

Strategies to survive in environments of varying ionic and osmotic characteristics

- **Ionoconformers** (all marine):
Have little control on the ionic content of their extracellular fluid
- **Ionoregulators**:
Control the ionic content of their extracellular fluid
- **Osmoconformers** (mostly marine invertebrates):
Let their internal osmolarity vary with changing environmental osmolarity
- **Osmoregulators**:
Maintain a nearly constant internal osmolarity as environmental osmolarity changes

Role of osmoregulatory systems:

Maintain **VOLUME** and **COMPOSITION** of internal fluids (cytoplasm, extracellular fluid)

Notions of **HOMEOSTASIS**
and maintenance of the
“**MILIEU INTERIEUR**”



Claude Bernard
(1813-1878)

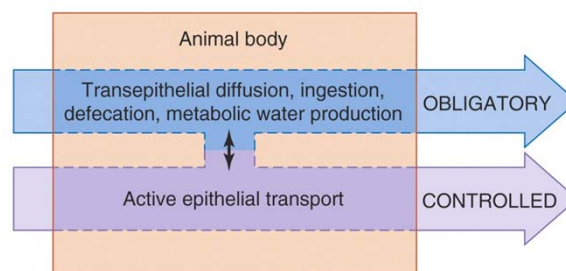
Obligatory and regulated exchanges

Obligatory exchanges:
are caused by physical factors over which the animal has little or no control

Regulated exchanges:
are physiologically controlled exchanges geared to maintain homeostasis

Factors affecting obligatory exchanges:

- Transepithelial gradients
- Surface to volume ratio
- Permeability
- Feeding
- Metabolism
(and its modulators
like temperature and
exercise)



Osmoregulatory strategies

Osmoregulatory organs are responsible for controlling (regulated) ion and water exchanges.

They vary tremendously between species (conformers vs regulators) and also depend a lot on environmental factors

Regulation problems are extremely different in FRESHWATER, SEAWATER, or on LAND

Euryhaline animals: tolerate large changes in salinity

Stenohaline animals: are adapted to live at a particular salinity

Euryhaline animals use different strategies to survive in their changing environment:

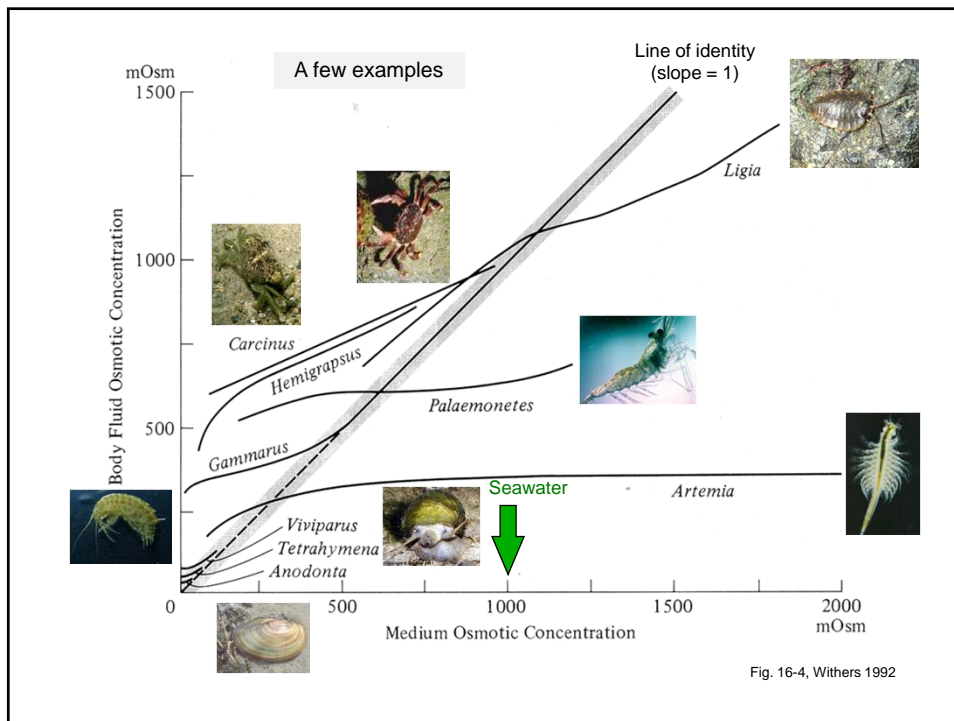
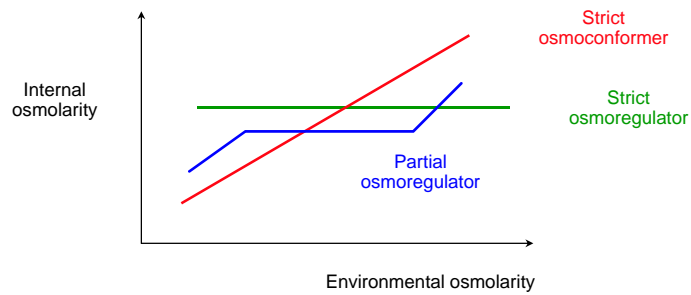
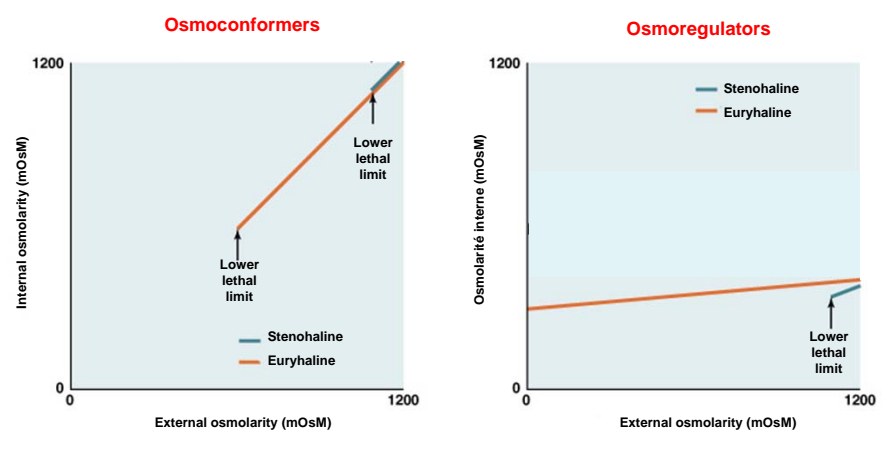


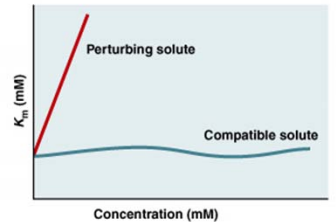
Fig. 16-4, Withers 1992

Osmoconformers and osmoregulators



Based on Fig. 13.3

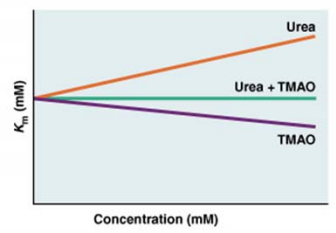
Various types of solutes have different effects on macromolecules



