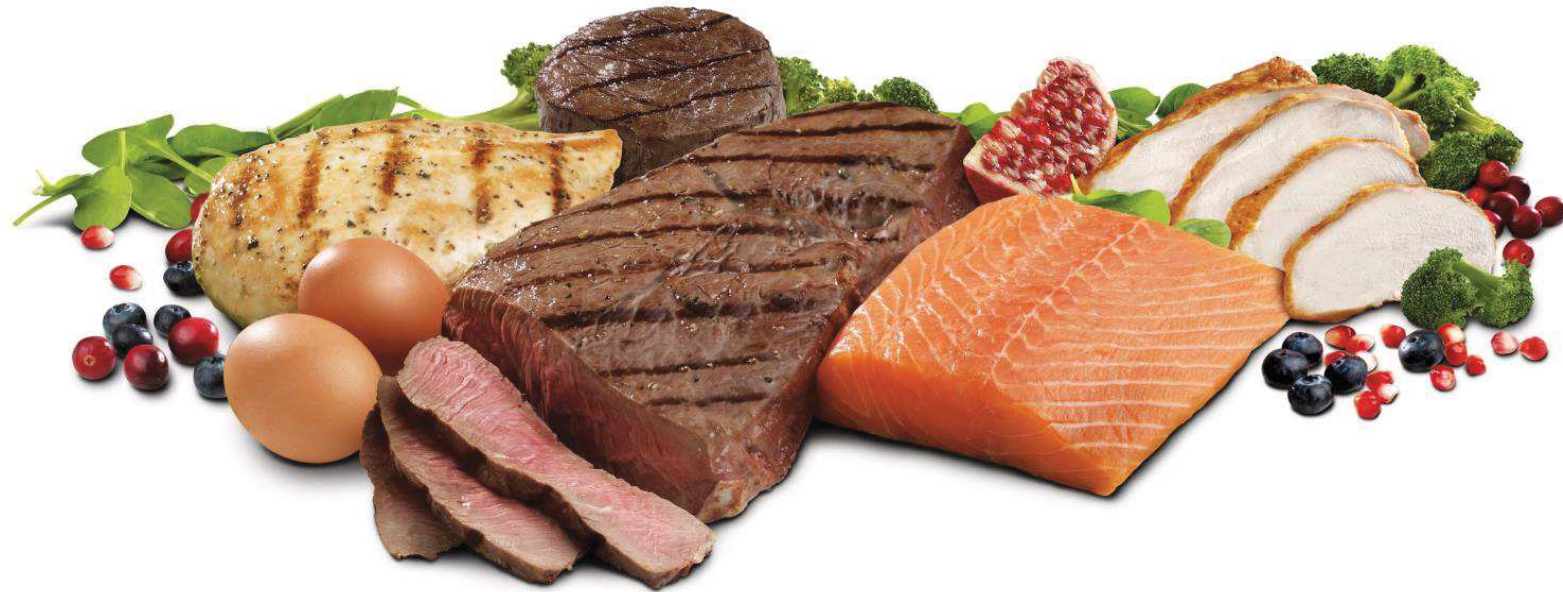
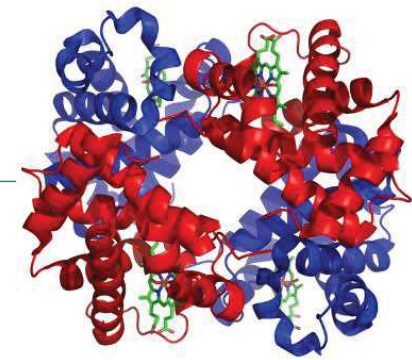


