21. URINE CONCENTRATION

The final concentration of the urine is very dependent on the amount of liquid ingested, the losses through respiration, faeces and skin, including sweating. When the intake far exceeds the losses, then, in order to maintain homeostasis the rest of the liquid is eliminated through urine. If the fluid intake is low and the losses are high, then the kidney has to concentrate as much as possible the urine in order to maintain homeostasis. As a result the concentration can range from as diluted as 65 to as concentrated as 1200 mOsm/kg. Producing diluted urine is not as problematic as to concentrating it. To achieve the higher concentrations the kidney depends on the juxtaglomerular nephrons that reach deep into the medulla and in the architectural relationship with the vasa recta. As mentioned before the concentration of the interstitial fluid increases in the medulla towards the tip of the renal pyramid. The higher concentrations of the interstitial fluid in the tip of the renal pyramid are achieved because the nephron has the capability of recirculate urea (Fig. 21-1). Urea in the filtrate is not completely reabsorbed and most of it goes into urine. A percentage of the urea in the filtrate diffuses out of the collecting duct into the interstitial fluid. Once in the interstitial fluid urea provides the increase in osmalality that makes the tip of the renal pyramid so concentrated.

The urea circulates between the collecting duct where it diffuses into the interstitial fluid and the thin descending segment of the loop of Henle which is also permeable to urea. At this point it diffuses into the tubule to reach again the collecting duct (Fig. 21-1).

All the concentration capacity of the nephron can be attributed to the fact that the loop of Henle is in close association or apposition with the extension of the peritubular capillaries which deep in the medulla are called the vasa recta (Fig. 19-10).

The association between these two structures is one that creates a counter current mechanism which permit the removal of all reabsorbed solutes and the water that follows by osmosis out of the medulla into the venous return of the kidney. Otherwise the interstitial fluid would be rapidly diluted or engorged with water and solutes.

Regulation of final urine volume and urine concentration.

The events taking place in the proximal convoluted tubule and the loop of Henle are standard and they account for the reabsorption of approximately 80 % of the filtrate volume (Fig 21-1).
21-2). These events are endocrine independent in terms of regulating final volume and concentrations. There are endocrine influences at the level of the renal corpuscle that regulate the GFR but not the concentration of the urine.

The later section of the distal convoluted tubule and the collecting duct operates under direct endocrine influence to regulate the amount of fluid and electrolytes to be discarded in urine (Fig. 21-2). This is not a locally made decision but it represent the needs of the entire organism as many baro and osmo receptors convey their information to the hypothalamus who in turn process it and organize the release of Anti Diuretic Hormone. The other hormonal system which contributes to the regulation of the concentration of the urine is the renin-angiotensin-aldosterone system (Fig. 21-3). Although both system work in conjunction to maintain homeostasis ADH release is more in response to changes in osmolality in blood while the renin-angiotensin-aldosterone system is more sensible to changes in blood pressure.

**Anti Diuretic Hormone**

Also known as vasopressin is a small protein hormone synthesized by neurones located in the supraoptical nuclei with axons projecting to the pars nervosa where the hormone is released (Fig. 21-4). Within the hypothalamus there are osmoreceptors that continuously monitor the osmolar concentration of the interstitial fluid. If these receptors detect a slight increase in osmolar concentration they send a signal to the secretory neurones located in the supraoptical nuclei which then depolarize. The depolarization triggers the release of stored ADH from the axon terminal into a capillary plexus situated in the pars distalis. These capillaries then connect with general circulation allowing ADH to reach the kidney.

In the kidney ADH bind membrane receptors located in the tubular cells of the distal convoluted tubules and the collecting ducts (Fig. 21-5A). The ADH receptors are linked to a g protein mechanism which, upon binding activates adenylate cyclase Fig 21-5B). Adenylate cyclase converts ATP to cAMP. The elevation of cAMP, in these particular cells triggers the insertion of specialized water channels named aquaporin-2 into the apical membrane and aquaporins-3 and 4 into the basal membrane (Fig. 21-5C).