(a) Perturbing and compatible solutes

- Perturbing solutes**
- Disturb macromolecule function
 - Ex: Na^+ , K^+ , Cl^- , SO_4^{2-} , charged amino acids

- Compatible solutes**
- Very little effect on macromolecules
 - Ex: glycerol, glucose, uncharged amino acids

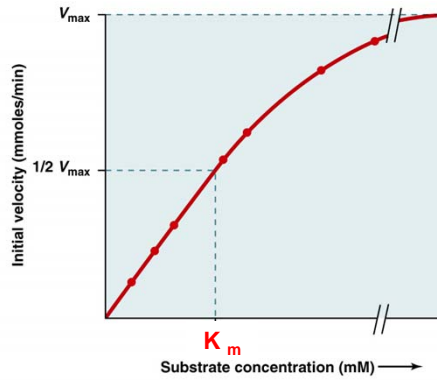


(b) Counteracting solutes

- Counteracting solutes**
- Disturb macromolecules on their own, but counterbalance perturbing effects of other solutes when combined
 - Ex: Urea weakens hydrophobic interactions whereas methylamines strengthen them; when combined: no effect

Fig. 13.4

Don't remember those first year chemistry classes ?.... K_m of a reaction (check Fig. 3.12)

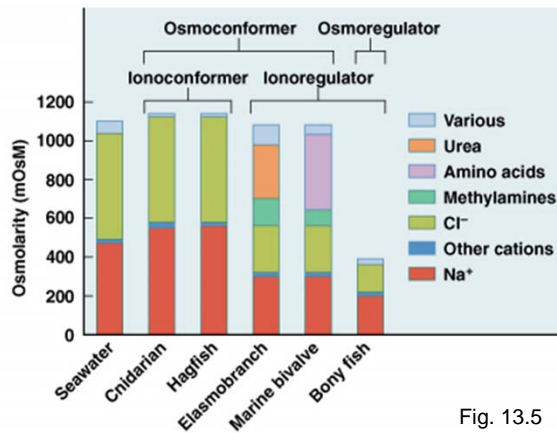


V = initial velocity of the reaction
 S = substrate
 V_{max} = maximal velocity
 K_m = index of enzyme affinity for the substrate

Michaelis-Menten curve



Main solutes present in different aquatic organisms



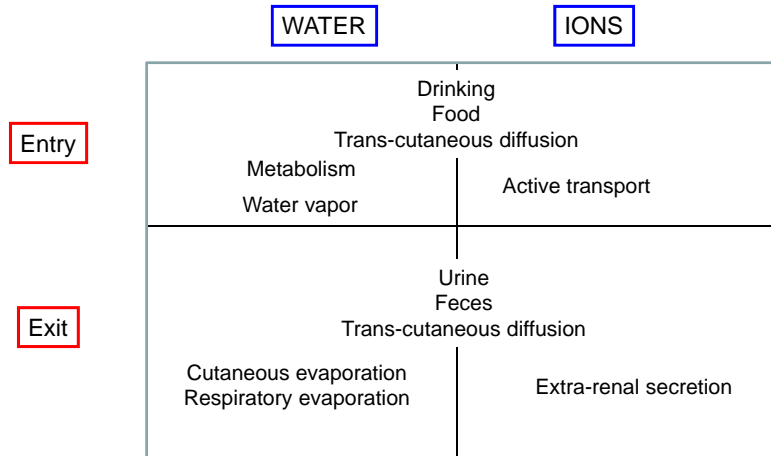
Hagfish is a very unusual vertebrate: **osmoconformer** and **ionoconformer**



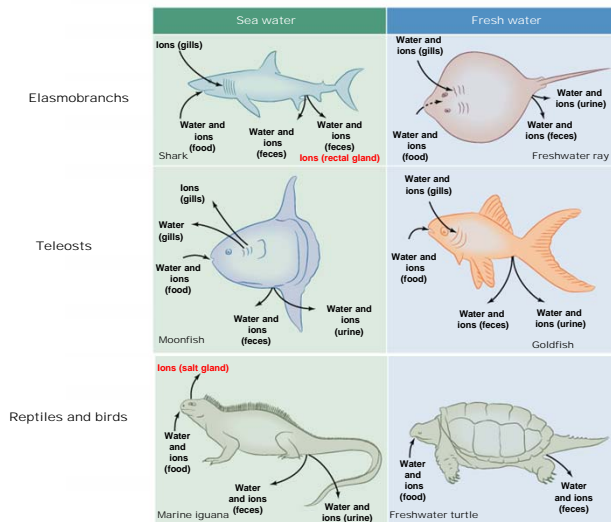
Fig. 13.5

To maintain balance: **Water entry = Water exit** and **Ion entry = Ion exit**

What are the possible avenues for water and ion exchanges ?



Summary of exchanges through epithelial tissues



- Epithelium forms a barrier between the animal and its environment

- This tissue is involved in many functions:

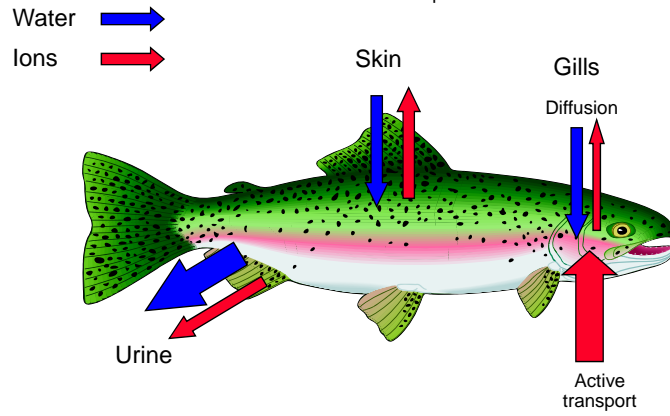
osmoregulation,
 ionoregulation,
 respiration
 digestion

Freshwater fish

2 problems: oedema and loss of ions
(tissues: 200-300 mOsm, water: < 5 mOsm)

Solutions:

- Skin very impermeable to water and ions
- Active transport (gills and digestive system)
- Diluted urine
- Renal reabsorption of ions

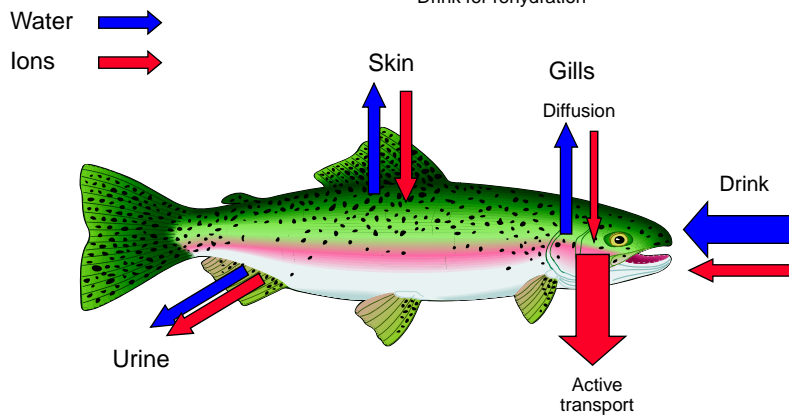


Sea water

2 problems: dehydration and gain of ions
(tissues: ~ 300 mOsm, water: ~ 1000 mOsm)

