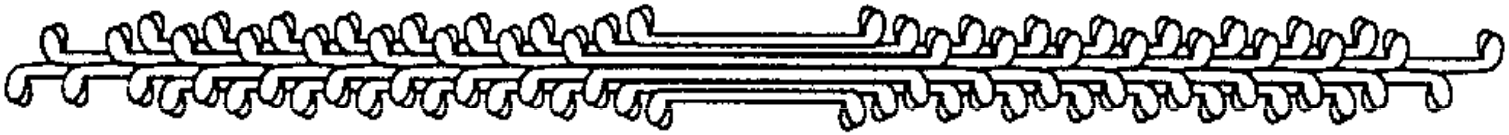
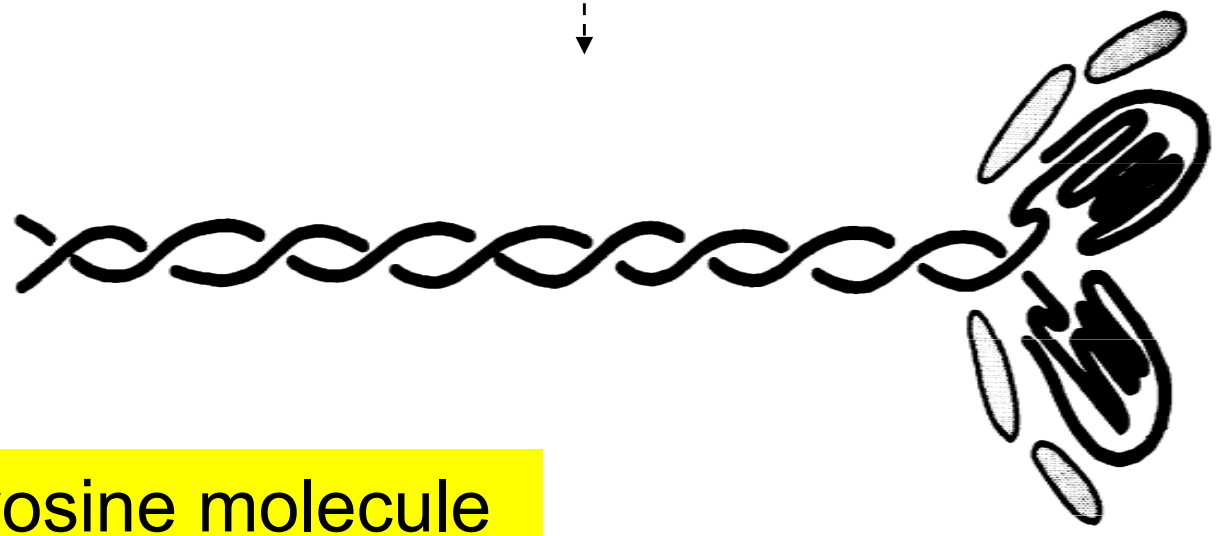
Biochemistry of muscles

Seminar No. 14

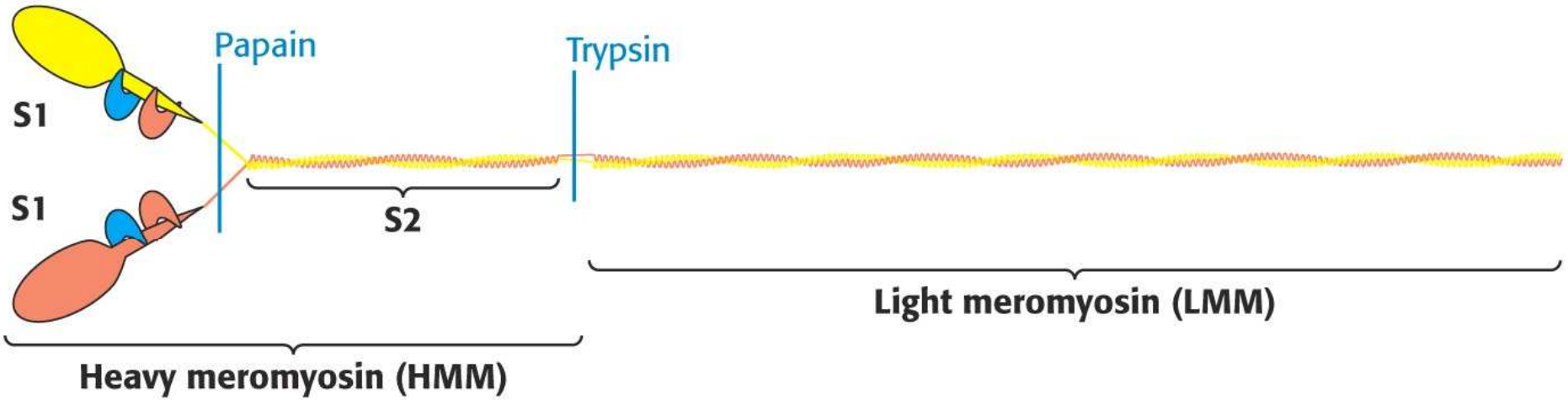
Thick filament is the myosin aggregate of cca 350 monomers