Once the aquaporins are inserted, water can move by osmosis from the lumen of the convoluted tubule and collecting duct into their respective cells and then move through to the interstitial fluid where it is picked up by the vasa recta and removed into circulation (Fig. 21-5D).

There are also many baroreceptors located in carotid sinuses, in the aortic arch, in the atria of the heart and in most large veins, which are constantly monitoring blood pressure. If they detect a drop in blood pressure of about 5 % they convey this signal to the hypothalamus to trigger secretion of ADH.

If the osmoreceptors detect a decreased concentration of interstitial fluid and blood, or if the baroreceptors detect an increase in blood pressure, they inhibit the production of ADH. Low concentration of ADH translates in an increase in water
excretion through the urine because this does not get reabsorbed in the distal convoluted tubule or the collecting ducts (Fig. 21-6).

**Aldosterone**

Aldosterone is a mineralocorticoid secreted by the adrenal gland cortex. It has a large effect in sodium retention, and as a result, in water by the kidney (Fig 21-7).

The mechanism to produce aldosterone starts with the detection of low pressure in the afferent arteriole or, if the concentration of sodium in the filtrate decreases, as the filtrate passes through the macula densa of the juxtaglomerular apparatus. The consequence of these events is the release of larger amounts of renin by the cells of the juxtaglomerular apparatus. Renin is an enzyme capable of converting a pro-hormone called angiotensinogen which is produced by the liver, into angiotensin I. This conversion takes place in circulation.
Figure 21-7.
(A) Aldosterone arriving through circulation. (B) Aldosterone binds a nuclear receptor in the cell. (C) In response to aldosterone binding, the cell produce several transport proteins. (D) ATPase are located in the basal membrane and help to actively reduce the concentration of intracellular Na+. (E) Antiports are placed in the apical membrane to increase rate of exchange of H⁺ and K⁺ for Na⁺ from the filtrate. (F) Increase intracellular Na⁺ pulls water and Cl⁻ into the cell by osmosis. (G) Na⁺ continues being actively pumped out of the cell by the ATPase. (H) Water follows Na⁺ into the interstitial fluid and the capillaries of the vasa recta where they are removed from the kidney.
Then, another enzyme found attached to capillary beds of the lungs principally called angiotensin-converting enzyme (ACE) acts on angiotensin I and convert it to angiotensin II. On its own, angiotensin II is a potent vasoconstrictor, capable of increasing peripheral resistance, and blood pressure. Unfortunately this is a very short acting compound because angiotensin II is metabolized rapidly. Angiotensin II also stimulates thirst, salt craving, release of ADH, but the most important role is to stimulate secretion of aldosterone by the adrenal gland.

Aldosterone reaches the cells of the distal convoluted tubule and the collecting ducts and, as all steroids, enter in the cells where it binds nuclear receptors. Activation of the receptor hormone complex in these cells results in the synthesis of transport proteins. The proteins produced include those that facilitate the removal of Na⁺ from the cells to the interstitial fluid (the ATPases), as well as those that form antiports in the apical membrane to exchange Na⁺ for K⁺ or H⁺ (Fig. 21-7).

The final effect is a larger retention of Na⁺ in the interstitial fluid and in circulation, and with this, a higher osmotic gradient to facilitate the transport of more water out of the filtrate into circulation.

**BODY FLUIDS**

The percentage of the body that is made by solids and fluids is relatively stable at any given time. As the animal grows the amount of water tend to decrease slightly. A small percentage of the water in the young animal is replaced by adipose tissue (Fig. 21-8).

If we want to classify the compartments holding the majority of the water in an animal, the obvious separation is between intracellular and extracellular pool of fluids (of which water is the main component) (Fig. 21-9). Intracellular fluid makes up approximately 40% of the total body weight. Extracellular fluid can be found in several compartments, but, its common characteristic is that, as the name indicates, is found outside the cell.