Solutions:

- Skin not very permeable to water and ions
- Active transport (gills)
- Drink for rehydration



Ion exchange in fish

Across complete body surface, the intestine, but mainly through the **gills**

Gills are involved in many functions:

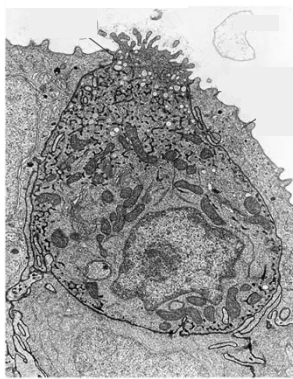
- Ion transport (osmoregulation, acid-base balance)
- Excretion of nitrogenous wastes
- Respiration



Because total gill surface is limited:

There is competition for the use of that surface, mainly between ion exchange (osmoregulation) and gas exchange (respiration)

Ionophores (= "mitochondria-rich cells" or previously "chloride cells")



Their **number, structure, position** et **metabolism** change as a function of salinity

- Many mitochondria
- High concentration of Cl^-
- Presence of many key enzymes:

Na^+/K^+ ATPase
(main transmembrane ion pump in animals)

Glutamate dehydrogenase (amine excretion)

Carbonic anhydrase (acid-base balance)

Gills of gobies acclimated to different salinities

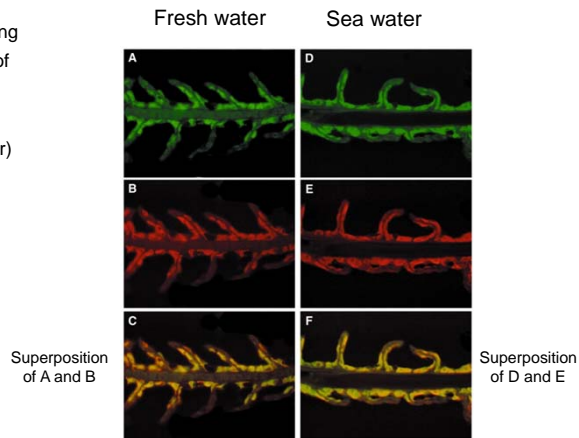


Immunoprecipitation experiment:
Specific transmembrane ion transporting proteins were labeled with antibodies of different colors:

green (Na⁺/K⁺ ATPase)
red (Na⁺/K⁺/2 Cl⁻ co-transporter)

Results show that the 2 proteins are clearly localized in the same cells

This co-localization is demonstrated by the color yellow seen in panels C and F (exact superposition of green and red = yellow)



McCormick, S. D. et al. J Exp Biol 2003;206:4575-4583

Extra-renal osmoregulation: Salt glands

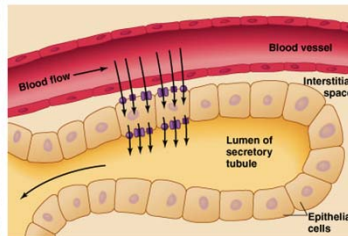


Fig. 13.39

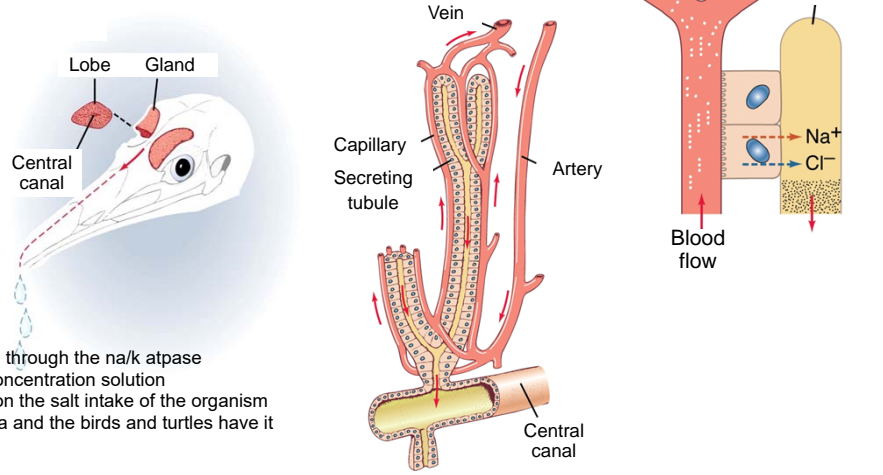
- Salt glands are found in marine reptiles and birds (usually in the head)
- Some salt water crocodiles have such glands in their tongue
- Sharks have a rectal gland

The kidneys of these marine animals are not able to produce urine that is concentrated enough to get rid of all the excess salt

The salt glands produce a highly concentrated solution of Na⁺ and Cl⁻

Ion pumps + transporters are used in conjunction with a counter-current exchange system

Salt glands in marine birds

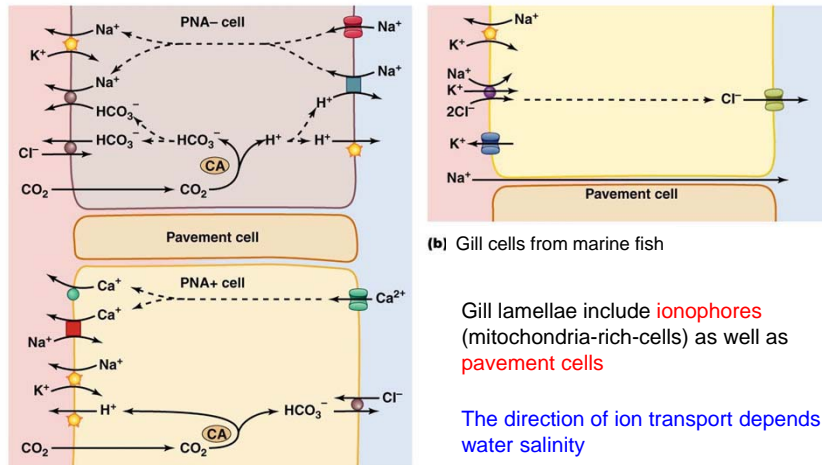


maintains salt balance, and basically take the salt from the blood through the na/k atpase where it is collected and excreted as a concentration solution --essentially the thickness is dependent on the salt intake of the organism the sharks have it around the rectum area and the birds and turtles have it around their eyes area.

