PHYSIOLOGY OF THE BLOOD

TOTAL BODY WATER

Males: 60 %
Females: 50 %

Extracellular fluid (ECF) – 20 %, 14 litres
1) intravascular (blood-plasma 5%, lymph)
2) extravascular – intersticial 15 %

Intracellular fluid (ICF) – 40 %, 28 l

BLOOD VOLUME

Adults: 7% of b.w.
- Males**:** 7.7 % b.w., 5.5 l; 70 ml/kg
- Females**:** 6.5-7 % (more fat)

Normovolemia

Hypovolemia:
- proportional
- hemoconcentration
- hemodilution

Hypervolemia

Measurement – methods: direct and indirect (radioisotopes, dyes)

BIOPHYSICAL CHARACTERISTICS OF THE BLOOD

1) Specific gravity
Blood - 1056 (152-1063) kg/m^3
- plasma 1027
- red blood cells 1090

2) Viscosity
- water = 1; blood 4-5.4x more
Syndrome of hyperviscosity – impairment of microcirculation, hypoxia,...

3) Hematocrit
- the percentage of red blood cells in the whole blood

Males: 0.44±0.05 (44±5 %)
Females: 0.39±0.04 (39±4 %)
Newborns: 50-60 %

ERYTHROCYTE SEDIMENTATION RATE (ESR)

Blood - suspension ⇒ electrical bilayer: RBC – negative charges
plasma proteins – positive charges

Factors influencing ESR

1) Plasma proteins
2) RBC count and size
3) Lipidemia
4) pH of plasma

Values: M: 2-5 mm/h
F: 3-8 mm/h (less RBC, more fibrinogen)

Determination: Fahraeus-Westergreen method (FW) - see practicals

Acceleration of ESR: physiological (gravidity, menstruation)
pathological (infect.diseases, tumors, liver diseases,...)

RED BLOOD CELLS (RBC)

- non-nucleated cells
- biconcave discs, diameter 7.2 μm, thickness 2.1 μm, volume 85 fl, surface 130 μm^2; surface of all RBC 50x60 m

RBC count
M: 4.3 – 5.3 x 10^{12}/l
F: 3.8 – 4.8 x 10^{12}/l
Newborns: 7-8 x 10^{12}/l

Hypererythrocytosis (polycytemia, polyglobulia) ↑ count
– physical activity, high altitude, hemoconcentration

Erythrocytopenia (anemia) ↓ cout: physiol.(sucklings), pathologic

Structure
- water –70% and dry subst.30%
membrane, stroma, hemoglobin
membrane: lipid bilayer with proteins (peripheral, integrat.)
other substances: hemoglobin, ions (K, Na, Ca), enzymes (40), glutathion, ...

Metabolism: RBCs have low metabolic needs
1) Embden-Mayerhof pathway (anaerobic - 90 %)
2) Hexose-monophosphate shunt (aerobic - 10%)

HEMOGLOBIN

Molecular structure: tetramer
M.W.: 64 kDa

Heme: ferrous protoporphyrin (Fe^{2+})
Globin: 4 polypeptide chains (HbA: 2 alpha + 2 beta)

Quantity of Hb
M: 135-170 g/l
F: 120-160 g/l
Newborns: 190, sucklings:110

Each gram of Hb can carry 1.34 ml O_2 $\Rightarrow$ one litre of blood cca 200 ml O_2

Measurement of Hb concentration: *spectrophotometry* – see practicals

Hemoglobin derivates

Physiological
1) OxyHb ($+O_2$)
2) DeoxyHb ($-O_2$)
3) CarbaminoHb ($+CO_2$)

Pathological
1) CarboxyHb (+CO)
2) MetHb (+OH): $Fe^{2+} \rightarrow Fe^{3+}$

Types of hemoglobin

1) Embryonic
   a) Gower I (2 zeta, 2 epsilon)
   b) Gower II (2 alpha, 2 epsilon)
   c) Portland (2 zeta, 2 gamma)

2) Fetal (2 alpha, 2 gamma)

3) Adult
   a) HbA (2 alpha, 2 beta)
   b) HbA_2 – minor component (2 alpha, 2 delta) – up to 2 % - in adults