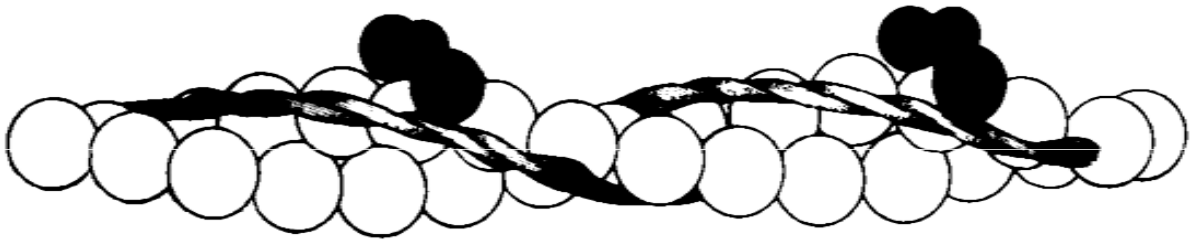
Describe myosine molecule

Myosin monomer



- two heavy chains (they make a double helix)
- four light chains (MLC – myosin light chains)
- *N*-terminal of a heavy chain forms a globular head with ATPase activity ($ATP + H_2O \rightarrow ADP + P_i$)
- treatment of myosin with proteases affords stable fragments (for research purposes).

Describe the thin filament



Thin filament – Actin

- globular monomer (G-actin) makes a double helix (F-actin)
- F-actin has other accessory proteins attached:
- tropomyosin (smaller double helix)
- troponin C – binds calcium ions
- troponin I – inhibits interaction actin-myosin
- troponin T – binds to tropomyosin and other troponins

Q. 10

(A) Relaxation: troponin I inhibits actin-myosin interaction, ATP (attached to myosin head) has been hydrolyzed ⇒ chemical energy is released and conserved in **high-energy conformation** of myosin head, concentration of Ca^{2+} in sarcoplasm is extremely low

Ca^{2+} is liberated from SR and attached to TnC, TnI is removed ⇒ myosin-ADP-P_i complex binds to actin **(B)**

ADP + P_i are liberated from myosin head, actin filament is pulled by cca 10 nm towards to sarcomere centre **(C) = contraction = chemical energy is transformed to mechanical work**

new ATP molecule binds to myosin head ⇒ dissociation of actin-myosin complex **(D)**

the liberation of Ca^{2+} ions from troponin C, insertion of TnI, and hydrolysis of ATP lead again to relaxation **(A)**

Q. 11

A. 11

The functions of ATP and calcium are **antagonistic:**

- ATP – separates actin from myosin
- Calcium ion – joins actin with myosin

Rigor mortis

Rigor mortis is a recognizable sign of death (L. *mors*, *mortis*, *f*.) that is caused by a chemical change in the muscles, causing the limbs of the corpse to become stiff (L. *rigor*, *oris*, *m*.) and difficult to move or manipulate.

Assuming mild temperatures, rigor usually sets in about 3-4 hours after clinical death, with full rigor being in effect at about 12 hours.

ATP supply from metabolic reactions is exhausted, the muscles remain contracted for ever.

Red and white filaments

Filament	Myoglobin	Mitochondria	Contraction	ATP source
Red	yes	many	slow	**aerobic** phosphorylation
White	no	few	fast	substrate level phosphorylation in **anaerobic** glycolysis

What is

- myoglobin
- aerobic phosphorylation
- substrate level phosphorylation

?

Phosphorylation:

substrate-OH + ATP → substrate-O-P + ADP

(e.g. glucose, protein, catalyzed by kinases)

Distinguish

Substrate level phosphorylation:

macroergic phosphate X~P + ADP → **ATP** + second product

X~P: 1,3-bisP-glycerate, phosphoenolpyruvate (glycolysis), succinyl phosphate (CAC)

Aerobic phosphorylation:

ADP + P_i + energy of H^+gradient → **ATP** + heat

(H^+gradient is made in respiratory chain by the oxidation of NADH+H^+ and $FADH_2$ from aerobic glycolysis, β-oxidation of FA, and citric acid cycle)