From Eckert, Animal Physiology, 2002

Ionoregulation through fish gills

Fig. 13.38



(a) Gill cells from freshwater trout

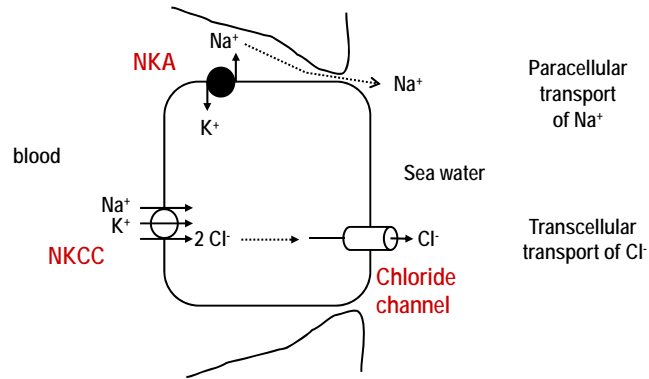
PNA = peanut lectin agglutinin

(b) Gill cells from marine fish

Gill lamellae include **ionophores** (mitochondria-rich-cells) as well as **pavement cells**

The direction of ion transport depends on water salinity

NaCl secretion through the gills of marine fish



This same mechanism is used in the salt glands of reptiles and birds (but not in the shark rectal gland)

Mechanism for NaCl secretion is different in the rectal gland of sharks

- This accessory gland is found in elasmobranchs
- Active transport to reduce [Na⁺] and [Cl⁻] in the blood
- Protein kinase A (PKA) is activated by a hormone (Vasoactive intestinal peptide: VIP). It causes the migration of chloride channels to the cell membrane and opens them

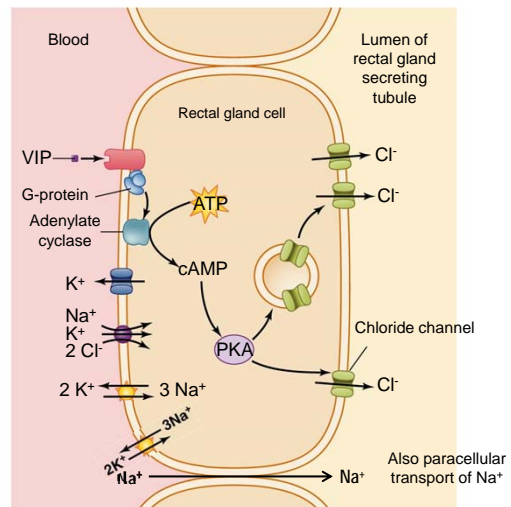


Fig. 13.40

Integument

- Animals out of water can modulate water flow through their body surface by changing the permeability of the integument
- A family of proteins, *aquaporines*, can alter membrane permeability by up to 100 times
- Most terrestrial animals need to reduce water outflow to avoid dehydration. They often do that by covering their external surface with hydrophobic molecules

- Mucus
- Waxes



- Cuticle covered with *chitin*
- Epidermis covered with *keratin*

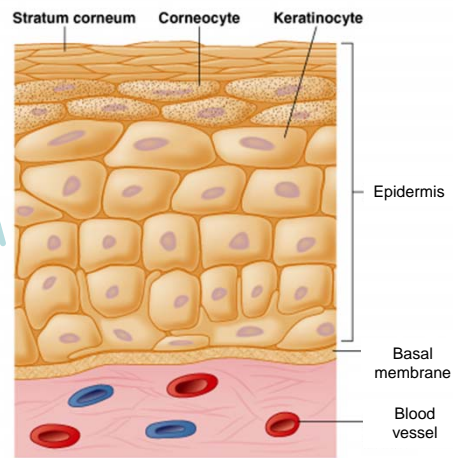


Figure 13.8

Animals adapted for life in the desert

- Urine concentration

Non-adapted: 1-2 Osm / L H₂O
Adapted: 5-9 Osm / L H₂O

This is achieved via very long
LOOPS of HENLE in their nephrons

- Dehydration of feces

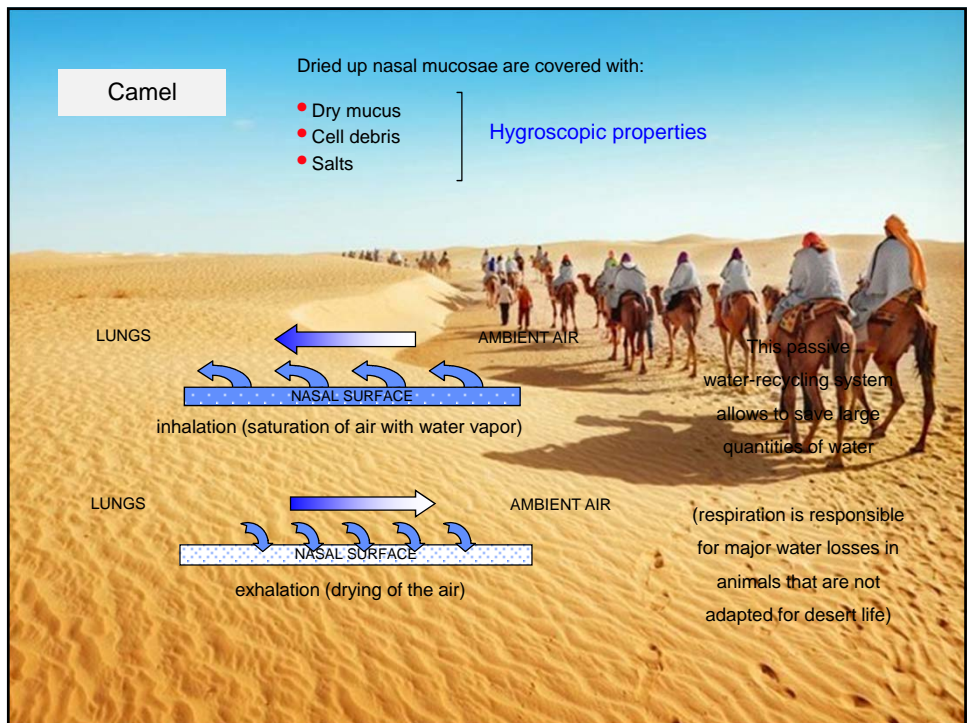
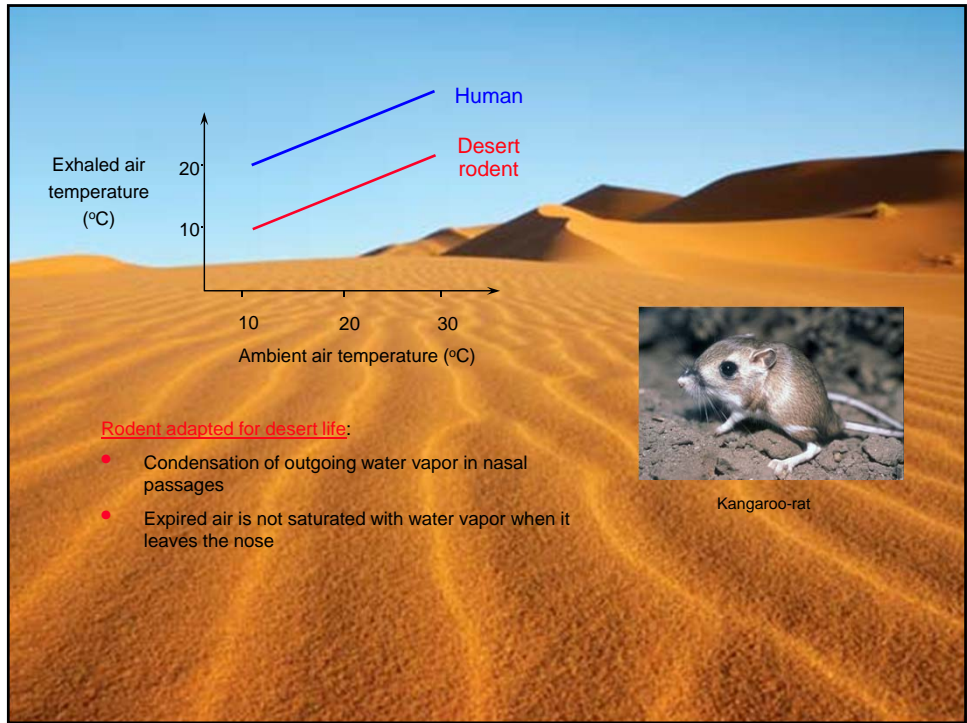
Active reabsorption of NaCl that drives passive reabsorption of water in the intestine