HEMOLYSIS

Destruction of the RBC membrane and release of Hb
1) Osmotic (hypotonic, hypertonic)
2) Physical (temperature, mechanical, radiation)
3) Chemical (saponin-see practicals)
4) Toxic (cobra venom – hemolysis)
5) Immunologic (mismatched transfusion)
6) Hereditary (deficiency of G-6PD)

BLOOD GROUPS

System AB0

Agglutinogens A, B – on membrane of RBC

A – acetyl-galactosamine
B – D-galactose
H – acetyl-glucosamine – maternal substance

6 blood types: A_1, A_2, B, A_1B, A_2B, 0(H)

Agglutinins anti-A and anti-B

Blood group	Agglutinogen (RBC)	Agglutinin (plasma)
0	0(H)	anti-A, anti-B
A	A	anti-B
B	B	anti-A
AB	A,B	no agglutinins

Other agglutinogens:
Antigens M,N
M(33%), N(15%), MN(52%)
- agglutinins anti-M, anti-N never occur spontaneously

S (56%), s(44%)

P. Lewis, Lutheran, Wright – familiar occurence

The Rh system

Antigens: C, **D**, E, c, d, e
Agglutinins in Rh system spontaneously never occur !

Formation of anti-Rh agglutinins after immunization:
1) transfusion of Rh^+ blood to Rh^- person
2) Rh^- mother has Rh^+ fetus

Effect of mothers antibodies on the fetus:
- agglutination of RBC
- occlusion of small vessels
- hemolysis of RBC
- hyperbilirubinemia

Clinical picture in newborn:
- jaundice, erythroblastosis, anemia, hepatoslenomegalia, hypoxia, edema, brain damage (bilirubin in CNS in basal ganglia – kernicterus)

Prevention:
to destroy Rh^+ RBC before they inniciate antibodies production
$\Rightarrow$ injection of anti-Rh agglutinins

Therapy:
- to decrease bilirubin level: exchange transfusion, phototherapy

Incompatibility in AB0 system
- already 1.child can be affected
- not serioously since A,B are weak antigens in newborns

TRANSFUSION

- blood, plasma, RBC, WBC, platelets, plasma clotting factors, ...

Indications: ↓ in blood volume
anemia
thrombocytopenia
hemophilia

Compatible transfusion ⇒ only the same group (A,B,0 and Rh)
Universal donor (0) vs.universal recipient (AB) (? – revisited)

Examination of blood prior transfusion
1) to determine blood groups - slide technique
2) cross-matching – RBC from donor + serum of recipient and vice versa
3) to determine blood groups „at the bed" – slide technique, determination of compatibility
4) biological experiment

Complications of mismatched transfusion

Transfusion reactions:
1) agglutination
2) hemolysis

Acute kidney failure:
1) renal vasoconstriction
2) obstruction of renal tubules by Hb

Pyrogenic reactions

Treatment:
- stimulation of diuresis
- alkalinization

Transfusion reactions resulting from anticoagulants:
- if anticoagulant combines with Ca^{2+} ⇒ hypocalcemia

WHITE BLOOD CELLS (WBC) – LEUKOCYTES

- true cells (nucleus + organels; active metabolism)

COUNT

- adults: 4-10 x 10^9/l of blood (no gender differences)
- newborns: 15-17 x 10^9/l

- **diurnal rhythm** ⇒ morning – decreased count

Changes in count:

1) ↑ count – leukocytosis
a) physiological (after food intake, physical activity, gravidity, ...)
b) pathological (inflammation)

2) ↓ count – leukopenia
a) physiological (fasting, cold environment)
b) pathological (depression of bone marrow)

Leukemia: overproduction of WBC – immature forms

Differential WBC count – characteristics, numbers and functions of different WBC classes - in details -see practicals !

