the possible mechanisms.
2. What happened to urine volume when 20% glucose solution was injected into the vein? Please describe the possible mechanisms.
3. What is the effect and mechanism of furosemide on urine volume?
4. What is the effect and mechanism of pituitrin on urine volume?
5. What is the effect and mechanism of norepinephrine on urine volume?