# Determination of total protein concentration in body fluids

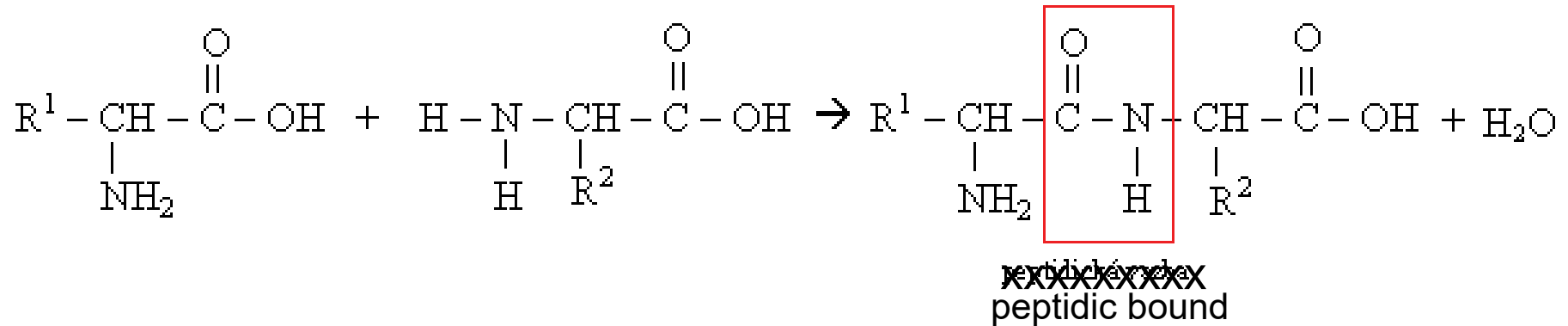


- proteins
- biuret reaction and its use in practice
- body fluids
- model organisms
- practical part: determination of protein concentration

# Proteins



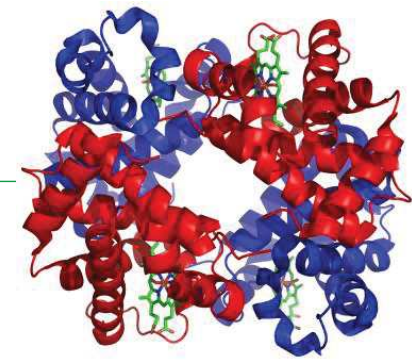
- Peptide-linked ~~AMKs~~ aminoacids (AMK)
- formation on ribosomes
- oligopeptides (2–10 AMK), polypeptides (11–50 / 100 AMK), own proteins (more than 50/100 AMK)



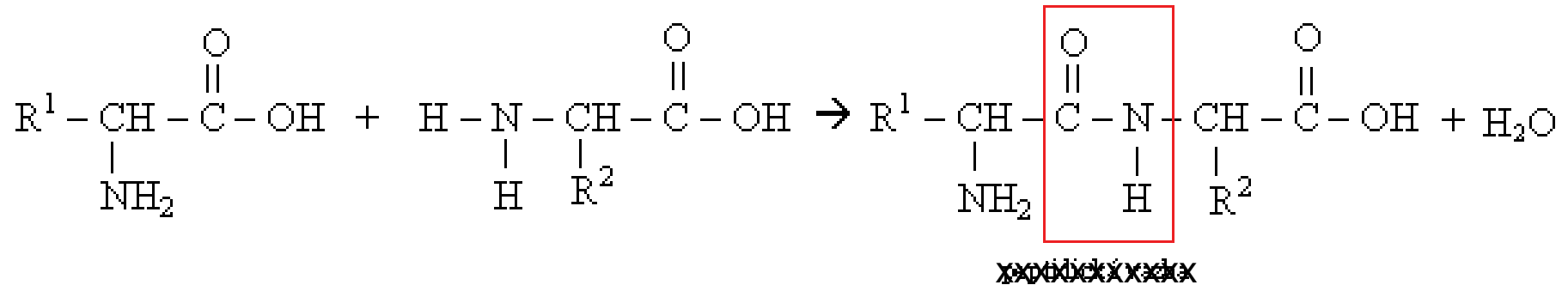
## General protein function:

- ~~xxxxxxxx~~  
building
- transport and storage
- ensuring movement
- catalytic, control and regulation
- protective and defensive

# Proteins



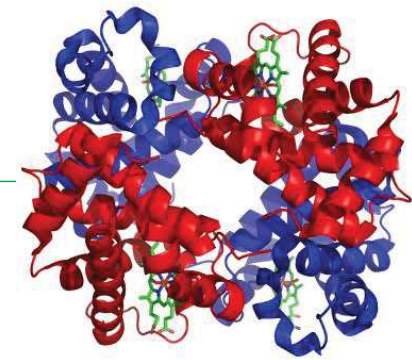
- ~~AMKs~~ Peptide-linked ~~AMKs~~
- formation on ribosomes
- oligopeptides (2–10 AMK), polypeptides (11–50 / 100 AMK), own proteins (more than 50/100 AMK)



