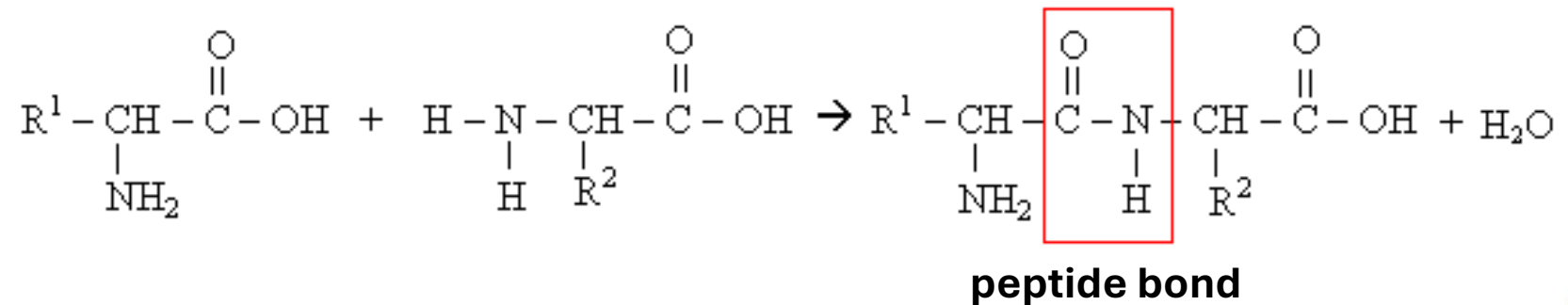


Determination of total protein concentration in body fluids

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

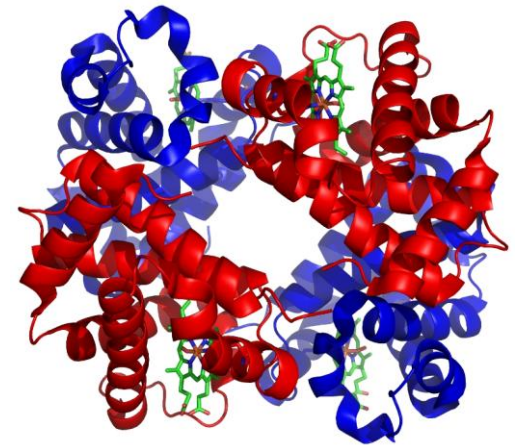
Proteins

- amino acids (AA) linked by peptide bond
- synthesis on ribosomes
- oligopeptides (2–10 AA), polypeptides (11–50/100 AA), proteins (more than 50/100 AA)



General function of proteins:

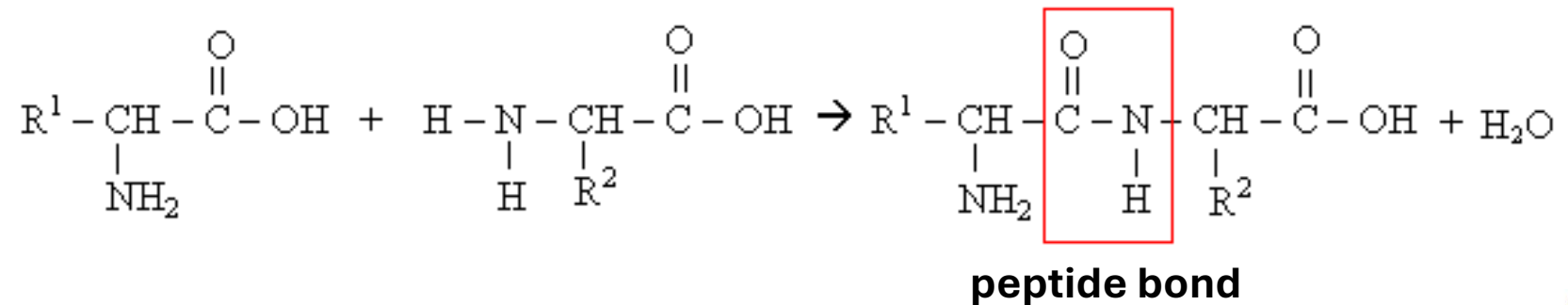
- structural
- transport and storage
- mediating movement
- catalytic, control and regulatory
- protective and defensive