Q. 16

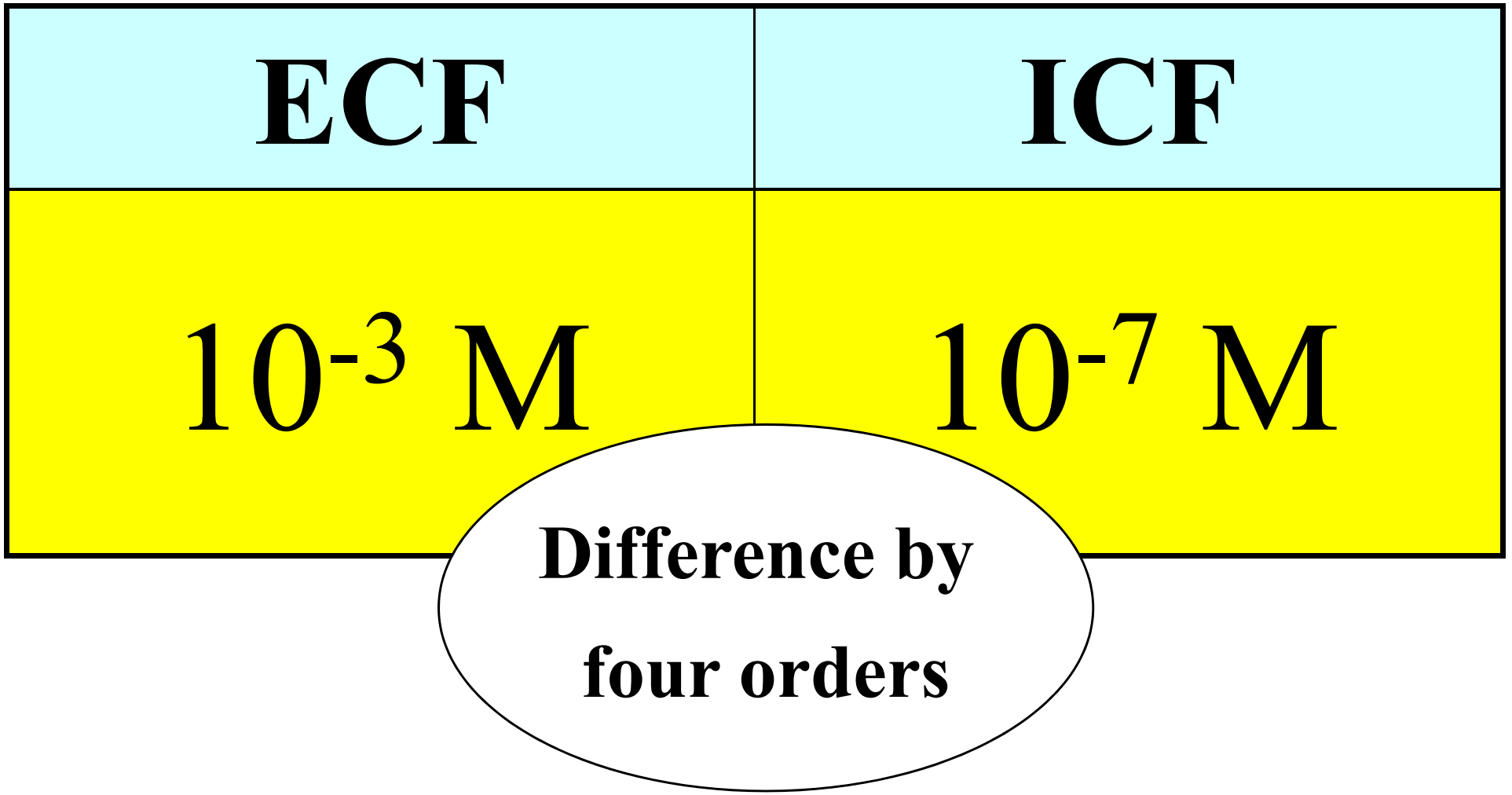
Calcium concentrations in sarcoplasm

Resting	Contraction
10^{-7} M	10^{-5} M

Difference by two orders

Q. 17

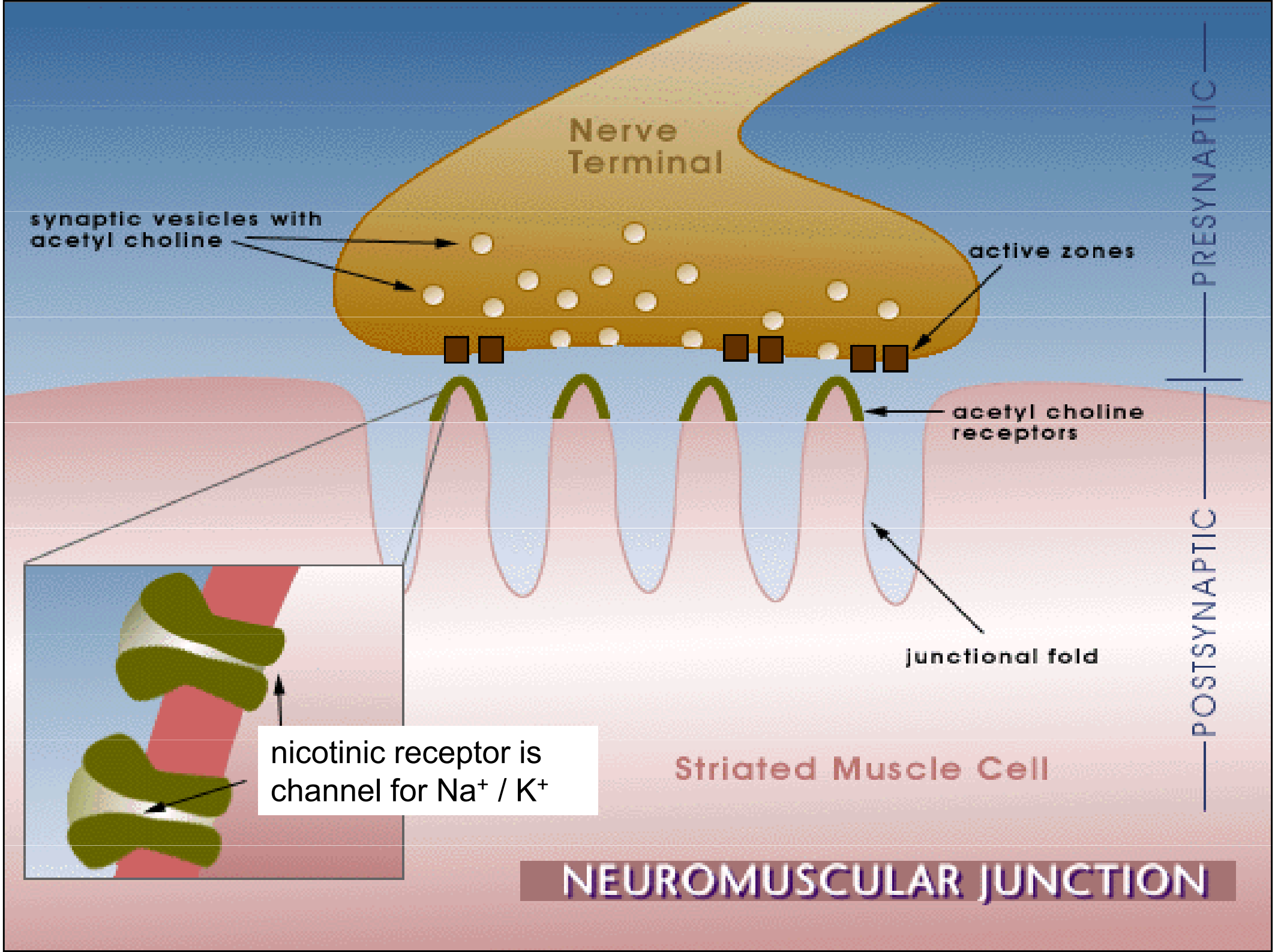
Calcium concentrations in body fluids



Q. 19

Events on neuromuscular junctions

- junction consists from nerve terminal separated from postsynaptic region by the synaptic cleft
- **acetylcholine** is released from presynaptic vesicles and binds to nicotinic receptors in muscle cell membrane ⇒ depolarization of membrane and T-tubules
- T-tubules are connected with sarcoplastic reticulum (SR) ⇒ **Ca^{2+} ions** are released from SR (where are associated with calsequestrin protein)
- calcium ions then bind to troponin C ⇒ **contraction**


Nerve Terminal
synaptic vesicles with acetyl choline
