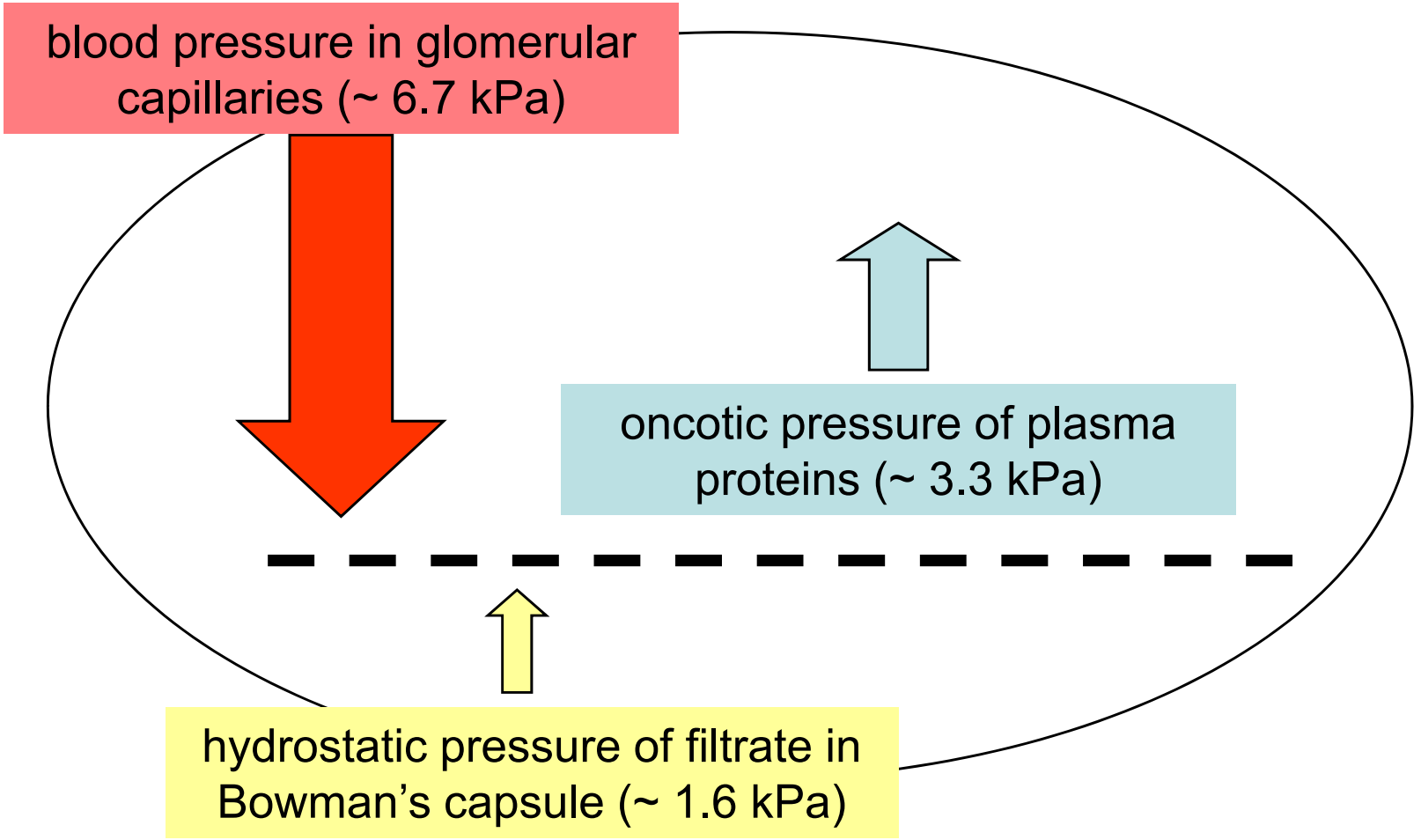
Biochemical functions of kidneys

Seminar No. 12

1	Arteria renalis
2	Arteria afferens
3	glomerulus
4	Arteria efferens
5	Bowman’s capsule
6	Proximal tubule
7	The loop of Henle
8	Distal tubule
9	Collecting duct

Q. 2

For details consult physiology



Effective filtration pressure ≈ 6.7 – 1.6 – 3.3 ≈1.8 kPa

Q.3 The volume of blood plasma that is completely cleared of creatinine in one second

Express:

- Molar amount of plasma creatinine filtered per 1 s:

  n_p =

- Molar amount of urine creatinine excreted by urine per 1 s:

  n_u =

- Clearance of creatinine:

- Molar amount of plasma creatinine filtered per 1 s:

  $n_p = c_p \times V_p$ **(mmol/s)**

- Molar amount of urine creatinine excreted by urine per 1 s:

  $n_u = c_u \times V_u$ **(mmol/s)**

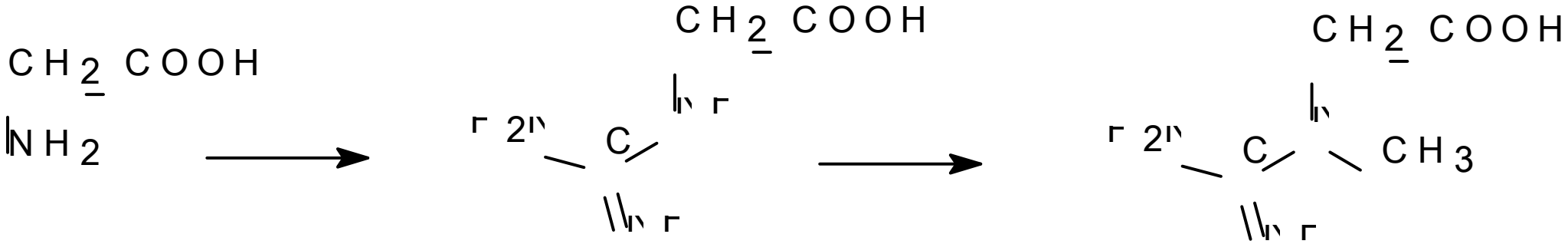
- Clearance of creatinine: $V_p = (c_u \times V_u) / c_p$ **(ml/s)**

Q. 4

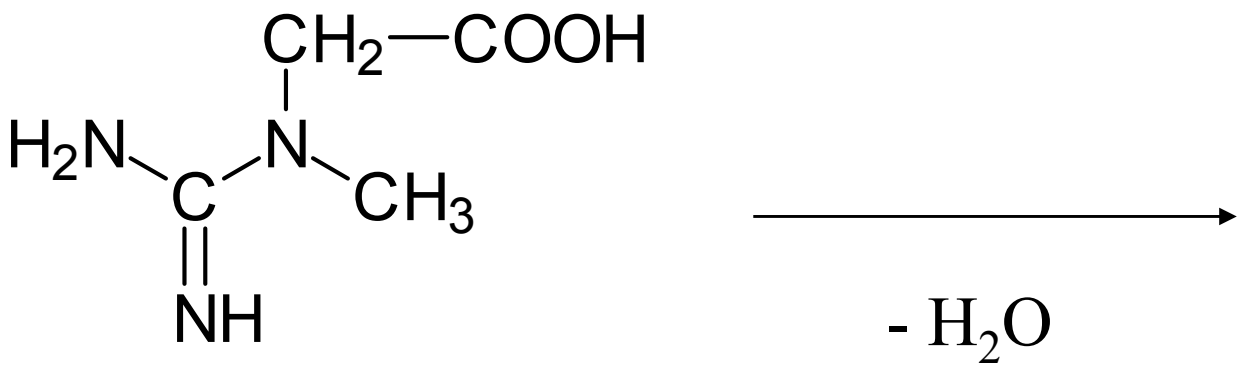
$$V_p = \frac{12(\text{mmol/l})}{0.106(\text{mmol/l})} \times \frac{1890(\text{ml})}{24 \times 60 \times 60(\text{s})} = 2.48 \text{ ml/s}$$