wiki: hemoglobine

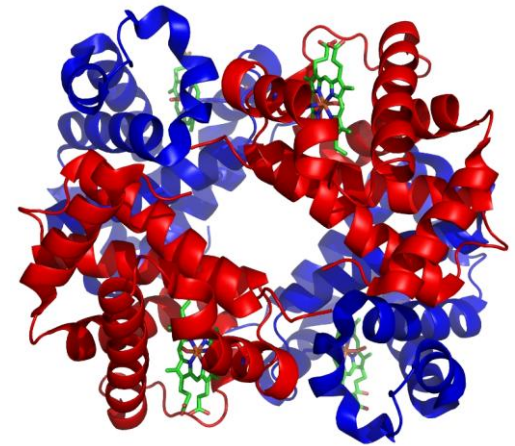
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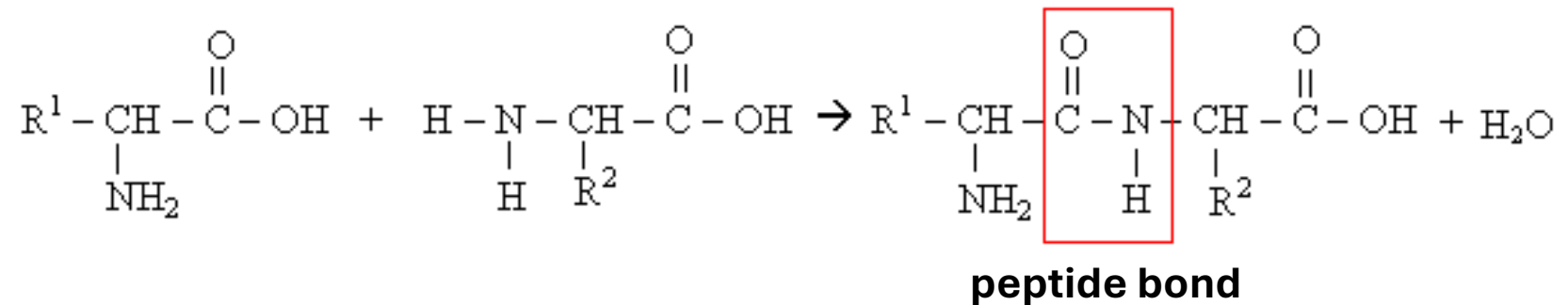
- structural (collagen, elastin, keratin...)
- transport and storage (hemoglobin, transferrin...)
- mediating movement (actin, myosin...)
- catalytic, control and regulatory (enzymes, hormones, receptors...)
- protective and defensive (immunoglobulin, fibrin, fibrinogen...)



wiki: hemoglobine

Proteins

- amino acids (AA) linked by peptide bond
- synthesis on ribosomes
- oligopeptides (2–10 AA), polypeptides (11–50/100 AA), proteins (more than 50/100 AA)



Function of plasma proteins:

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

Proteins in animal body fluids

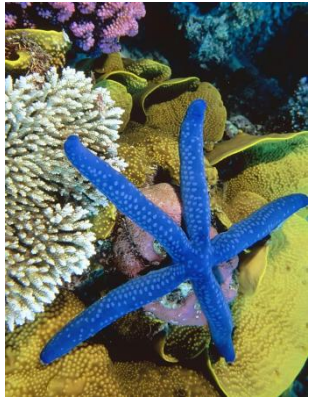
- transport, storage, support function, etc. (matching the function of proteins)

Hydrolymph (echinoderms)

- salt solution, few proteins and minimum of free cells

Hemolymph (insects, crustaceans, molluscs)

- open vascular system, hemolymph washes the organs
- population of free cells (hemocytes), total protein around 6 %
- various proteins:
 - storage proteins (vitellogenins = proteins that form the main component of yolk sac; more abundant in females compared to males)
 - transport proteins (lipophorins, transferrin etc.)
 - hormones (adipokinetic, prothoracicotropic, bursikon etc.)
 - immune proteins (lysozyme, coagulation proteins etc.)