- Strong reduction of evaporative losses through respiration

Mechanisms for cooling and drying exhaled air

Drinking = 0
for days to weeks





Excretion of nitrogenous wastes

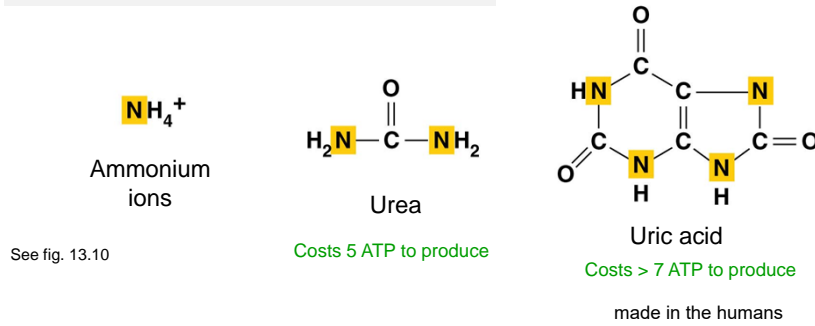
Protein catabolism produces NH_4^+ (toxic !)

- waste product increases pH
- blocks some ion channels and ion pumps (NH_4^+ is very similar to K^+ !!...)

NH_4^+ must therefore be excreted, either diluted or somewhat detoxified

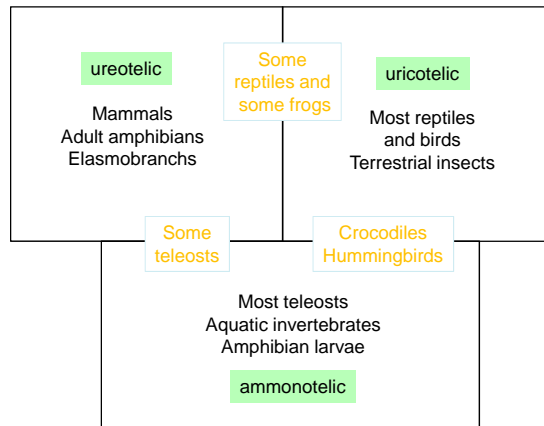
In terrestrial animals: there must be a compromise between **excretion** and **water balance**

Chemical structure of nitrogenous waste products



Animals excreting most of their **nitrogen wastes** as:

- | | | | |
|-------------------|--------------------|-----------------|--------------------------------------|
| • NH_4^+ | ammonotelic | very toxic | 500 ml $\text{H}_2\text{O g}^{-1}$ N |
| • Urea | ureotelic | less toxic | 50 ml $\text{H}_2\text{O g}^{-1}$ N |
| • Uric acid | uricotelic | much less toxic | 1 ml $\text{H}_2\text{O g}^{-1}$ N |



The choice of strategy depends on the environment (particularly **water availability**)

NH₄⁺ production and transport via glutamine

To lower toxicity NH₄⁺ is converted to urea or uric acid (in the liver)
 Glutamine is used as a "NH₄⁺ transporter" to bring it to the liver

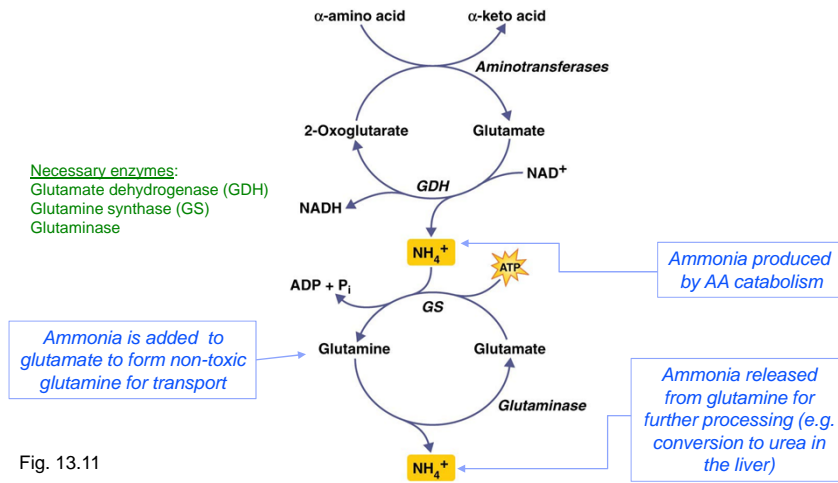
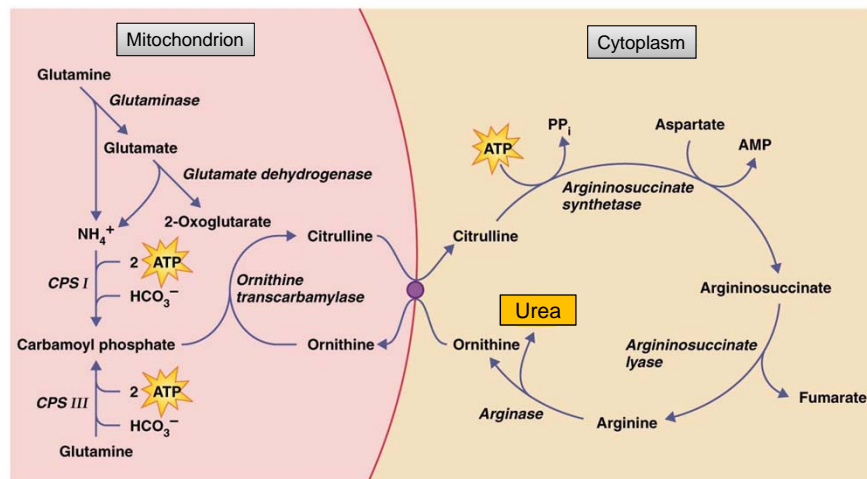