diuresis per one second

Q. 6

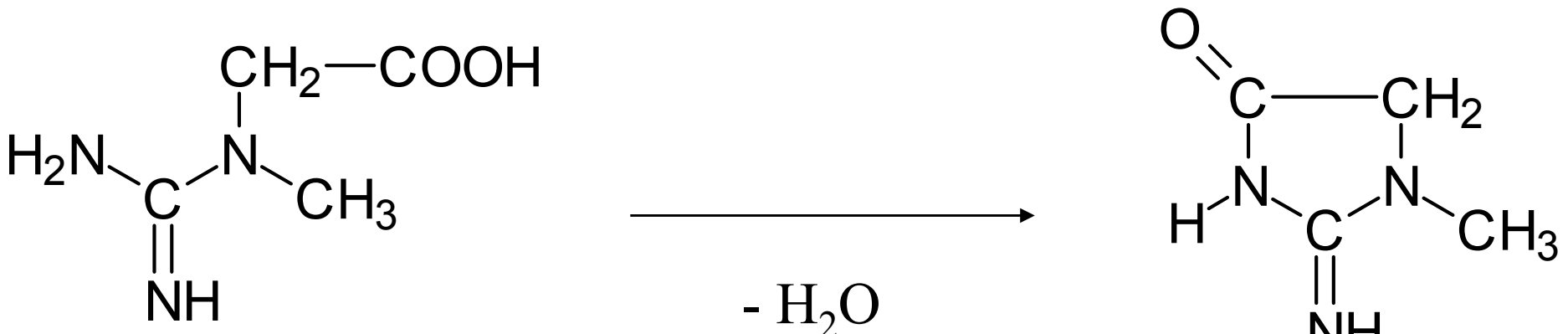

CH2 COOH
NH2
CH2 COOH
C
CH2 COOH
C
CH3

Creatinine formation



creatine

Creatinine formation



creatine

creatinine

Filtration of plasma

- glomerular membrane is a filter system
- **structural barrier** (collagen IV)
- **electrostatic barrier** (negative charges of sialic acid in glycoproteins repulse anionic proteins)
- the basement membrane allows free movement of electrolytes, water and small molecules (urea, glucose, AA, creatinine ...)

The filtration of proteins strongly depends on their molecular mass

- **Proteins with M_r < 60 000 (microproteins)**
  pass easily into urine regardless of their charge

- **Proteins with M_r 60 000 – 150 000**
  only very small amount is filtered into urine

- **Proteins with M_r > 150 000**
  do not pass into urine regardless of their charge

Which enzyme is readily filtered into urine?

Q. 8

A. 8

total proteins: < 150 mg/day

albumin: < 20 mg/day

Q. 10

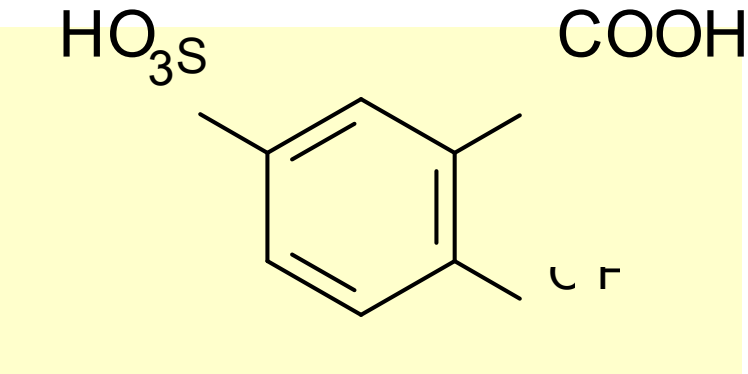
1. **Test strips based on protein error of acid-base indicators**

   primarily specific for albumin

   (albuPhan, Pliva-Lachema, Brno)

2. **Precipitation tests**

   proteins are precipitated (denaturated) by 5-sulfosalicylic acid as fine particles (turbidity)



see lab manual

Microalbuminuria

- urine excretion of albumin 20 – 300 mg/day
- an early indicator of diabetic nephropathy

- **immunochemical tests using antibody against human albumin**
- antibody can be **gold-labelled** ⇒ red coloured zone on a strip corresponds with albumin concentration in urine sample (test strips Micral)
- antibody **freely soluble** ⇒ nephelometry

see lab manual

Q. 11

(see chapter 1)

SDS-PAGE

- **s**odium **d**odecyl **s**ulfate **p**olyacryl**a**mide **g**el **e**lectrophoresis
- separation is carried out in the presence of SDS, oligoproteins are separated into their subunits, SDS binds to proteins and gives them a large uniform negative charge, thus proteins are separated according to their M_r.

Glomerular proteinuria

- consequence of the loss of glomerular membrane integrity
- typical protein: albumin

- **selective glomerular proteinuria:**

  proteins with M_r 60 000 – 100 000 pass into urine

- **non selective glomerular proteinuria:**

  more severe glomerular lessions

  proteins of all sizes M_r > 60 000 pass into urine

Tubular proteinuria

- tubules are unable to reabsorb proteins
- small proteins molecules (microproteins) $M_r < 60\,000$ appear in the urine
- typical protein: β_2-microglobulin

Q. 13

β_2-microglobulin	
Hemoglobin	
RBP	
α_1-acidic glycoprot.	
α_1-antitrypsin	
albumin	
transferrin	
IgA, IgG	

β_2-microglobulin	Immune defense, **indicator of tubular dysfunction**
Hemoglobin	Transport of O_2, small amount of CO_2, buffer system
RBP	Transport of retinol
α_1-acidic glycoprot.	Physiological role not known Transports some alkaline xenobiotics (drugs)
α_1-antitrypsin	antiprotease
albumin	Transport of various species, oncotic pressure, buffer
transferrin	Transport of Fe^{3+}, **acute phase protein**
IgA, IgG	Immune defense