Proteins in animal body fluids

Blood (vertebrates)

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

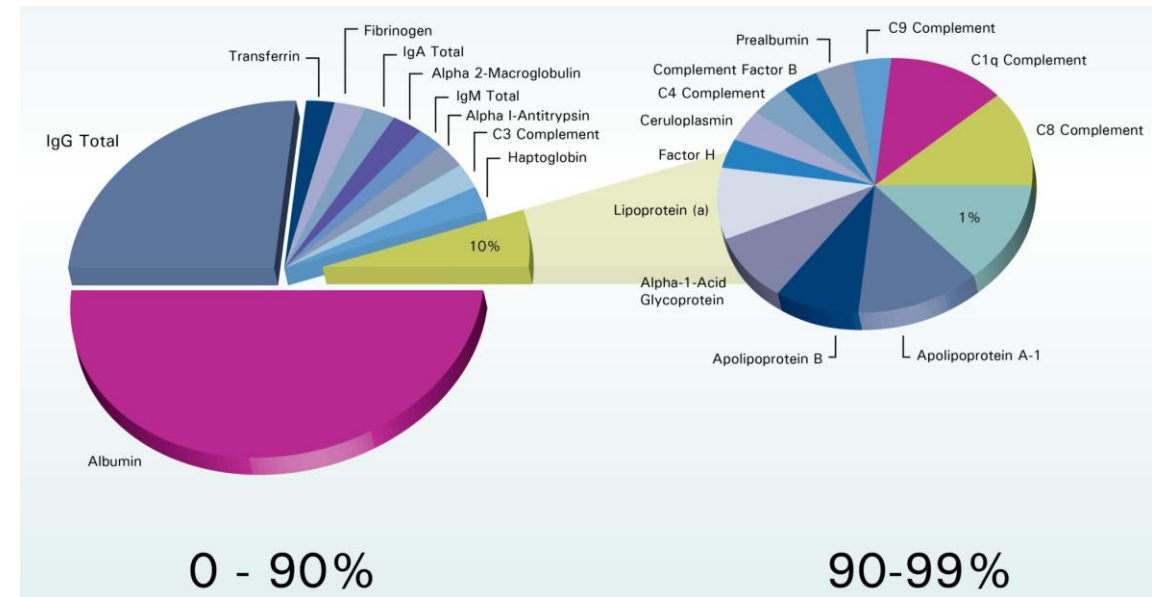
Tissue fluid

- extracellular fluid; free of plasma proteins

Lymph

- derived from tissue fluid
- immune and transport functions

Amniotic fluid, cerebrospinal fluid, perilymph and endolymph in the ear, aqueous humor in the eye and others.