A) **GRANULOCYTES**
(polymorphonuclear cells) – specific granules in cytoplasm

1) **Neutrophils** – 56-64%; 1-5 nuclei; Arneth, Hynek ⇒ age of Neu
- circulating
- marginating
- tissue compartment
- reserve (bone marrow)

2) **Eosinophils** – 1-3%, ↑ in allergic and parasitic diseases

3) **Basophils** – 0,5-1%, heparin, histamin

B) **AGRANULOCYTES**
(without specific granules)

a) lymphocytes (T, B, NK)
b) monocytes (histiocytes – in tissues) (see Immunophysiology)

PROPERTIES OF WBC

1) **Ameboid motion** - movememt in tissues
2) **Chemotaxis** – response to chemical substances
3) **Tigmotaxis** – ability to adhere at the surface of foreign bodies

4) **Diapedesis** – ability to pass through the capillary wall
5) **Phagocytosis** – destruction of foreign particles

before phagocytosis:
- **opsonization:** opsonins on the surface of the „target" (bacteria)
- **adherence:** at the surface by means of pseudopodia

PHAGOCYTOSIS ⇒ phagosome, phagolysosome, digestion – enzymes

Capacity to phagocyte: Microphages – Neu (5-20), Eo (Ag-Ab)
Macrophages – Mono (100)

OTHER FUNCTIONS OF WBC

1) in immune mechanisms:
a) Eo – in allergic reactions
b) Baso – histamin – immediate-type of hyperresponsivness
c) Lymphocytes – see Basics in Immunophysiology
d) Monocytes – macrophages, secretory function

2) in blood coagulation
- basophils – heparin
- eosinophils – active in fibrinolysis (profibrinolysin)

P L A T E L E T S

= round/oval disc, diameter 2-4 _m/, thickness 0.5 – 1 _m, volume 4-8 fl (1/10 of ery).
Nonnucleated cells, granulated cytoplasm

They contain: - actin –- myosin – similar to those in muscle cells – contraction of the platelets
- ATP, ADP (on surface)
- enzyme system for prostaglandins (thromboxane A2)
- fibrin – stabillizing factor
- serotonin (from GIT)
- platelet – derived growth factor
- platelet – clotting factors

Platelet – Clotting Factors:
N.l = plasmatic f.5 = Proaccelarin – acts on
Prothrombin → Thrombin
N.2 = beta-thromboglobulin - Fibrinogen→ Fibrin
N.3 = Phospholipid – thromboplastic f. – Prothrombin →
- Thrombin
N.4 = anti-heparin factor
N.5 = platelet fibrinogen

Half-life: 8 – 12 days

Count – 150 – 300 x 109/l = 150 – 300 000/_l
2/3 in circulating blood, 1/3 in the spleen

Decreased count = **thrombocytopenia**,
Increased count = **thrombocytosis**

Platelets counting
Methods – direct – Procain sol. – Burker´s chamber - indirect – blood film – stain Brilant – Krezyl blue, count Tr/1000 Ery (recalculation to volume by erythrocyte count)

ROLES OF PLATELETS in:

1) Hemostasis
a) mechanical
b) humoral

2) Wound healing
Platelet – derived growth f. (PDGF) – polypeptide (+ macrophages and endothelial cells) – mitogen for vascular smooth muscle.
Role in atherosclerosis – abnormal grow of endothelial cells – narrowing of the vessels

3) Inflammation – changes in capillary permeability (histamin)

HEMOSTASIS

Reactions to stop bleeding:
1) contraction of the injured vessels
2) Accumulation of platelets in the lesion Hemostasis
3) Hemocoagulation
4) Activation of fibrinolysis

1) Vascular Constriction occurs immediately after injury

a) from direct effect of the injury upon vascular smooth muscle cells (sec)
b) reflex vasoconstriction – sympathicus (min)
c) humoral (serotonin fibrinopeptides, thromboxan A2 – from platelets) – 0.5 h,
2) Accumulation of platelets - formation of a temporary hemostatic plug.