The most voluminous compartments are the plasma fluid which, is the volume contained by the structures of the circulatory system, and the interstitial fluid which is the fluid found between cells and blood vessels. Smaller volumes are made by synovial fluid, ocular fluid, cerebrospinal fluid, and lymph. Each of these compartments contains slightly different composition of but they are all in constant exchange of water and small ions. Proteins, which characterize the composition of each fluid, are normally not exchanged with adjacent pools because the membranes separating them prevent such exchange. Despite composition differences among pools the osmotic pressure of most pools are very similar.

**Water content**

To maintain homeostasis the organism has to maintain a relatively stable body fluid content. Any deviation from the balance trigger changes in osmolality or blood pressure which, once detected, activates different mechanisms aimed at correcting it.
To maintain such balance the animal has to acquire as much water as it eliminates (Fig. 21-10). The majority of the water is acquired through the diet as fluid or food (90%) the other 10% is acquired as a result of cell metabolism within the organism. The modes through which an animal can lose water are more varied and, to a great extent, they depend on environmental conditions.

Most of the fluids are lost in urine (60%). The second largest drain is through evaporation (35%) which includes both mechanisms of sweating (insensible which are those loses that evaporate directly into the air without condensing in the hair or surface and the sensible form which is what we recognize as sweating). The other evaporative losses are through the respiratory system and the tongue. Depending on the species this form of evaporating can be crucial to maintain homoeothermia. This mechanism is very important for dogs that do not sweat, thus depend of respiratory evaporation for cooling. The last route of water losses is through faeces. This mechanism can be very important in ruminant species as large volumes of water are discarded in the excreta.

**Thirst**

Incorporation of ingested water is done by osmosis through the GIT. The amount of water ingested is partially regulated through thirst. Thirst is triggered as a result of an elevation in the osmotic concentration of the interstitial fluid (which is detected mainly by cells of the supraoptical nucleus, and or a reduction in plasma volume which is detected by baroreceptors located in multiple organs).

To prevent over drinking when an animal is dehydrated the mechanism of thirst is titrated to induce the consumption of the proper amount in small increments. The first controlling mechanism is mediated by water in contact with the oral mucosa which conveys this information to the hypothalamus which in turn reduces temporarily the sensation of thirst (Fig. 21-11). This allows some time for the water to be absorbed in the GIT and decrease the osmolality of the interstitial fluid. The second mechanism is directly mechanical as the volume of ingested water distend the stomach which in turn informs the hypothalamus and this temporarily reduce the sensation of thirst. As the water is absorbed the sensation of thirst is re-established if further water ingestion is required.
Regulation of fluid volume

Intracellular fluid

The makeup of the intracellular fluid is maintained different from the interstitial fluid by the plasma membrane. This barrier is not very permeable to proteins and closely regulates the permeability of ions and smaller molecules (Fig. 21-13). The large proteins synthesized in a cell are normally not secreted (except in specialized cells such as enzyme or hormone secretory cells) and ions are actively transported across the membrane depending on the needs of the cell. Their transport is also determined by the electric charge across the membrane. Water moves in and out of cells by osmosis, thus under dehydration the osmotic pressure in the interstitial fluid increases, drawing water out of the cells. After the interstitial water volume has been replenished the osmotic concentration is higher inside the cell, thus water moves into the cells.

Extracellular fluid

There are several mechanisms that contribute to the regulation of water volume in the extracellular fluid. There are baroreceptors located in several of the large arteries such as the carotid sinus and the aortic arch. In the juxtaglomerular apparatus there are receptors monitoring pressure in the afferent arterioles, furthermore in the wall of the atria and in large veins there are also very sensible baroreceptors. All of these receptors use slightly different mechanism to eventually compensate for any variation of the expected normal pressure. There are at least three well known hormonal mechanisms to regulate the volume of extracellular fluid. A fourth mechanism involves direct neural activation. Some of the mechanisms, as it is the case of ADH, operate indirectly through the regulation of blood osmolality (Fig. 21-12).