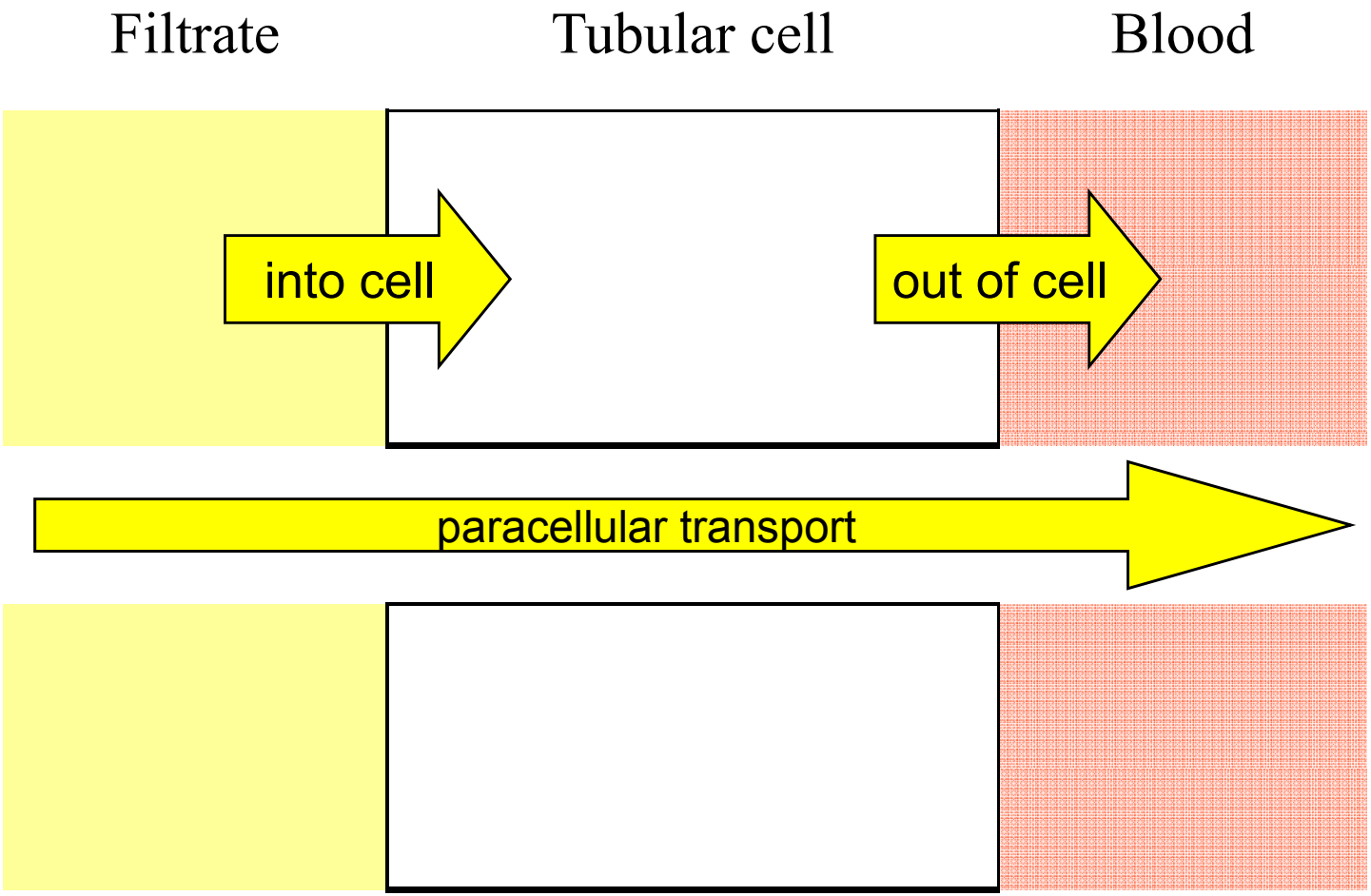
Q. 14

A. 14 Resorption of proteins

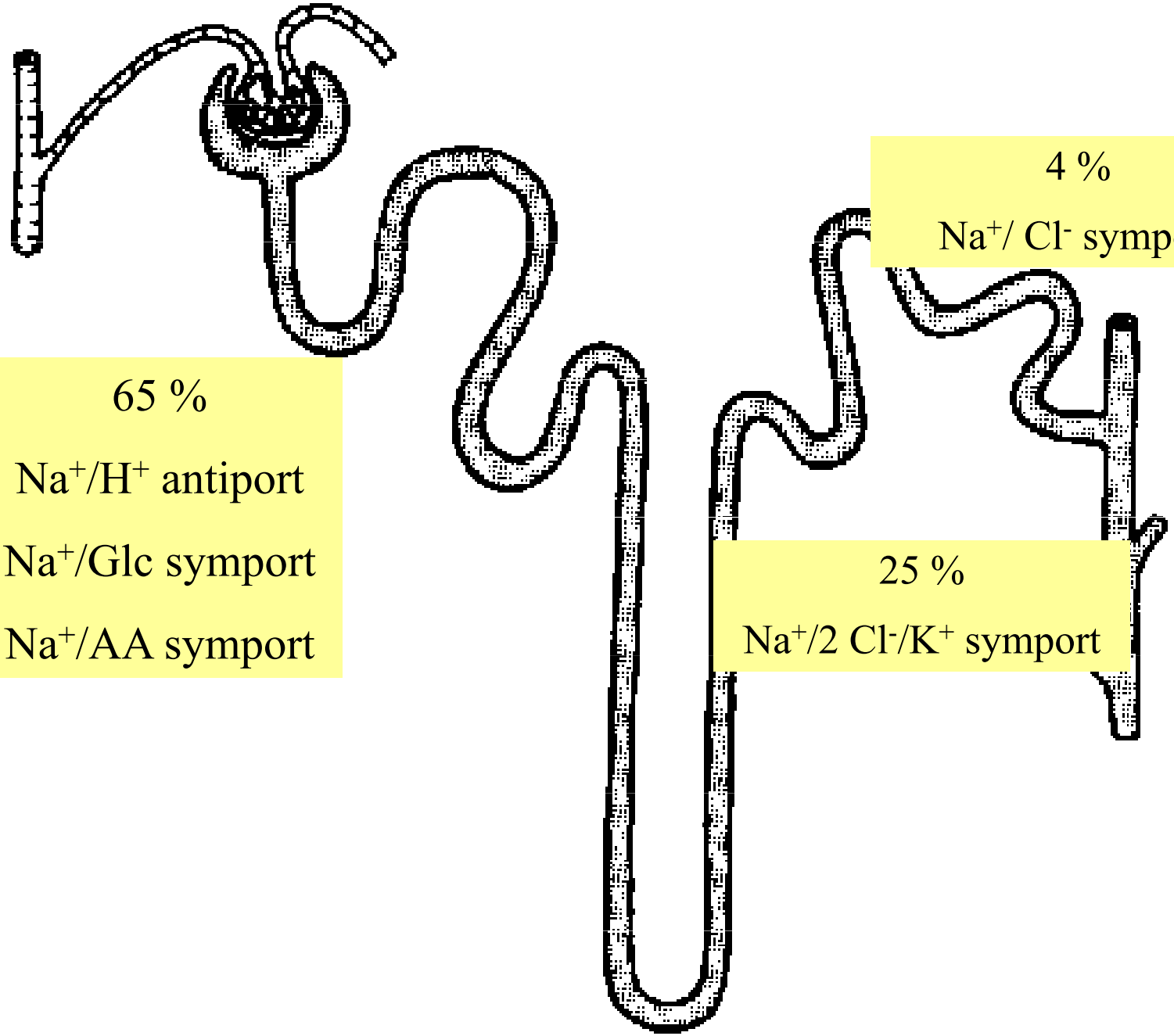
- microproteins (M_r < 60 000) are resorbed from the primary urine by **receptor-mediated endocytosis**
- after hydrolysis in secondary lysosome in tubular cells are returned into AA pool