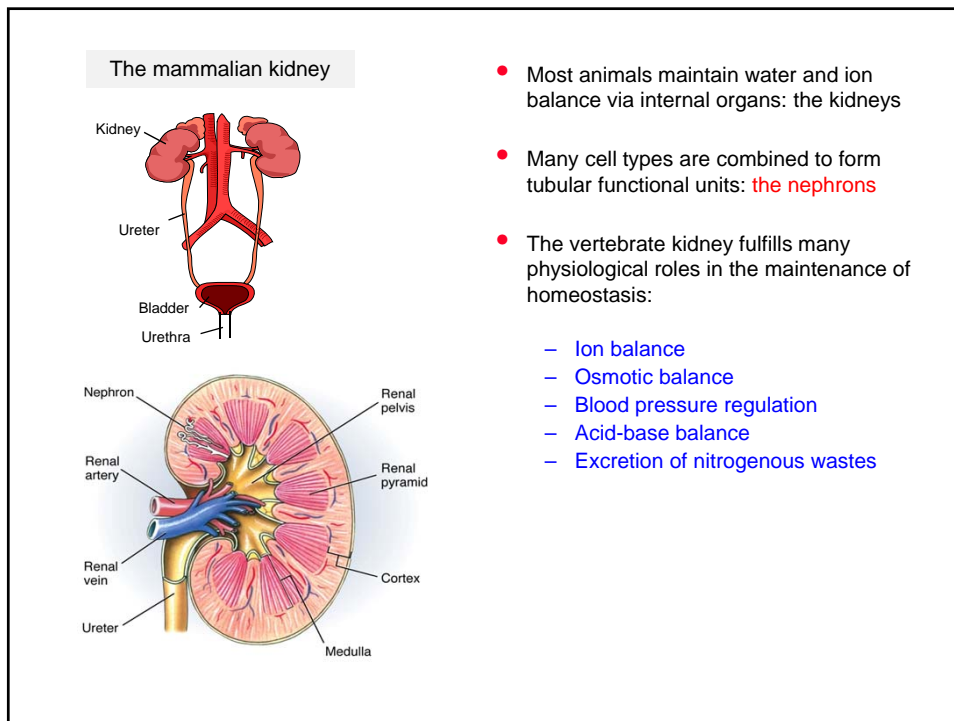
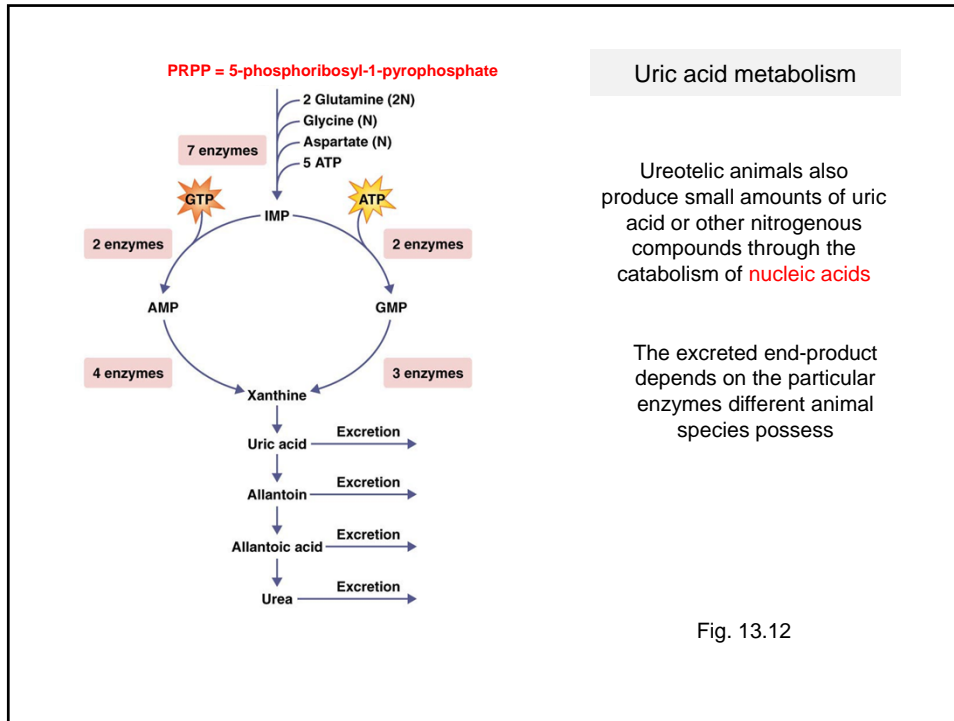
Fig. 13.11

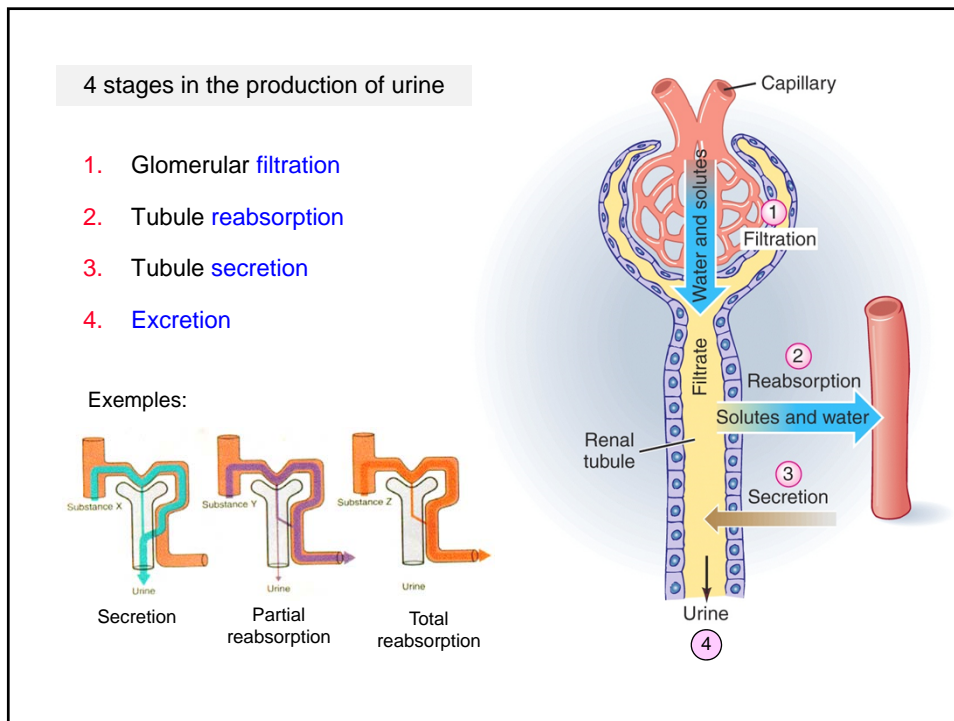
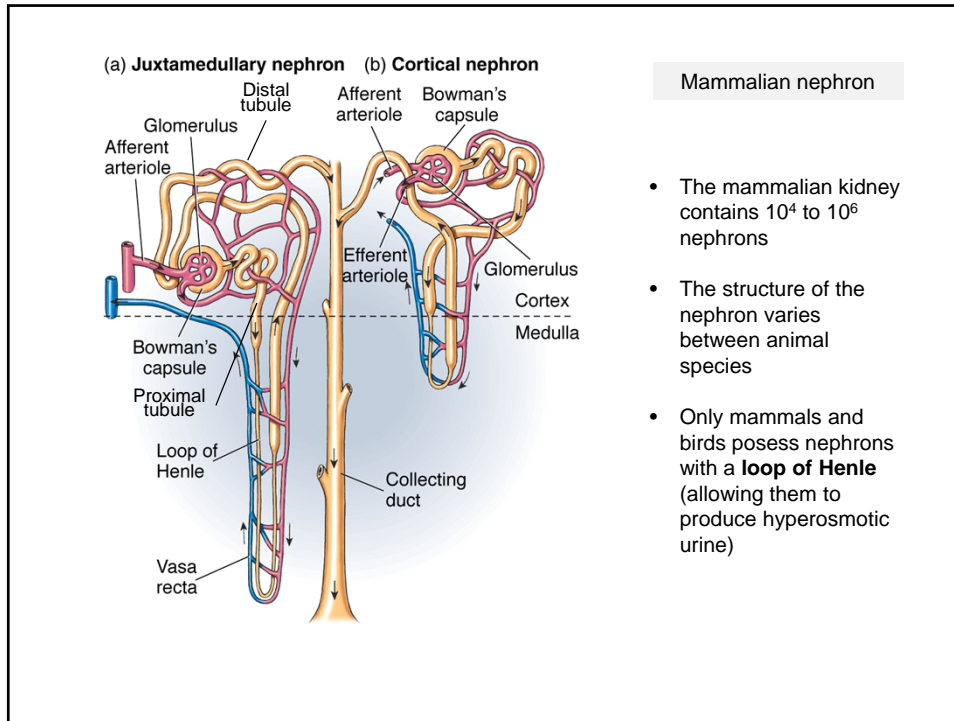
Urea is produced through the **ornithine cycle** (also called the **urea cycle**)