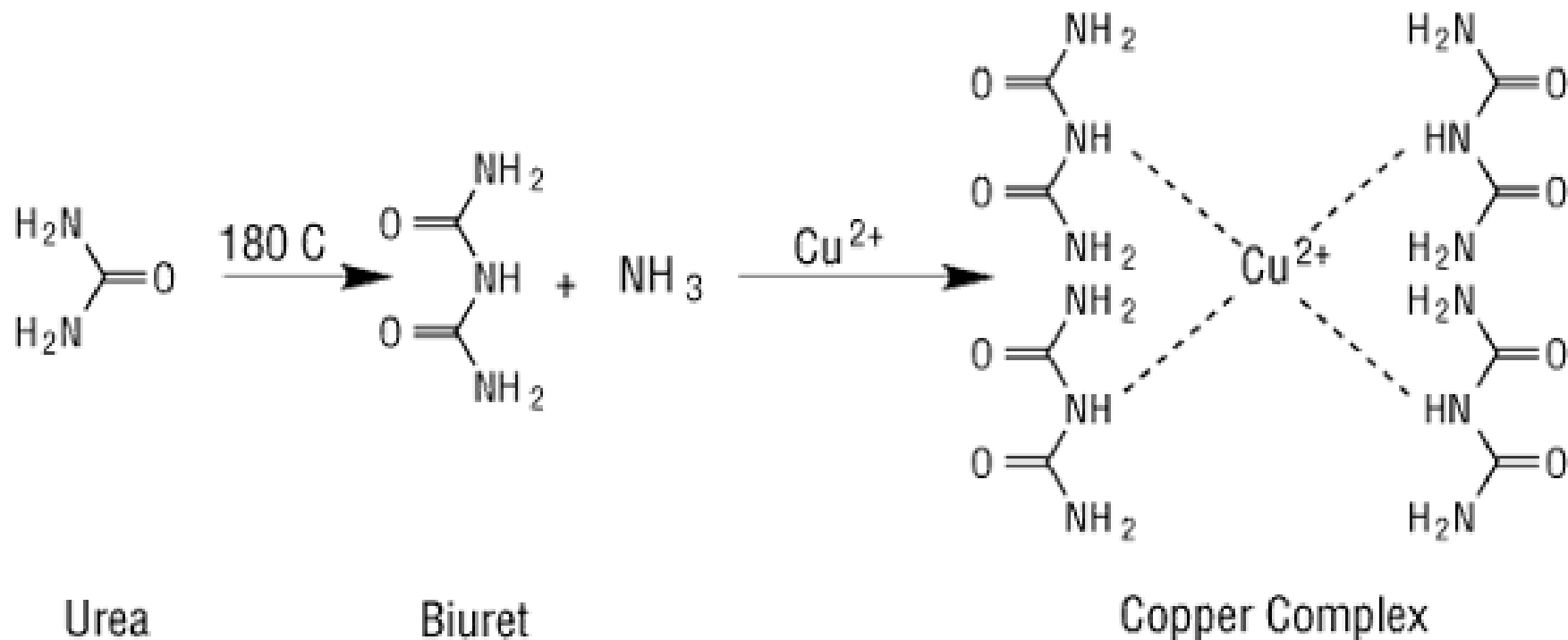
Total protein concentration in vertebrate plasma

- indicative values only (selected from specific studies; relatively small number of individuals measured etc.)

Species	Total protein [g/l]	Species	Total protein [g/l]
Grey parrot (<i>Psittacus erithacus</i>)	35-45	Cayman (<i>Caiman crocodilus</i>)	47
Cuban amazon (<i>Amazona leucocephala</i>)	29-51	Crocodile (<i>Crocodylus niloticus</i>)	65
Scarlet macaw (<i>Ara macao</i>)	26-43	Grass snake (<i>Natrix natrix</i>)	43
Parakeet (<i>Melopsittacus undulatus</i>)	14-36	Common European viper (<i>Vipera berus</i>)	55
Eurasian Goshawk (<i>Astur gentilis</i>)	24-31	Bengal monitor (<i>Varanus bengalensis</i>)	69
Pigeon (<i>Columba livia</i>)	15-35		
Wild duck (<i>Anas platyrhynchos</i>)	35-45	Cattle (<i>Bos taurus</i>)	65-80
		Pig (<i>Sus domesticus</i>)	65-85
Sturgeon (<i>Acipenser sturio</i>)	45	Horse (<i>Equus ferus caballus</i>)	46-70
Common carp (<i>Cyprinus carpio</i>)	41.5	Cat (<i>Felis catus</i>)	60-80
Rainbow trout (<i>Oncorhynchus mykiss</i>)	34.6	Dog (<i>Canis familiaris</i>)	60-80