Disruption of the endothelial cells lining the vessel brings platelets into contact with tissues. Platelets adhere to – the subendothelial tissues - each other to form the platelet hemostatic plug

a) Platelets Adhesion
Initial step in the formation of the plugs is adhesion of platelets to the disrupted subendothelium – on the collagen fibers in the vascular wall. The release of platelets granules containing multiple active factors. Platelets stick to the collagen fibers – by means of ADP, von Willebrand factor (vWF, VIII.) – through GPIb receptors (glycoprotein) – latter through GPIIb and III. a receptors.

b) Platelets Activation
After adhesion, platelets become activated.
Stimuli for platelets activation: - thrombin membrane
- collagen receptors

through messengers (diacylglycerol, inositol TP):
- Activation of myosin – platelet shape change, secretion, contraction
- Activation of protease called calpain – activates platelet enzymes
- Activation of phospholipase A2 – liberates arachidonic acid

c) Platelets Aggregation
Though binding to fibrinogen receptors (GP II b, III a), fibrinogen forms „bridges“ between platelets – primary phase of platelet aggregation – reversible.
Secondary – irreversible phase- connection by means of thrombospondin (alpha granule protein) – reinforces the fibrinogen „glue“ of aggregation.
Activation and aggregation of successively increasing numbers of platelets that themselves attract more and more additional platelets – thus forming a platelet plug. If the vascular injury is small, plug by itself can stop blood loss completly.

Pathology – hereditary disorder – thrombastenia – Patients cannot form functional GP II b, II b, III a receptors – their platelets fail to aggregate – the serious bleeding from mild injury.

BLOOD COAGULATION
Conversion of the soluble plasma plasma protein fibrinogen to insoluble fibrin.

Over 50 important substances affect blood coagulation
- promoting coagulations = procoagulants
- inhibiting coagulation = anticoagulants

Procoagulants:
1) Tissue thromboplastin
2) Plasma factors

<u>Plasma coagulation factors</u>
I. Fibrinogen – plasma protein, m.w. 340 000, liver, 3 g/l
II. Prothrombin – alpha 2 globulin, liver, vit. K dependent
III. Thromboplastin – membrane of endothelial cells ...
IV. Ca2+
V. Proaccelerin, labile factor, liver
VI. 0
VII. Proconvertin, stable factor, liver, vit. K dep.
VIII. Antihemophilic factor, globulin (AHG) – f. VIII. c + f.vW
IX. Plasma thromboplastin component (PTC), Christmas f. antihemophilic f.B, alpha globulin, liver, vit. K dep.
X. Stuart –Prower f., liver, vit. K dep.
XI. Plasma thromboplastin antecedent (PTA), antihemphilic f.C, Rosenthal f., liver
XII. Hageman f., glass f., liver
XIII. Fibrin – stabilizing factor, Laki – Lorand f.

Prekallikrein – Fletcher f.
HMW kininogen – Fitzgerald f.
Blood Coagulation Reaction

Three steps:

1) Reactions resulting in the generation of activator of prothrombin
2) The prothrombin activator cleaves prothrombin to form thrombin
3) Reactions of thrombin with fibrinogen and f. XIII lead to the deposition of cross-linked polymers of fibrin.

Anticoagulants

I. Intravascular Anticoagulants

1) Endothelial Surface Factors – the most important factors for preventing clotting in the normal vascular system – smoothness of the endothelium („nonwettable" – like siliconized surface) + severalproteins bound to the endothelial cells.
2) Blood flow – continual flow – without an accumulation of clotting factors.
3) The antithrombin action of thrombin and fibrin; negative feed-back-thrombin becomes adsorbed to the prothrombin and fibrin acts as antithrombin.
4) Antithrombin III – cofactor of heparin – plasma f., liver, inactives f. IIa, IXa, Xa, XIIa, kallikrein
5) Heparin – polysaccharide-basophils, mast cells, concentration 5 mg/l of blood. It combines with antithrombin III and increases as much as a hundred – to a thousandfold the effectiveness of antithrombin

II. Anticoagulants for Clinical Use
1) Siliconized surface – „nonwettable" – of tubes, containers for blood, test – tubes ...
2) Decalcification – clotting can be prevented in vitro if Ca2+ is removed from the blood by the addition of substances such as oxalates
3) Heparin – effective with antithrombin III.
4) Coumarin derivates - dicumarol, warfarin – antivitamin K – decrease the plasma level of f. II, VII, IX, X.
5) Hirudin – substance from Hirudo medicinalis (leech)

FIBRINOLYSIS

After the clot has stopped the bleeding – it is necessary to lyse fibrin = process – fibrinolysis.

The active component of the fibrinolytic system = PLASMIN (fibrinolysin).
The plasma proteins contain a globulin called PLASMINOGEN (profibrinolysin), which when activated, becomes a substance called plasmin = proteolytic enzym.
It digests the fibrin threads.