active zones
acetyl choline receptors
junctional fold
PRESYNAPTIC
POSTSYNAPTIC
nicotinic receptor is channel for Na+ / K+
Striated Muscle Cell
NEUROMUSCULAR JUNCTION

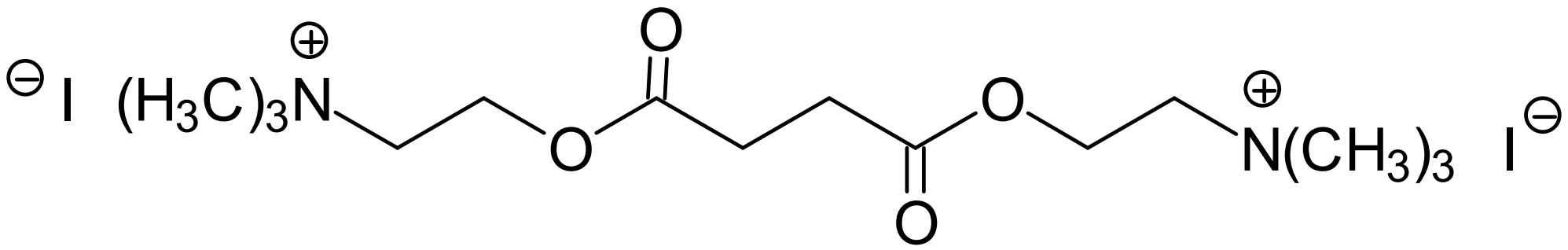
Q. 20

Inhibitors of skeletal muscle contraction

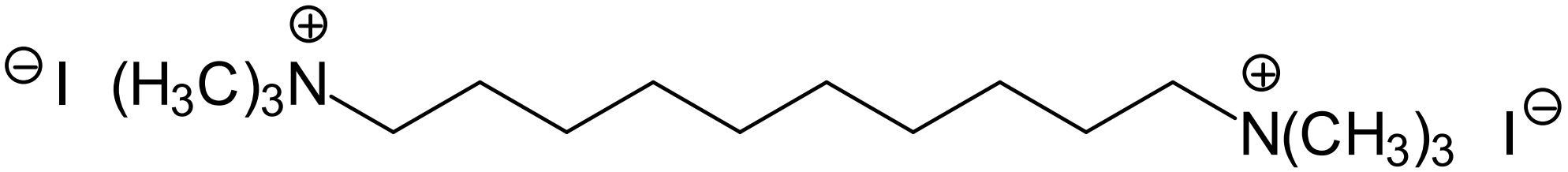
Substance	Action
Succinyl choline*	agonist of nicotinic receptor, not hydrolyzed by acetylcholinesterase, depolarization lasts longer – the result is myorelaxation
Decamethonium	agonist of nicotinic receptor, not hydrolyzed by acetylcholinesterase
Botulotoxin	inhibits the release of acetylcholine at presynaptic membrane
Bungarotoxin*	antagonist of nicotinic receptor
Curare*	tubocurarine is antagonist of nicotinic receptor
Dantrolene	inhibits intracellular Ca^{2+} release from SR