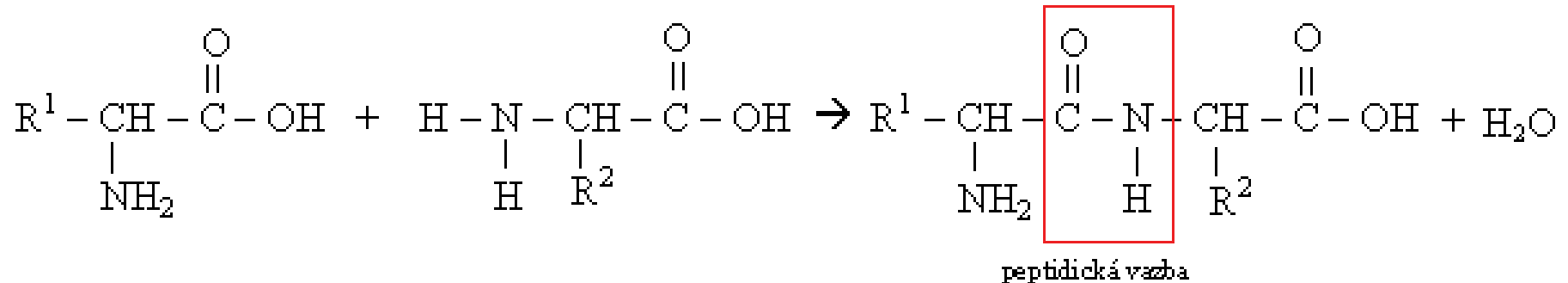
## General protein function:

- building (collagen, elastin, keratin...)
- transport and storage (hemoglobin, transferrin...)
- ensuring movement (actin, myosin...)
- catalytic, controlling and regulatory (enzymes, hormones, receptors)
- protective and defensive (immunoglobulin, fibrin, fibrinogen...)

# Proteins



- Peptide-linked AMKs
- formation on ribosomes
- oligopeptides (2–10 AMK), polypeptides (11–50 / 100 AMK), own proteins (more than 50/100 AMK)



## Plasma protein function:

- immune (globulins)
- hemostatic (fibrinogen)
- transport (albumins - non-polar fats, cholesterol, steroid hormones)
- oncotic pressure maintaining function (albumins)
- pH maintaining (buffering) functions
- control and catalytic (hormones, enzymes)

# Body fluids

---

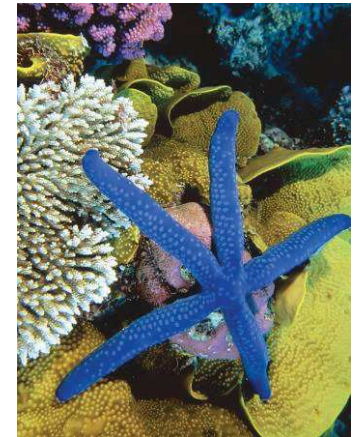
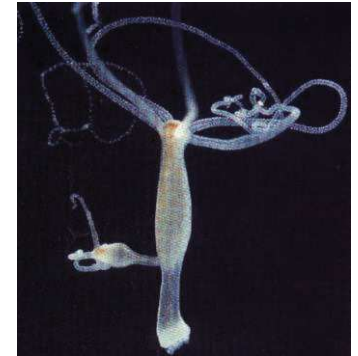
- transport, storage, support functions, etc.

## Hydrolymph (lychees, echinoderms)

- solution of salts, low protein and free cells

## Hemolymph (insects, crustaceans, molluscs)

- total protein about 6%
- dyes, free cells, proteins :
  - storage proteins (especially larval stages)
  - transport proteins (lipophorins, transferrin...)
  - hormones (adipokinetic, prothoracicotropic, bursikon...)
  - vitellogenins (female proteins that form the main part of the yolk sac)
  - immune proteins (lysozyme, coagulation proteins...)



# Body fluids

---

**Blood** (vertebrates) = plasma + blood elements

- transport of cholesterol, glucose, fats, ions (Fe, Cl and others)
- blood protein (total protein 6-8%):
  - albumins (60% plasma proteins) - bind water, transport of Cu, Zn, fatty acids, hormones
  - globulins (40% of plasma proteins) - bind fat, hormones, immune reactions (Ig)
  - fibrinogen , etc. (<1%)

**Tissue fluid** (extracellular fluid; without plasma proteins)

**Lymph** (from tissue fluid; immune and transport functions)

Amniotic fluid, cerebrospinal fluid, perilymph and endolymph in the ear, ventricular water and more.