Q. 15

Distinguish transcellular *vs.* paracellular transport



15a) Sodium transport into tubular cells



65 %

Na^+/H^+ antiport

Na^+/Glc symport

Na^+/AA symport

4 %

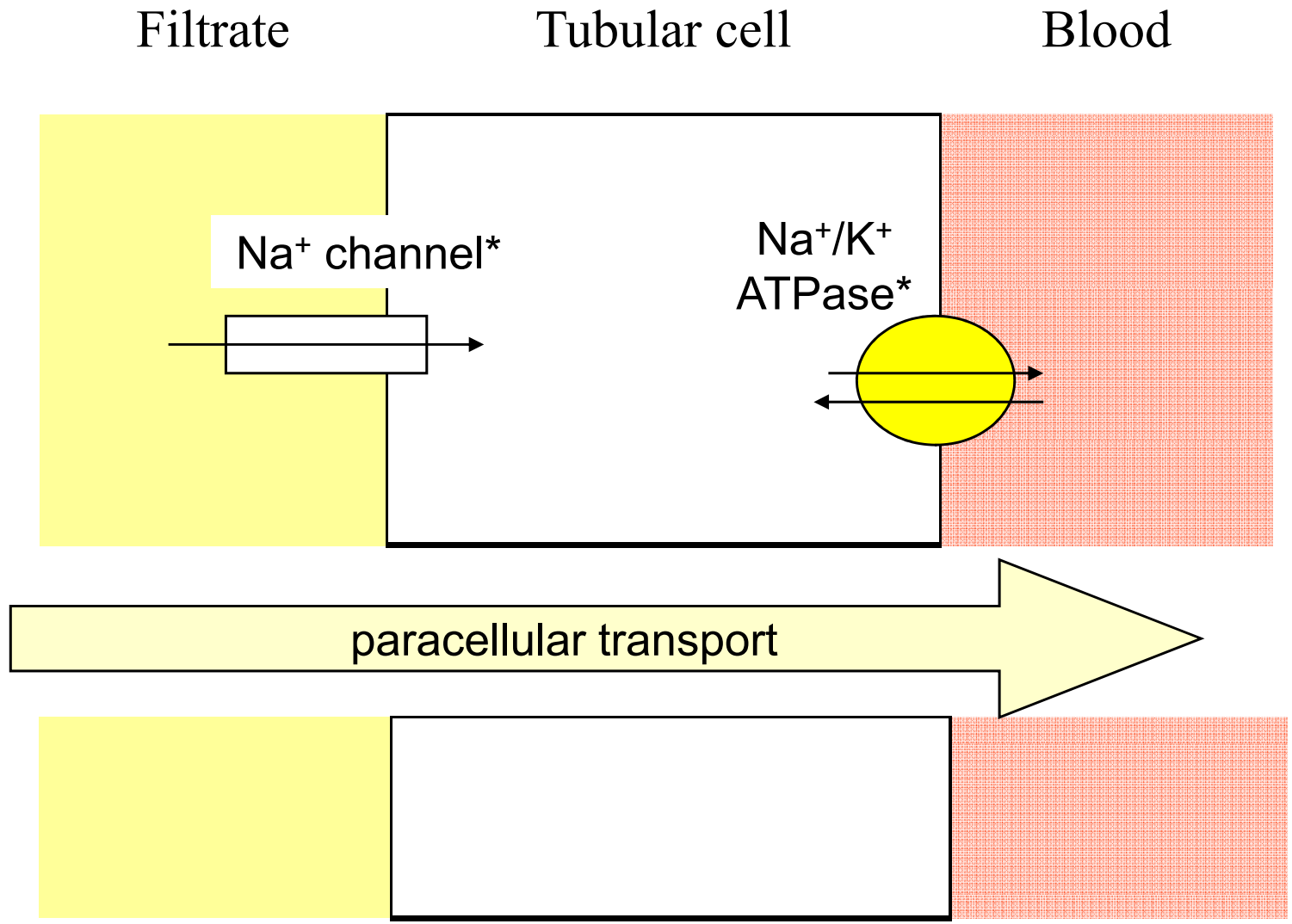
Na^+/ Cl^- symport

25 %

$Na^+/2\ Cl^-/K^+$ symport

Na^+ channels activated by aldosterone (ADH)

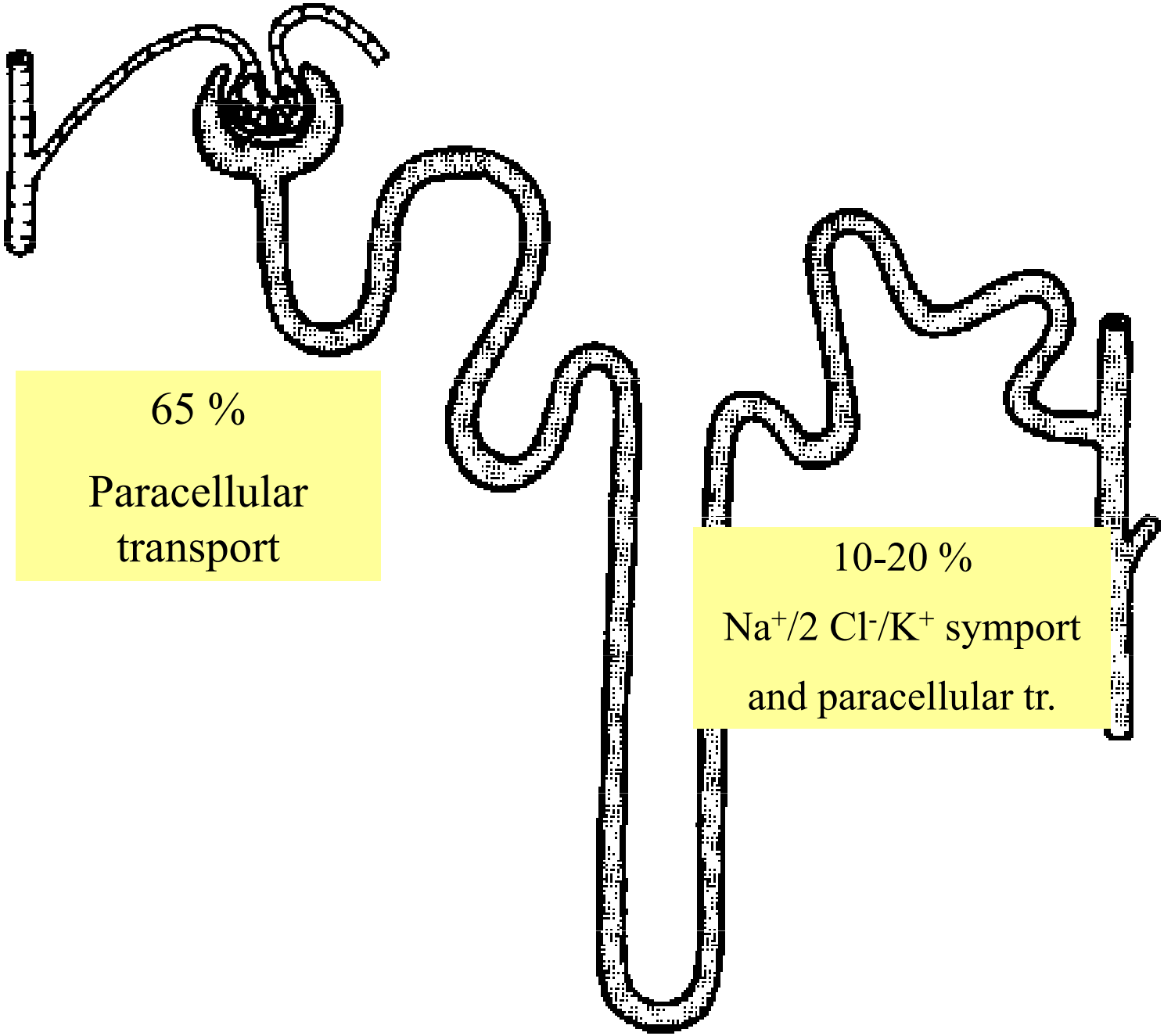
15b)