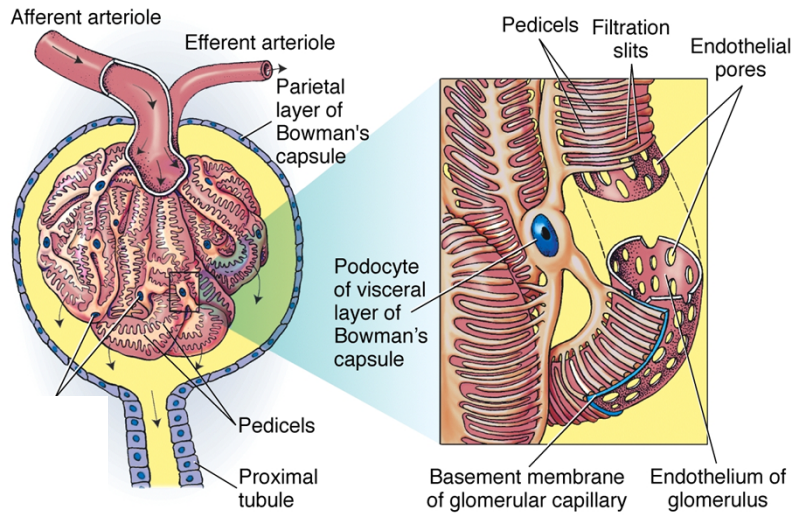
The enzymes of this cycle are present in liver (and kidney) cells

Fig. 13.13





Glomerular filtration



Glomerular filtration

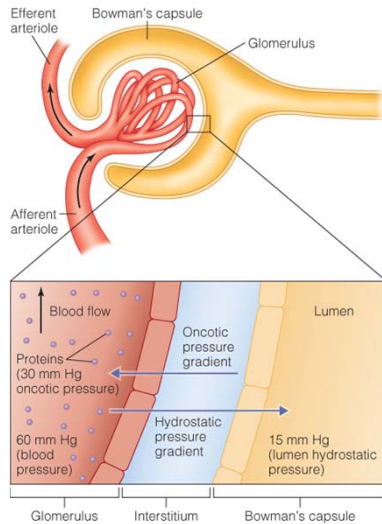
	Molecular weight	% going through filter
water	18	100
urea	60	100
glucose	180	100
sucrose	342	100
Inulin	5500	98
myoglobin	17000	75
hemoglobin	68000	3
albumin	69000	<1

Human: about 200 liters of fluid enter the proximal tubules every day !

Composition of filtrate = composition of the blood without the cells and proteins

Glomerular filtration rate (GFR)

= Total volume of filtrate passing through all glomerules of both kidneys per unit time



Effective pressure of glomerular filtration

GFR is affected by:

1. Hydrostatic pressure in the glomerular capillaries
2. Hydrostatic pressure in Bowman's capsules
3. Oncotic pressure = osmotic pressure associated with the difference in protein concentration between blood and lumen of Bowman's capsules

Fig. 13.18

How to measure GFR ?

Use of substances with the following properties:

- filtered by kidney in free form
- not reabsorbed or secreted in the nephron
- not produced or metabolized by the kidney itself
- having no effect on renal function

Examples of compounds used for practical assessment of GFR: **Inulin** and **creatinine**

$$[\text{creatinine}]_{\text{plasma}} \times \text{GFR} = [\text{creatinine}]_{\text{urine}} \times \dot{V}_{\text{urine}}$$

$$\text{GFR} = \frac{[\text{creatinine}]_{\text{urine}} \times \dot{V}_{\text{urine}}}{[\text{creatinine}]_{\text{plasma}}}$$

Examples of units:

Concentrations in micromoles / ml

GFR et \dot{V}_{urine} in ml / min

Renal clearance

Clearance of a substance =
volume of plasma from which a substance is completely removed (cleared) per unit time

For inulin and creatinine: renal clearance = GFR

For other substances:

$$\text{Renal clearance} = \frac{[\text{substance}]_{\text{urine}} \times \dot{V}_{\text{urine}}}{[\text{substance}]_{\text{plasma}}}$$

$$\text{Fractional elimination of a substance (FE)} = \frac{\text{Renal clearance of this substance}}{\text{Renal clearance of inulin (= GFR!)}}$$

if $FE < 1$ substance in question is **reabsorbed**

if $FE > 1$ substance is **secreted**

Reabsorption of glucose and sodium

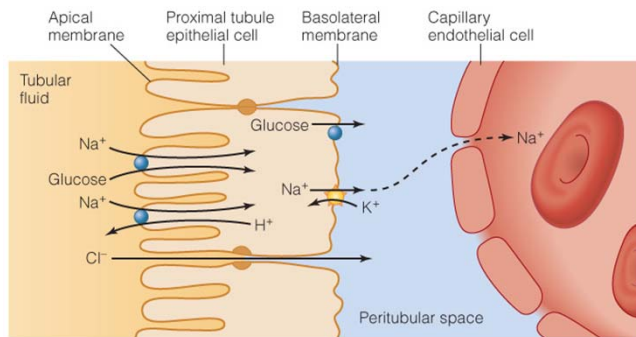
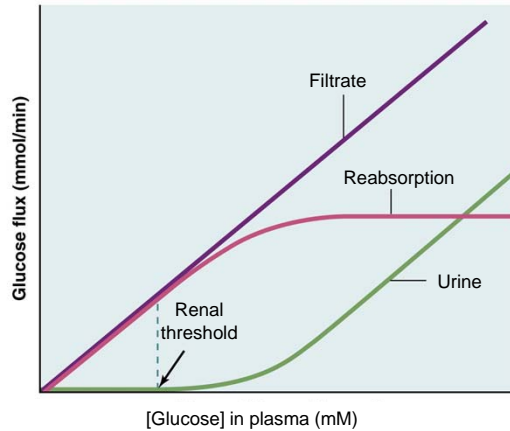