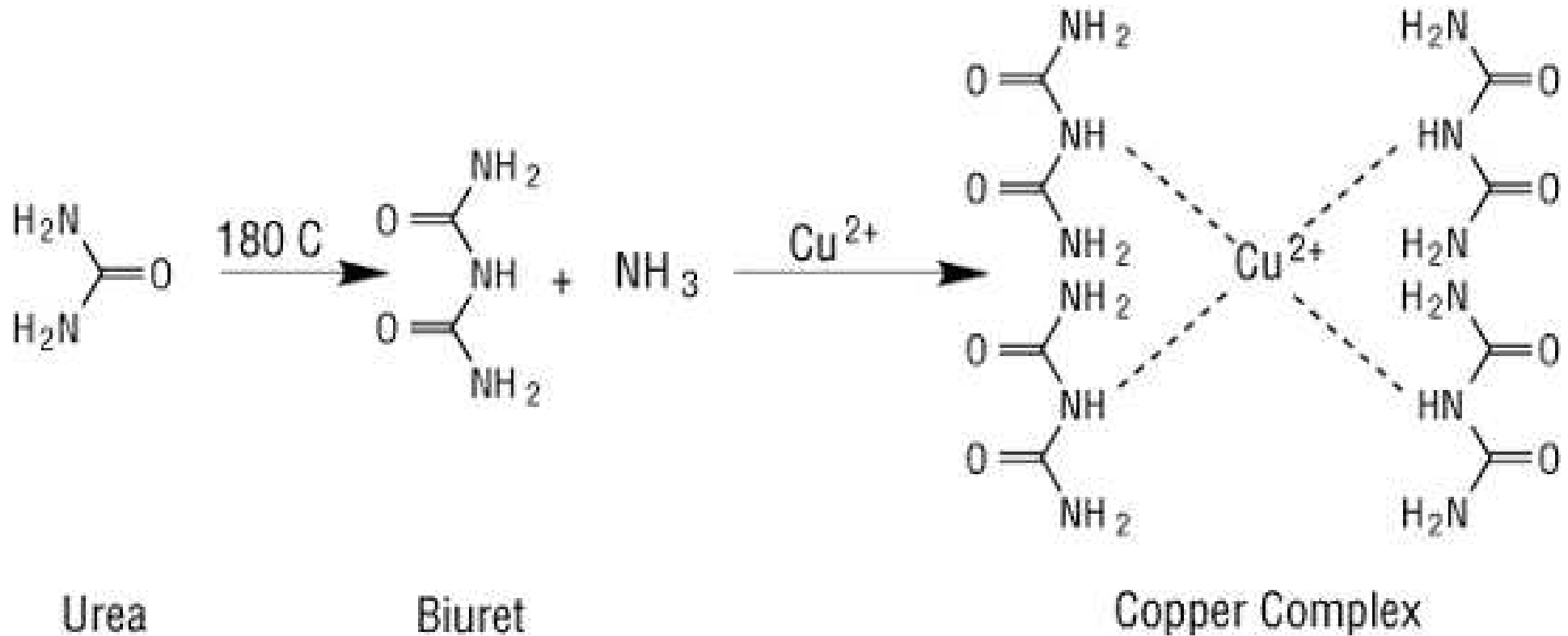
# Total protein in vertebrate plasma

	Total protein [g / l]		Total protein [g / l]
<del>zako</del> <del>zako</del>	35-45	caiman	47
Amazon	29-51	grass snake	43
ara	26-43	viper	55
parakeet	14-36	crocodile	65
hawk	24-31	varan	69
pigeon	15-35		
duck	35-45	<del>soot</del> cattle	65-80
		pig	65-85
sturgeon	45	horse	46-70
carp	41.5	cat	60-80
trout	34.6	dog	60-80

Note: Indicative values (reptiles, fish selected from specific studies, relatively small *n*).

# Determination of proteins - principle of biuret reaction

- in the alkaline environment, the protein chain forms a "matrix" around  $\text{Cu}^{2+}$  > purple color
- the intensity of the staining is proportional to the number of peptide bonds





# Protein determination - laboratory practice

---

- commercial kits, eg Bio-Rad Protein Assay:
  - modification to microtiter plate
  - 5  $\mu$ l sample / standard + 25  $\mu$ l reagent solution A + 200  $\mu$ l reagent solution B
  - measured at 700 nm after 15 minutes of incubation
  - calibration curve required



# Model organisms

## **silkworm - silk processing**

- before the butterfly hatches, it is necessary to dry the cocoons with pupae
- cooking cocoons
- fiber (fibroins)
- silk proteins (sericins)



# Model organisms



## silkworm - interesting facts

- monophagy
- sexual dimorphism (larger female, targets)



- hybrids - monovoltic species (~~monocotyledons~~) with a white cocoon
- - polyvoltine species having eg yellow cocoon (xanthophylls)
- the adult does not fly and does not eat
- bred in China (3000 BC); today SE Asia, Japan, Brazil
- fiber up to 1.5 km long
- 2 t leaves > 120 kg cocoons > 20 kg silk