* See Chapter 9, p. 2

Skeletal muscle relaxants bind to nicotinic receptor, but are not hydrolyzed by acetylcholinesterase



succinylcholine iodide



decamethonium iodide

Botulotoxin

- Botulinum toxin is produced by bacterium *Clostridium botulinum*. The toxin is a two-chain polypeptide with a heavy chain joined by a disulphide bond to a light chain.
- The light chain is a protease that attacks one of the fusion proteins at a neuromuscular junction, preventing vesicles from anchoring to the membrane to release acetylcholine.
  By **inhibiting acetylcholine release**, the toxin interferes with nerve impulses and causes **paralysis of muscles** (botulism).
- no action potential is generated $\Rightarrow$ permanent relaxation

Medical uses of botulinum toxin

- Currently, Botox (= trade name) is finding enormous potential in several therapeutic areas including the treatment of migraine headaches, **cervical dystonia** (a neuromuscular disorder involving the head and neck), blepharospasm (involuntary contraction of the eye muscles), and severe primary axillary hyperhidrosis (excessive sweating).
- Other uses of botulinum toxin include urinary incontinence, anal fissure, **spastic disorders** associated with injury or disease of the central nervous system including trauma, stroke, multiple sclerosis, or cerebral palsy and focal dystonias affecting the limbs, face, jaw etc.

Botulinum toxin injections are applied in cosmetics to vanish facial wrinklers

Bungarotoxin is the antagonist of nicotinic receptor (blocks opening the Na^+/K^+ channel)

Bungarus multicinctus

Cardiac muscle: Three sources of calcium

- Extracellular Ca^{2+} (~ 10 %) enters by voltage operated channels (VOC)
- This influx of calcium triggers the release of calcium ions from SR and mitochondria (~ 90 %)

CICR = calcium-induced calcium release

Cardiac muscles - Contraction

- In sarcoplasm, Ca^{2+} ions bind to:

troponin C $\Rightarrow$ **contraction**

calmodulin $\Rightarrow$ autoregul. - **relaxation**

Cardiac muscles - Relaxation

- Ca^{2+} ions are liberated from troponin C and removed from sarcoplasm
- there are **four systems** how to vanish Ca^{2+} in sarcoplasm

1. Ca^{2+}-ATPase in SR
2. Ca^{2+}-ATPase in sarcolemma
3. Na^{+}/Ca^{2+} antiport in sarcolemma
4. Ca^{2+} re-entry to mitochondria

Autoregulation in cardiac muscle (scheme p. 4)

- intracellular calcium is in the complex with protein calmodulin: CM-4Ca^{2+}
- Ca^{2+}-CM stimulates **all** Ca^{2+}-pumps (some by phosphorylation) which decrease the Ca^{2+} concentration in sarcoplasm
- **the increase of intracellular [Ca^{2+}] triggers contraction but, at the same time, stimulates relaxation processes**

Q. 25

Modulatory effect of cAMP

Modulatory effect of cAMP on cardiac muscles

- cAMP is the second messenger produced after the activation of G_s-protein-linked-receptors (β-adrenergic receptors)
- such receptors are activated by catecholamines (nor/adrenaline)
- cAMP activates protein kinase A
- protein kinase A catalyzes the phosphorylation of:

calciductin of VOC ⇒ influx of Ca^{2+} ⇒ **contraction**

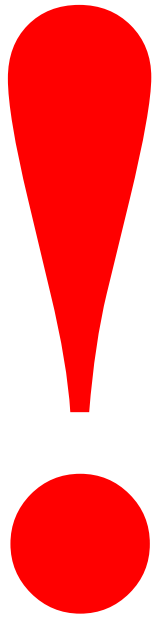
Ca^{2+}-ATPase in sarcolemma ⇒ eflux of Ca^{2+} ⇒ **relaxation**

Ca^{2+}-ATPase in SR ⇒ eflux of Ca^{2+} ⇒ **relaxation**

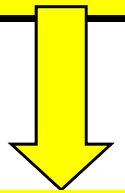
troponin I ⇒ conformation change - contact of actin-myosin ⇒ **contraction**

Q. 26

Compare Chapter 9, p. 8



Feature	Adrenergic Receptors			
	α_1	α_2	β_1	β_2
Hormone	adrenaline	adrenaline	adrenaline	adrenaline
G-protein	G_q	G_i	G_s	G_s
2nd messenger	DG, IP_3	cAMP ↓	cAMP ↑	cAMP ↑
Occurence	smooth m.	brain	cardiac m.	smooth m.



increased pulse rate + contractility

as the result of modulatory effect of cAMP

Metabolic background of MI

- ischemia (lack of oxygen in tissues) leads to anaerobic metabolism ⇒ glucose is converted to lactate
- lactate accumulates in ICF and alters intracellular environment ⇒ prolonged acidosis causes irreversible cell damage (necrosis)
- permeability of cell membrane increases ⇒ cytoplasmatic/mitochondrial/contractile proteins are released into ECF
- the best markers of MI are: myoglobin, CK-MB, cardial troponins (T or I) – this triple combination is recommended
- LD isoforms are no longer used

Smooth muscles - Contraction

- source of Ca^{2+}: ECF (VOC, ROC), SR
- there is no troponine C, but two other regulatory proteins binding calcium – calmodulin + caldesmon
- calcium-calmodulin complex (Ca^{2+}-CM) activates MLCK (myosin light chain kinase)
- activated MLCK catalyzes the phosphorylation of myosin
- phosphorylated myosin is capable to make complex with actin $\Rightarrow$ **contraction**