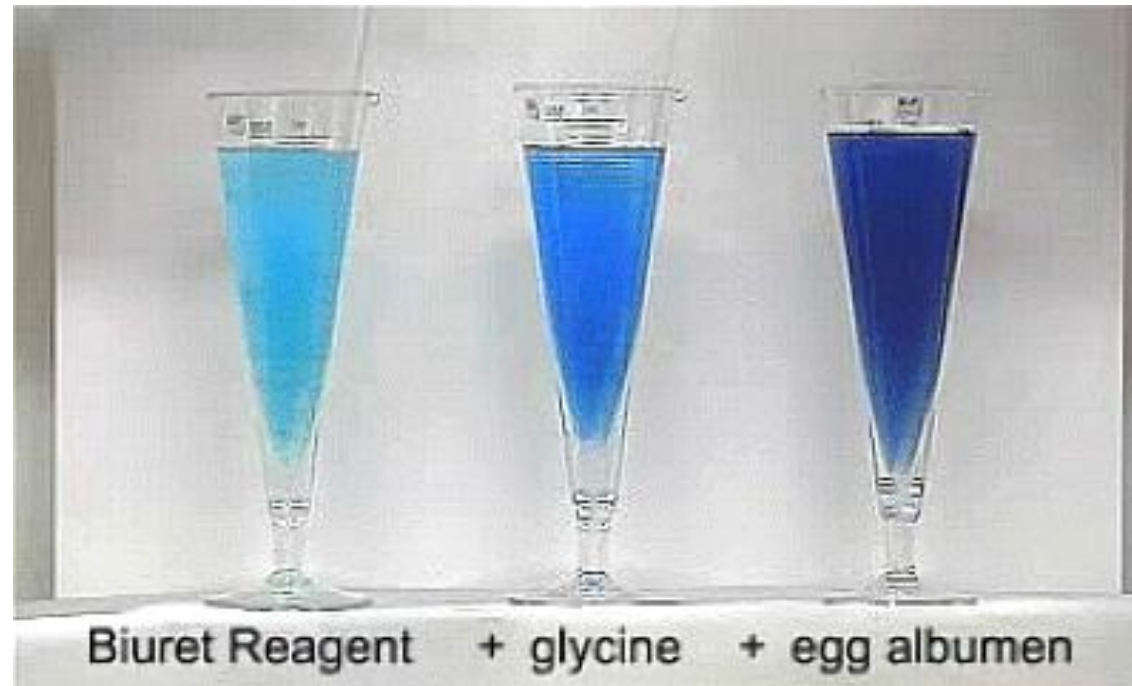
Protein determination – biuret test

- evidence of proteins in the sample
- reaction with peptides formed by at least three AA
- **biuret reagent:** 6mM CuSO_4
5M NaOH
21mM $\text{C}_4\text{H}_4\text{KNaO}_6$ (chelatone 3)



Protein determination – biuret test

- in an alkaline environment, peptide bonds form a characteristic blue/violet-colored complex with copper salts = biuret
- **the intensity of the color is proportional to the number of peptide bonds**
- determination of specific proteins (electrophoresis, western blot, mass spectrometry etc.)



Protein determination – lab practice

- sample limitations > scale-down
- commercial kits, e.g. Bio-Rad Protein Assay (Lowry method):
 - modification to microtiter plate
 - 5 μ l sample/standard + 25 μ l reagent A + 200 μ l reagent B
 - after 15 min incubation measured at 700nm
 - calibration curve required



Practical part

- **1 ml biuret reagent + 20 µl blank/standard/sample** > mix
 - **blank** (water) to eliminate the absorbance of biuret reagent itself
 - **standard** (commercial human serum with total protein concentration 70 g/l) to calculate total protein of other samples
 - **samples:** hemolymph of silkworms (female, male), serum or plasma of vertebrates (eg. mouse, cattle, dog, cat, carp, crucian carp, or others)
- **10-12 min incubation** > visual assessment
- spectrophotometric **absorbance measurement at 544nm**
- Lambert–Beer's law: the concentration of a substance in a sample corresponds to its absorbance (directly proportional)

Total protein concentration in the measured sample → $c \text{ (g/l)} = c_s \times \frac{A}{A_s}$

Protein concentration of the standard (70 g/l) ↑

Sample absorbance ↙ A

↘ A_s Absorbance of the standard

Practical part – What to pipette?

- name tubes > pipette according to example in the table:

Tube name	standard	blank	BM♂	BM♀	FBS	fish
Biuret reagent	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Standard	20 µl					
Blank (water)		20 µl				
Silkworm hemolymph – male			20 µl			
Silkworm hemolymph – female				20 µl		
Fetal bovine serum (FBS)					20 µl	
Fish serum/plasma						20 µl
...as well as other samples						