If the osmolality of blood increases towards the upper range of the normal range the osmoreceptors in the hypothalamus detect the change and stimulate release of ADH by the cells in the supraoptical nuclei which discharge in the pars distalis. This also translates in an increase in the sensation of thirst. The result of these two events is an increase in water intake through drinking and the increase in permeability of the cells in the distal convoluted tubule and the collecting duct so less water is lost in urine. This water is retained in circulation decreasing the osmolality. If the osmoreceptors in the hypothalamus detect a reduction in the osmolality in blood the production of ADH is reduced and the sensation of thirst diminishes. As a result less water is drunk and the permeability of the cells of the distal convoluted tubule and collecting duct decreases. In this fashion more water is eliminated in the urine and the osmolality of blood increases.

### Regulation of Blood Osmolality

<table>
<thead>
<tr>
<th>HIGH OSMOLALITY</th>
<th>LOW OSMOLALITY</th>
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<tbody>
<tr>
<td>Detected by hypothalamic osmoreceptors</td>
<td>Detected by hypothalamic osmoreceptors</td>
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<tr>
<td>Increase in thirst sensation</td>
<td>Increase in thirst sensation</td>
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<tr>
<td>Increase ADH secretion</td>
<td>Increase ADH secretion</td>
</tr>
<tr>
<td>Increase fluid intake</td>
<td>Decrease fluid intake</td>
</tr>
<tr>
<td>Increase permeability of renal cells</td>
<td>Decrease permeability of renal cells</td>
</tr>
<tr>
<td>More water move into blood</td>
<td>More water move into blood</td>
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<tr>
<td>Decrease blood osmolality</td>
<td>Increase osmolality</td>
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Figure 21-13. Mechanism used to control blood osmolality within normal range
Regulation of extracellular fluid by modifying blood volume

Renin-angiotensin-aldosterone system.

As indicated in the section dealing with endocrine regulation of kidney function and depicted in figures 34 and 35, this system regulates extracellular volume by modifying the absorption of Na\(^+\) in the distal convoluted tubule and collecting ducts of the kidney.

Antidiuretic hormone mechanism.

This system works in the same manner as explained for regulation of blood osmolality. The only difference is that ADH is also secreted in response to relatively large changes in blood pressure (between 5 and 10%) (Fig. 21-13).

Atrial Natriuretic Hormone (ANH).

An elevation in atrial pressure, normally produced by an increase in blood volume, triggers the release of ANH. This hormone works in agonistic competition with Aldosterone as it decreased the reabsorption of Na\(^+\) in the distal convoluted tubule and collecting ducts. As a result, the osmolality of the filtrate is higher and less water is reabsorbed into circulation. The net result is that larger amount of Na\(^+\) and water is secreted in urine. A decrease in blood volume does not appear to trigger the opposite effect but, a decrease in atrial pressure appears to inhibit the release of ANH (Fig. 21-14).

Urodilatin

Urodilatin is a non-glycosilated peptide of the family of ANH. This particular compound is produced by cells of the distal convoluted tubule in response to an elevation in mean arterial pressure and it is secreted into the filtrate. It does not travel in circulation and it works exclusively in a paracrine fashion. Through the filtrate urodilatin reaches cells of the collecting duct where it activates a system that reduces the rate of sodium absorption. The net result is higher osmolality in the filtrate and, as a consequence less reabsorption of water in the collecting ducts, thus, larger volumes of urine secreted and, consequently a reduction in mean arterial pressure.

Nervous system

An increase in blood pressure, which is detected by baroreceptors in several vessels, reduces the rate of sympathetic stimulation to the afferent arterioles. As a result, these dilate allowing an increase in glomerular capillary pressure. The consequence is an increase in glomerular filtration rate, with the consequent elevation in the volume of the filtrate. If a decrease in blood pressure is detected, then there is an elevation in sympathetic stimulation to the afferent arterioles, thus reducing filtration pressure, GFR and volume of filtrate.