Smooth muscles - Relaxation

Two relaxing processes occur:

1. Removing intracellular Ca^{2+} from ICF (like in cardiac m.)
2. MLC-phosphatase catalyzes the hydrolysis of phosphorylated myosin:

$$MLC\text{-}P + H_2O \rightarrow P_i + MLC$$

MLC **does not** bind to actin $\Rightarrow$ relaxation

The influence of cAMP on smooth muscles

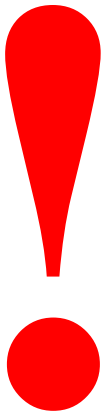
- cAMP activates protein kinase A (PK-A)
- PK-A phosphorylates MLC-kinase:

  MLCK $\rightarrow$ MLCK-P
- MLCK-P is inactive, does not phosphorylates MLC $\Rightarrow$

  no interaction between actin and myosin $\Rightarrow$ **relaxation**

Compare: Influence of cAMP on muscles

Skeletal muscle	Cardiac muscle	Smooth muscle
none	modulation	relaxation



Q. 30

Activation through	**Effect on smooth muscle**
β-receptor	$G_s \Rightarrow \uparrow$ cAMP $\Rightarrow$ relaxation
α_2-receptor	$G_i \Rightarrow \downarrow$ cAMP $\Rightarrow$ contraction
α_1-receptor	$PIP_2 \Rightarrow \uparrow Ca^{2+} \Rightarrow$ contraction
NO	relaxation

Different actions mediated through different adrenergic receptors

Feature	Adrenergic Receptors			
	α_1	α_2	β_1	β_2
Hormone	adrenaline	adrenaline	adrenaline	adrenaline
G-protein	G_q	G_i	G_s	G_s
2nd messenger	DG/IP_3/**Ca^{2+}**	**cAMP ↓**	cAMP ↑	**cAMP ↑**
Muscle action	**contraction**	**contraction**	↑ contractility	**relaxation**
Muscle type	**smooth**	**smooth**	cardiac	**smooth**

Q. 32

A. 32

- nitric oxide (NO) is a relaxant of smooth muscles

  (e.g. arterial myocytes)
- activates guanylate cyclase in cytosol: GTP $\rightarrow$ cGMP + PP_i
- cGMP activates protein kinase G (PK-G)
- PK-G phosphorylates MLC-kinase: MLCK $\rightarrow$ MLCK-P
- MLCK-P is inactive, does not phosphorylate MLC $\Rightarrow$

  no interaction between actin and myosin $\Rightarrow$ **relaxation**

Q. 33

NO releasing compounds

- **Endogenous**:

  L-arginine (the imino nitrogen of guanidine part)

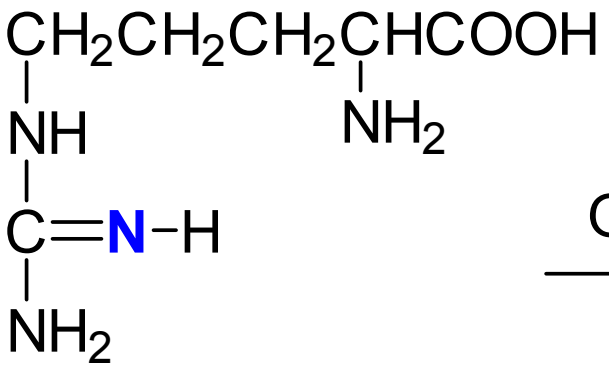
- **Exogenous:**

  organic nitrates = esters of nitric acid (R-O-NO_2)

  organic nitrites = esters of nitrous acid (R-O-N=O)

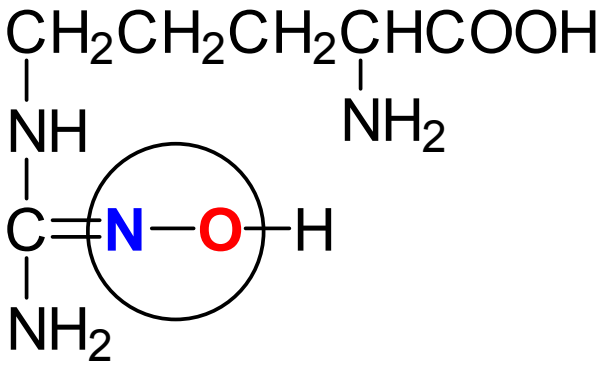
  sodium nitroprusside = a complex of Fe^{3+} with CN^- and NO