# Model organisms

---

## house mouse (*Mus musculus*)

- traditional model organism
- blood (blood plasma / serum + blood cells)
- major proteins
  - 3-5% albumin
  - 1-3% globulin
  - 0.5% fibrinogen



domestic dog (*Canis lupus f. Familiaris*)

domestic cat (*Felis silvestris f. Catus*)

domestic tur (*Bos taurus*) common carp

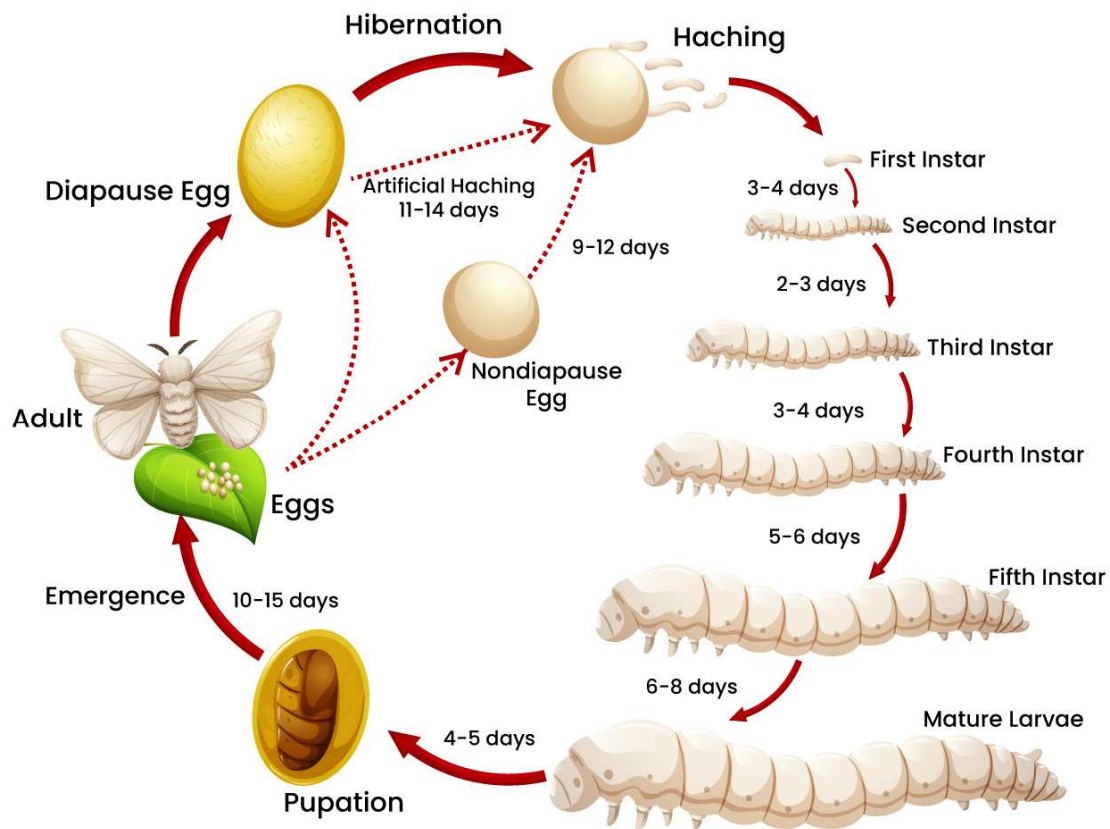
(*Cyprinus carpio*) tench (*Tinca tinca*)

crucian carp (*Carassius carassius*)



# Silkworm - *Bombyx mori*

- Silkworm (*Lepidoptera, Bombycidae*)
- Silk production



# Wax moth - *Galleria mellonella*

- Wax moth (*Lepidoptera*, *Pyralidae*, *Gallerinae*)
- Bee pests - feeds on wax, creates corridors in wax plates lined with fiber
- Excellent model organism - metamorphosis, immunity, metabolism, ...



	1	2	3	4	5	6
Biuret reagent	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Standard	20 µl					
Blank - H <sub>2</sub> O		20 µl				
Hemolymph BM			20 µl			
Hemolymph BM				20 µl		
FBS					20 µl	
Carp / linseed serum						20 µl
...						

Total protein (g / l) = a.



70 (protein concentration in the standard)

A<sub>1</sub> ... Absorbance of the sample

A<sub>2</sub> ... Absorbance of the standard