* activated by aldosterone in distal tubule

Q. 16

16a) Potassium transport



65 %

Paracellular transport

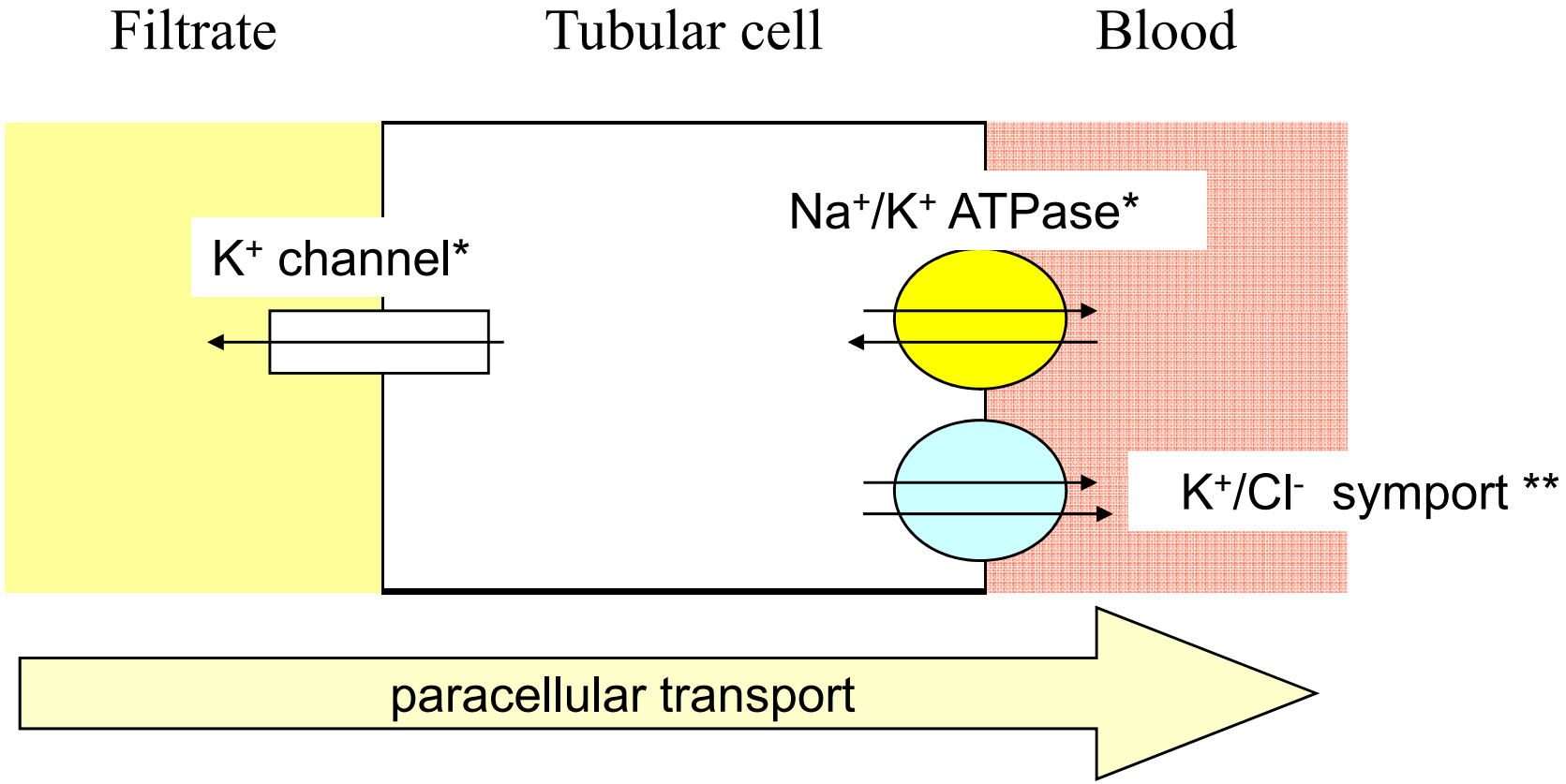
10-20 %

$Na^+/2\ Cl^-/K^+$ symport and paracellular tr.

Secretion

final decesion about K^+ output (aldosterone + other factors)

16b)



* activated by aldosterone (distal tubule)

** ascending limp of H. loop

Potassium secretion

Factor	K^+ secretion (↑ or ↓)
K intake	
Aldosterone	
Alkalosis	
Acidosis	
Anion comp.	

Potassium secretion

Factor	K^+ secretion (↑ or ↓)
K intake	↑
Aldosterone	↑
Alkalosis	↑
Acidosis	↓
Anion comp.	↑