NO originates from imino nitrogen of L-arginine



L-arginine

O_2, NADPH

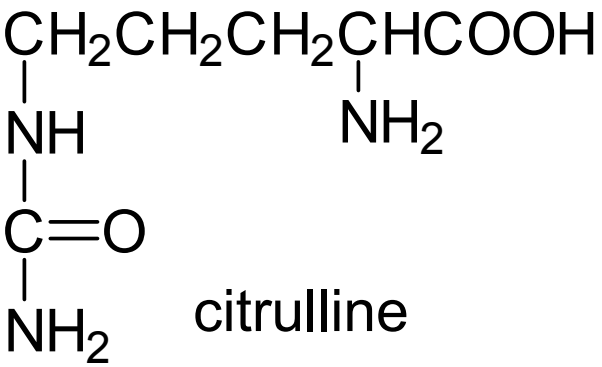


N-hydroxy-L-arginine

O_2, NADPH

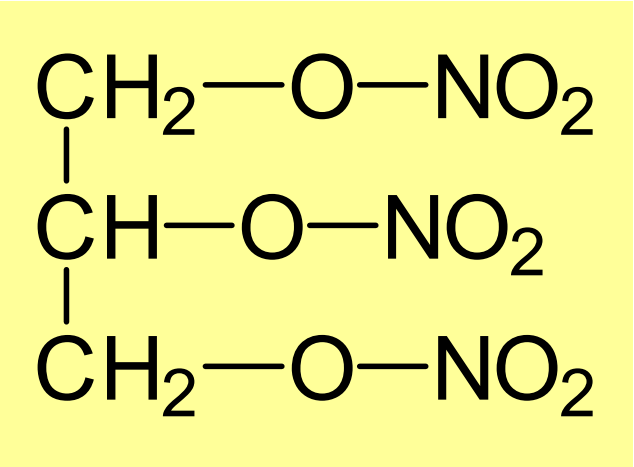
nitric oxide radical

+

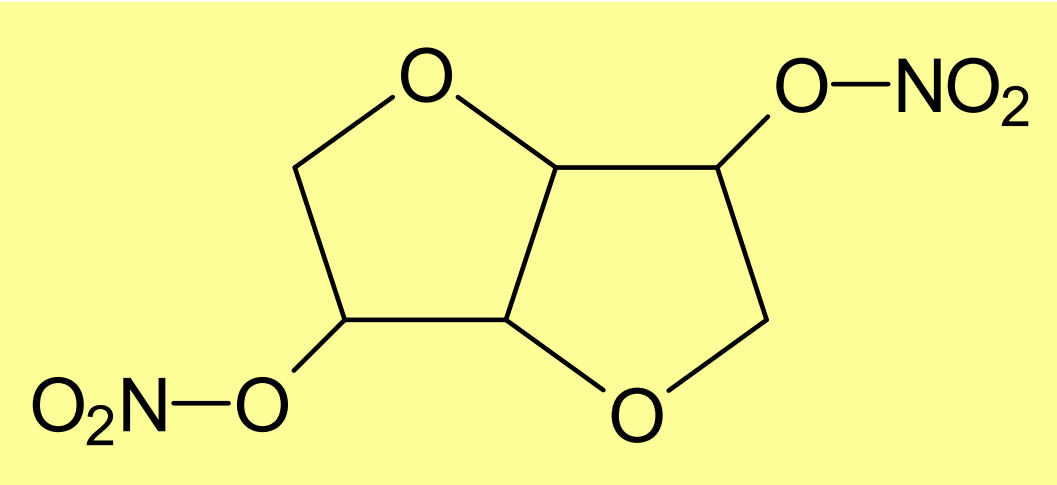


citrulline

Organic nitrates (alkyl nitrates)



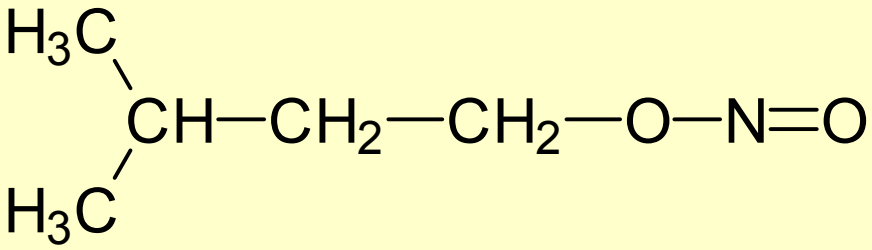
glycerol trinitrate
(glyceroli trinitras)



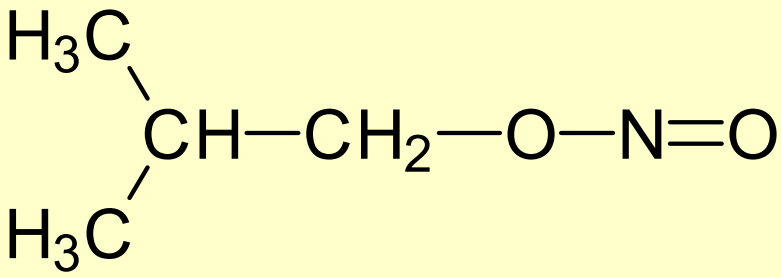
isosorbide dinitrate (isosorbidi dinitras)

In myocytes, they are reduced by glutathion
and subsequently release NO - **vasodilators**

Organic nitrites (alkyl nitrites)



isoamyl nitrite
(amylis nitris)



isobutyl nitrite
volatile liquid, new drug
(poppers, rush, liquid aroma ...)