Fig. 13.21

- *Primary urine* – initial filtrate from the glomerular capsule that is isoosmotic with the blood
- Most solutes found in the primary urine are then reabsorbed via transport proteins that use ATP (=pumps)
- Each region of the nephron tubules has a particular complement of specific transporters

Renal threshold for reabsorption

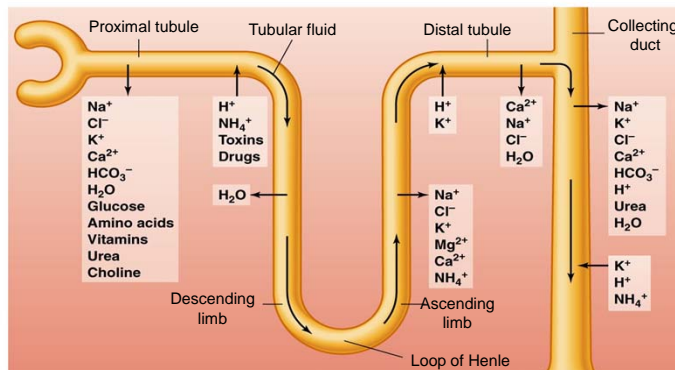
Ex: glucose reabsorption



When reabsorption rate reaches a maximum for a given substance (i.e. when the specific transporters are saturated), that substance is no longer 100% reabsorbed and some of it appears in urine

Fig. 13.22

Ion and water transport in the different regions of the nephron tubules



- **Proximal tubule** – reabsorption starts
- **Anse de Henle** – most of the reabsorption takes place here: divided in descending limb (water reabsorption) and ascending limb (ion reabsorption)
- **Distal tubule** – reabsorption is completed here for most solutes
- **Collecting duct** – drains several nephrons

Fig. 13.23

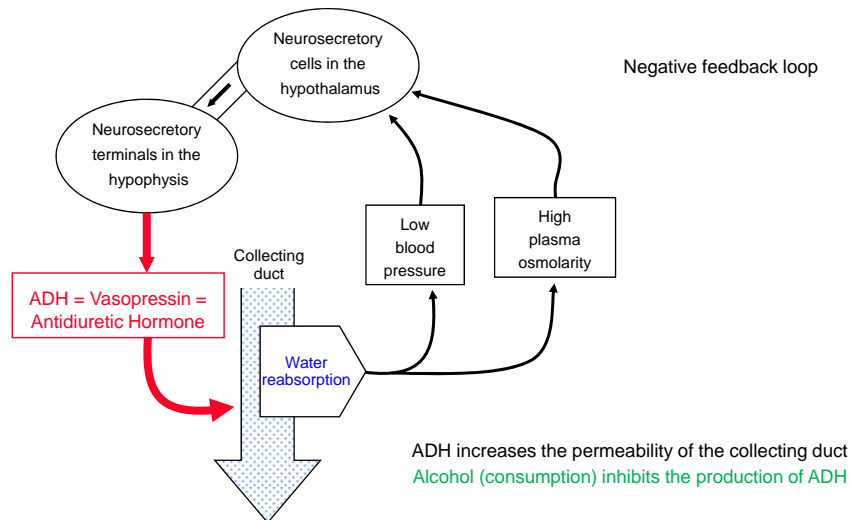
Renal secretion

- Works similarly to reabsorption, but in the opposite direction
- Also requires transport proteins (pumps) that use ATP
- Secreted substances include: K^+ , NH_4^+ , H^+ , organic acids and bases (HCO_3^-), drugs, toxins, and water-soluble vitamins
Therefore, the kidney plays a central role in [acide-base balance](#)
- The liver and kidney work together to [eliminate toxins](#):
 1. Conjugation of toxins with glucuronic acid or sulfate groups in the liver
 2. Conjugated compounds are then recognized by transporters in the kidney to be secreted

Hormonal regulation of kidney function

Diuretic hormones:	stimulate water excretion
Antidiuretic hormones:	reduce water excretion
Steroid hormones:	slow response
Peptide hormones:	more rapid response

Hormonal regulation of water reabsorption



Vasopressin (ADH)

- Peptide hormone produced in the hypothalamus and released in the circulation by the hypophysis
- Increase water reabsorption at the level of the collecting duct by translocating water transporting proteins **AQUAPORINS** from intracellular vesicles to the cell membrane
- **ADH production is stimulated**: when plasma osmolarity is increased (a signal detected by osmoreceptors in the hypothalamus)
- **ADH production is inhibited**: when arterial blood pressure is increased (a signal detected by baroreceptors in the atrium and in the carotid bodies of the aorta)

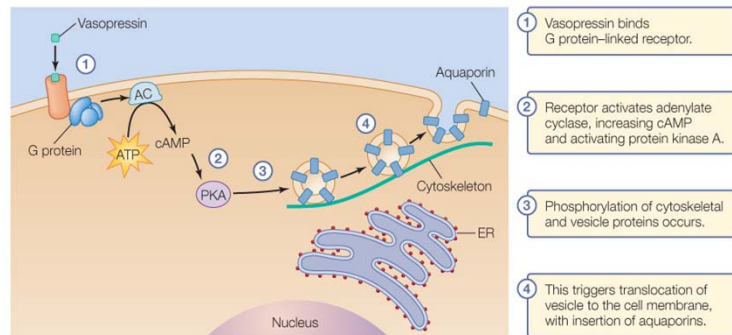


Fig. 13.30

Aldosterone

- Steroid hormone produced in the cortex of the adrenal gland
- Target cells are in the distal tubules and collecting ducts of nephrons
- Hormone stimulates Na^+ reabsorption (and water that follows), as well as K^+ secretion
- Aldosterone production is stimulated by an increase in circulating $[\text{K}^+]$

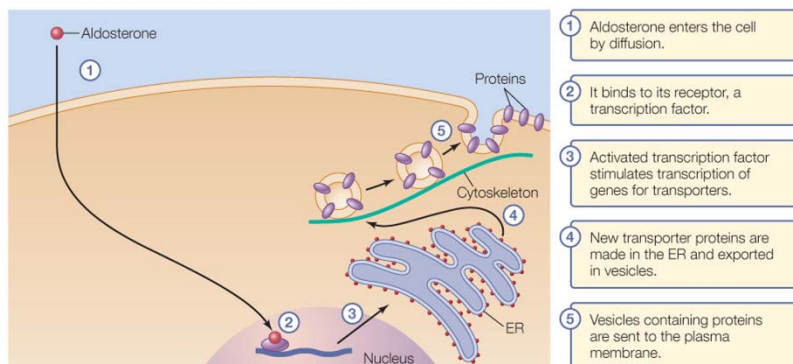


Fig. 13.31