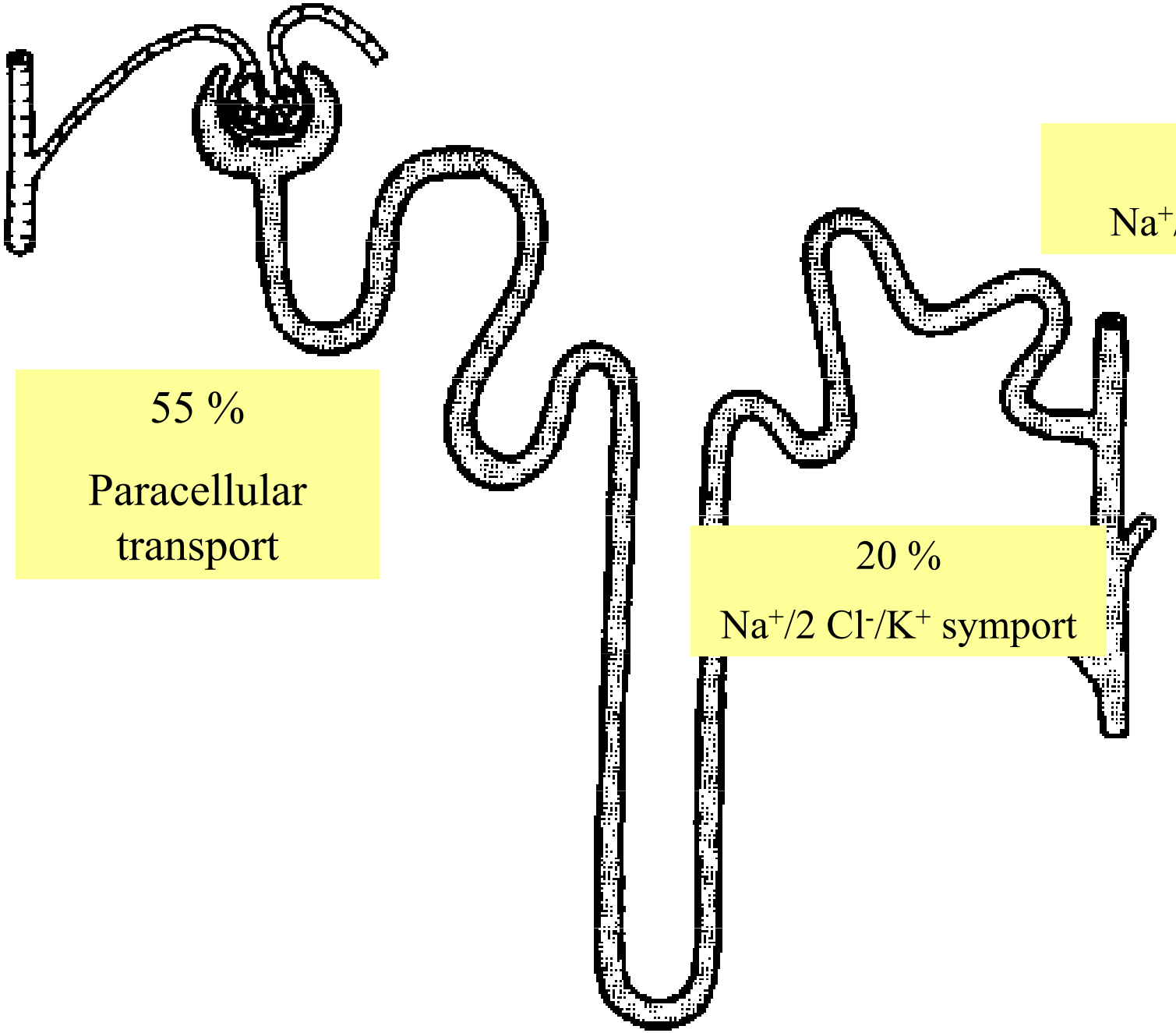
Compare chapter 11, p. 7

K change in intracell. content correlates with excretion change

Organic anions (drugs, ATB)