Alkyl nitrites as well as inorganic nitrites ($NaNO_2$) have oxidation properties $\Rightarrow$ oxidize Fe^{2+} in hemoglobin to Fe^{3+} $\Rightarrow$ they cause **methemoglobinemia**

Other NO releasing compounds

$Na_2[Fe(CN)_5NO]$

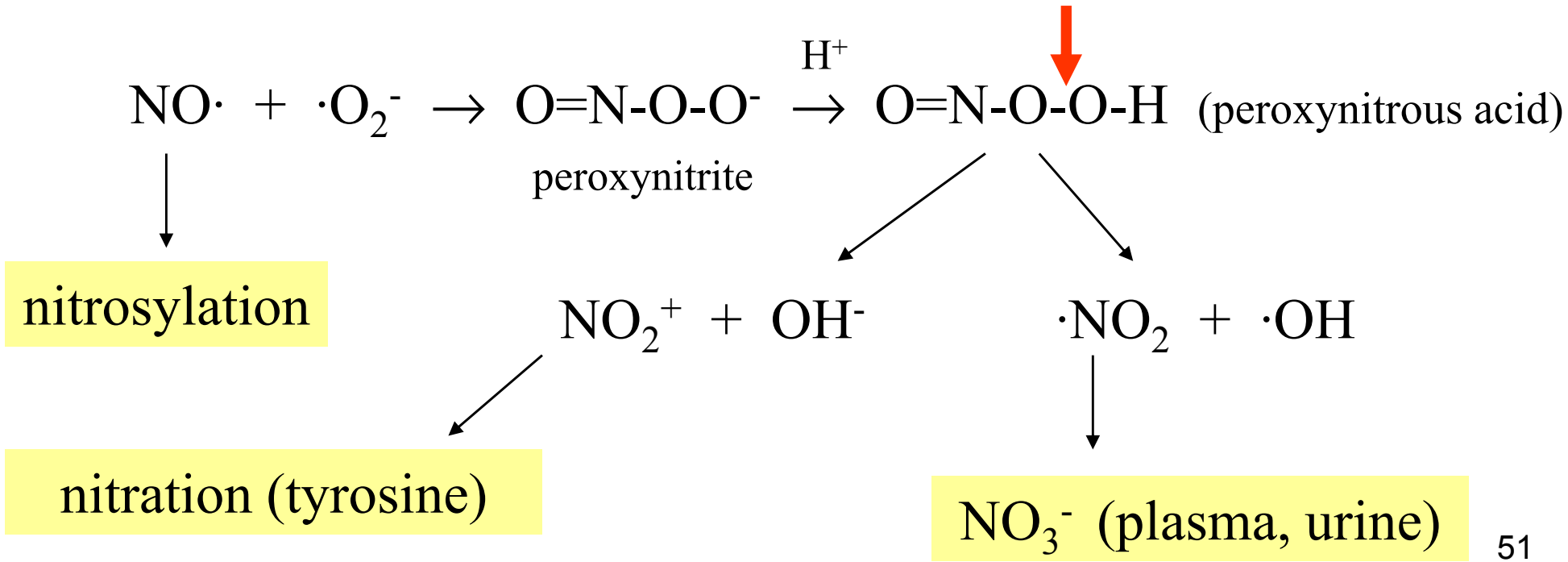
sodium nitroprusside (natrii nitroprussias)

sodium pentacyanonitrosylferrate(III)

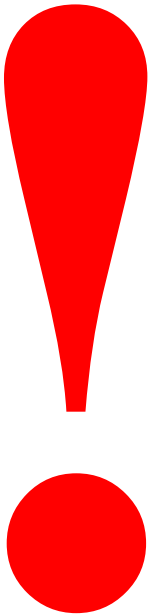
extremely potent vasodilator

Other metabolic pathways of NO

- nitric oxide is a radical (·N=O)
- reacts with superoxide to yield peroxynitrite
- the cleavage of peroxy bond (O-O) can occur in two ways



Q. 34



Different actions of the same signal molecule

Feature	Skeletal muscle	Smooth muscle
Signal molecule	acetylcholine	acetylcholine
Receptor	nicotinic	muscarinic (M_1/G_q)
2^{nd} messenger	none $\Delta\psi$ of membrane potential	IP_3, Ca^{2+}
Effect	$\uparrow Ca^{2+} \Rightarrow$ contraction	$\uparrow NO \Rightarrow$ relaxation
Scheme on page	3	7

Maximal intesity of muscle work

- anaerobic phase
- 30 sec – 2 min
- muscles use glucose ⇒ metabolized to lactate
- lactate goes to liver ⇒ substrate of gluconeogenesis
- small portion of lactate becomes **metabolic fuel** for resting muscles and myocardium

Prolonged muscle work/exercise

- working muscles are adapted to aerobic metabolism of glucose and FA
- resting muscles utilize FA and KB
- glycerol from lipolysis is the substrate for liver gluconeogenesis

Q. 35

A. 35

Type of glycolysis	ATP / Glc
Aerobic from glucose	36 – 38*
Anaerobic from glucose	2
Anaerobic from glycogen	3

* Depends on the type of transport of NADH from cytosol to mitochondria.

Q. 38

A. 38

- **in the first 10 sec** – ATP itself and creatine phosphate currently present in muscle cell

- **After 30 sec** – mainly anaerobic glycolysis
  glucose → 2 lactate + **2 ATP**

- **After 10 min** – aerobic oxidation of glucose
  glucose → 2 pyruvate → 2 acetyl-CoA → **38 ATP**

- **After 2 hours** – aerobic oxidation of FA
  stearic acid → 9 acetyl-CoA → **146 ATP**
  palmitic acid → 8 acetyl-CoA → **129 ATP**

Monday June 2, 13:00

Credit test (30 Q / 35 min)

- all seminar chapters
- all practical chapters
- reference values: **YES**
- calculations: **NO**

Limit for credit
12 / 30