Q. 17

17a) Chloride transport



20 %

Na^+/ Cl^- symport

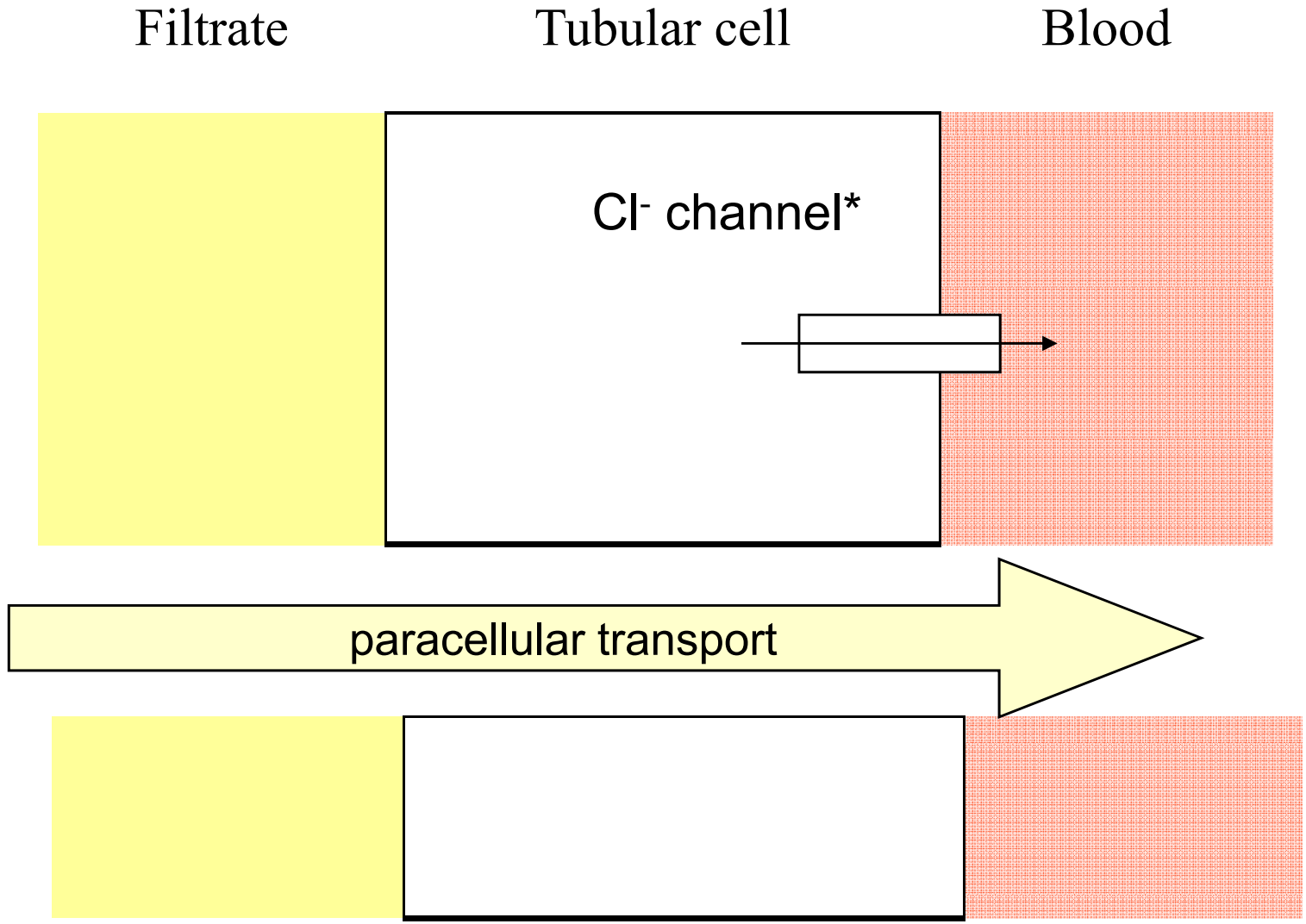
55 %

Paracellular transport

20 %

Na^+/2 Cl^-/K^+ symport

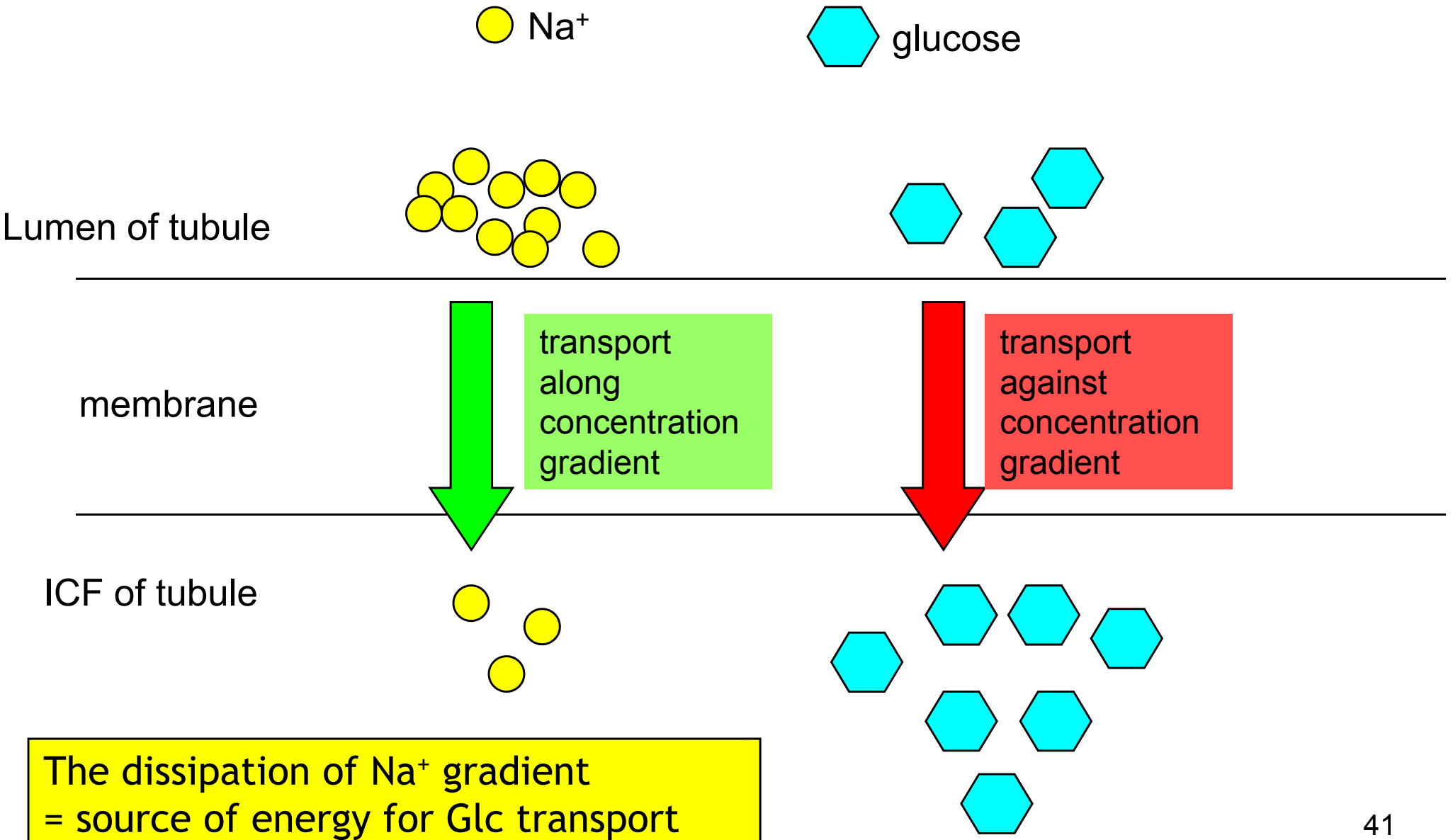
17b)



* activated by ADH

Q. 18

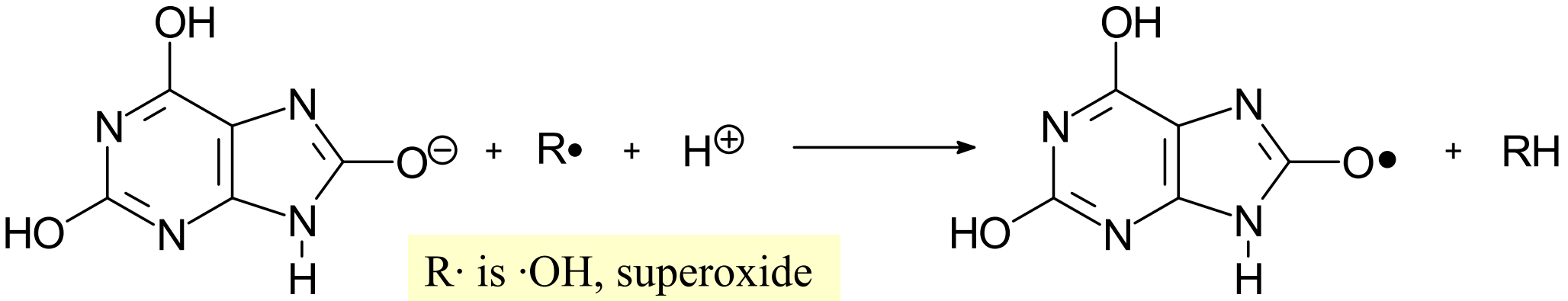
Symport of glucose with Na^+ is secondary active transport



The dissipation of Na^+ gradient
= source of energy for Glc transport

Q. 19

Uric acid is the most abundant plasma antioxidant



R· is ·OH, superoxide

hydrogen urate
(reduced form)

stable radical
(oxidized form)

Compare plasma concentrations

Ascorbic acid: 10 - 100 µmol/l

Uric acid (urate): 200 - 420 µmol/l

Q. 20 + 21

Compartment	Resorption mechanism
Proximal tubule	•Most part of water - through AQP1 •Also paracellular transport together with salts ⇒ tubular liquid is isotonic
Descending limb of H. loop	•Greatly permeable for water (AQP1) •Impermeable for Na, Cl ⇒ tubular liquid becomes hypertonic
Ascending limb of H. loop	•Impermeable for water •Na, Cl reabsorbed to great extent ⇒ tubular liquid hypotonic
Distal tubule + coll. duct	•AQP2 (ADH dependent) •Final concentration of urine

Q. 22

Compartment	Resorption mechanism
Proximal tubule	About 50 % of urea resorbed
Ascending limb + distal tubule	Impermeable for urea
Collecting duct	• Permeable under influence of ADH (UT1) • Urea diffuses back to: • Interstitial fluid (⇒ **contributes to osmotic gradient**) • Descending limb of H. loop • Vasa recta } urea recycling

Q. 23

Osmolarity =

Osmolality =

Osmolarity = i c = i × molarity **(mmol/l)**

Osmolality = i c_m = i × molality **(mmol/kg H_2O)**

Compound	**i**
NaCl	
urea	
$CaCl_2$	

Q. 24

- Plasma osmolality:

- Urine osmolality:

Q. 27

Hormone	Produced by	Action on kidney
Aldosterone	Adrenal cortex zona glomerulosa	Stimulates reabsorption of Na, secretion of K Induces synt. of Na and K channels, Na/K-ATPase
ADH	Posterior pituitary gland	Stimulates reabsorption of water and urea (Na, K, Cl) Stimulates exposition of aquaporines (AQP2)
Natriuretic peptides	Atrial myocytes of heart	Antagonists of RAAS Stimulate diuresis + excretion of Na, K vasodilation
Parathormone	Parathyroid glands	Stimulates calcium resorption ⇒ Ca in blood ↑ Stimulates phosphate excretion to urine Stimulates hydroxylation of calcidiol → calcitriol

Endocrine functions of kidney

Compound	Functions
EPO	Stimulates the production of erythrocytes
Renin	It is an **enzyme** (proteinase), secreted by juxtaglom. cells, starts RAAS, catalyzes hydrolytic cleavage: angiotensinogen → angiotensin I
Calcitriol	•Made in kidney by hydroxylation of calcidiol •Stimulates intestinal Ca resorption by inducing the synthesis of Ca binding proteins •Stimulates bone mineralization (but also bone resorption) •Stimulates the action of parathormone on kidney

Q. 29

- ammonogenesis
- gluconeogenesis

Q. 30

Condition	Prevailing fuel
After meal	
Fasting	
Long starvation	

Condition	Prevailing fuel
After meal	Glucose, FA, glutamine
Fasting	FA, ketone bodies, glutamine
Long starvation	FA, glutamine

Q. 32

Feature	Urea	Uric acid
Chemical name		
Behaviour in water		
pH of aqueous solution		
Solubility in water		
Reducing properties		
Catabolite of		
Formation in cell		
Formation in body		
Serum concentration		
Urine excretion		
Catabolic nitrogen portion		

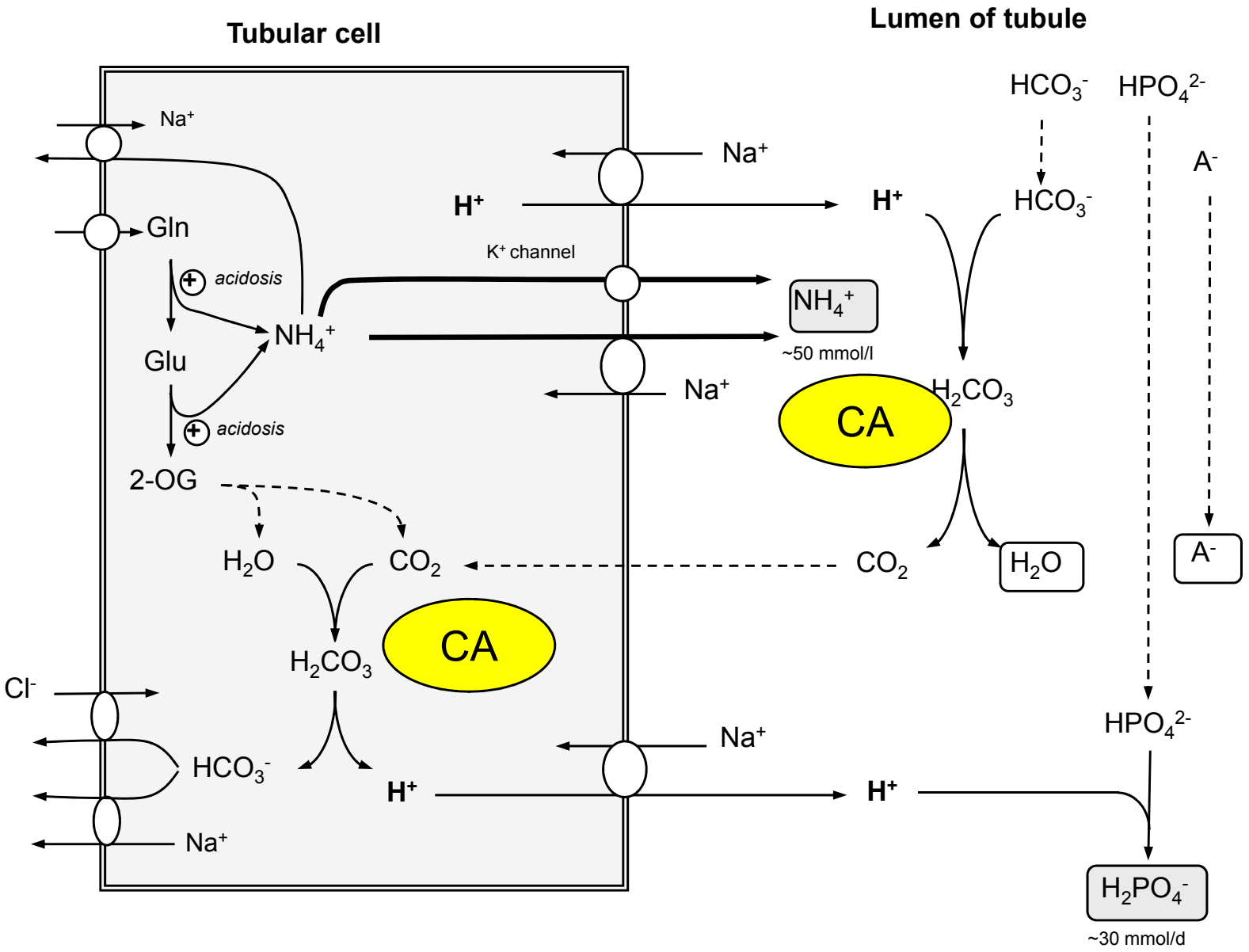
Feature	Urea	Uric acid
Chemical name	diamide of carbonic acid	2,6,8-trihydroxypurine
Behaviour in water	non-electrolyte	weak diprotic acid
pH of aqueous solution	neutral	weakly acidic
Solubility in water	perfect	poor
Reducing properties	no	yes (= antioxidant)
Catabolite of	amino acids	adenine + guanine
Formation in cell	mitochondria + cytosol	cytosol
Formation in body	liver	liver + other tissues
Serum concentration	3 – 8 mmol/l	200 - 420 μmol/l
Urine excretion	330 - 600 mmol/d	2.4 - 3.5 mmol/d
Catabolic nitrogen portion	80 - 90 %	1 - 2 %

Q. 33

Quantity	The way of excretion
Major portion	
Minor portion	
Traces	

Quantity	**The way of excretion**
Major portion	NH_4^+
Minor portion	$H_2PO_4^-$
Traces	free protons (H^+)

Q. 34 – see chapter 10, p. 7



Q. 35

Ion	Excretion (mmol/d)	Metabolic origin
Free H^+		
HPO_4^- $H_2PO_4^-$		
NH_4^+		
HCO_3^-		

Ion	Excretion (mmol/d)	Metabolic origin
Free H^+	0.01-0.02 *	• Origin: H_2CO_3 dissociation in tubules • H^+ get into urine by Na^+/H^+ antiport
HPO_4^- $H_2PO_4^-$	10-30	• Origin: food, catabolism of PL, DNA, RNA • filtered as HPO_4^-, binds H^+ to make prevaling $H_2PO_4^-$ • up to 97 % of HPO_4^- is resorbed by Na^+/P_i antiport in prox. t.
NH_4^+	30-50	• Origin: glutaminase + GMD reactions in tubules • NH_4^+ ions get into urine by K^+ channel or NH_4^+/Na^+ antiport
HCO_3^-	1-2 *	• Origin: H_2CO_3 dissociation in tubules • 98-99 % of HCO_3^- is resorbed by Cl^- antiport or Na^+ symport • HCO_3^- excretion is much greater in alkalosis

* If urine pH ≈ 5.0 and diuresis 1-2 litres.

Next seminar: 3rd revision test (15 Q / 20 min)

- Seminar chapters 8 – 12
- Practical chapters 8 – 10

Wednesday students are not allowed to come on Monday

Limit for credit **30 / 45**