Plasminogen Activators
Thrombin
Tissue plasminogen activator (tPA) - also for clinical use
Kallikrein
HMW kininogen
Streptokinase
Urokinase
Plasmin Inhibitors

Alpha 2 antiplasmin (made by the liver) – inactivates plasmin by combining with lysine and serine binding sites.

TESTS OF HEMOSTASIS AND HEMOCOAGULATION

1) The bleeding time – measures the time it takes to form platelet plugs that stop bleeding (screening test)
2) The coagulation time – the time needed for the coagulation of the blood in test-tube
3) The prothrombin time – measures the adequacy of the reactions with a high concentration of tissue factor (Quick´s test)

HEMATOPOESIS

- production of the blood cells

Intensity of hematopoiesis:
•RBC: 3.5x1011 /day
•Neutrophils: 1011 /day
•Monocytes: 8.4x109 /day
•Trombocytes: 1011 /day

Location of hematopoiesis
- ontogeny

Periods:
- mesoblastic
- hepatic
- myeloid

Embryo – 19th day - 6th week: blood islands in the yolk sac
– mesoblastic period from 6th week – liver
From 3th-6th mo: main hemopoietic organ – hepatic period

Fetus – from 12th week: + spleen 5th month - + bone marrow - later the main hemopoietic organ myeloid period

Newborns, children – bone marrow of all bones

From 20th year – mostly in humerus, femur, pelvis, sternum (flat bones)....

Bone marrow – cca 3.5 kg
– 60 - 75% myeloid cells
- 25% erythroid cells
- 10% lymphoid cells

Erythropoesis

Pluripotential stem cell
Multipotential stem cell (for RBC, granulocytes, monocytes, megakaryocytes)
Unipotential stem cell (erythrocyte colony forming cell)
Proerythroblast (15-20 μm)
Basophilic normoblast (1.x Hb)

Polychromatic n.
Ortochromatic n. (nucleus ex)
Reticulocyte
Erythrocyte (RBC)

Requirements for erythropoesis

Iron - 10-20 mg/day is needed (only 1 mg resorbed)
in food: Fe3+(ferric form)• in stomach: HCl ..... Fe2+(ferrous form)• in cells: Fe + appoferitin – ferritin (ferric form) ...storage• in plasma: Fe + beta 1 glob. = transferin → erythroblast
• *iron defficiency = hypochromic anemia*•••

Vit. B12, B6, follic acid- deficiency: pernicious anemia

Copper (in plasma bound on ceruloplasmin) - in mobilisation of Fe from ferritin,

Cobalt (part of the B12 mollecule)

AA, proteins, pyrroles, etc....

Regulation of erythropoesis
- Neural - hypothalamus
- Humoral - specific – erythropoietin (kidneys)
- Nonospecific - + androgens, thyroxin, GH, corticoids, - estrogens

Regulation of leucopoesis
- Nonspecific – bact. endotoxins – directly of through GM CSF
- Specific - Colony stimulating factors CSF
  - GM CSF – granulopoietin (Mo, endothelial cells, fibroblasts, T-ly) - stimulation of granulocytes and monocytes formationG - CSF (Mo, endot., fibro)
  M - CSF (Mo,endot.,fibro,T-ly)
  Multi-CSF = IL 3 (T ly)

Immunohormons: thymus: thymosins, thymopoietins; IL - 2, IL - 4, IL – 5, IL – 9...

Thrombopoesis
Formation:
- stem cells.
- megakaryoblast
- promegakaryocyte
- megakaryocyte
- thrombocyte (platelet)

Regulation of thrombopoesis
Feedback (through metabolites and degradation products)
Thrombocytopoietin (kidneys) – mostly formation of megakaryocytes,
Factors actively stimulating colonies of megakaryocytes (Meg-CSF) and IL – 3

ONTOGENY OF /HYSIOLOGY OF BLOOD

Body fluid

Total body water:
- fetus 90%
- newborn 78 – 86 %
- young adult 55 – 60 %
- older adults 50 – 55 % body mass

ECF : ICF -fetus - mostly ECF
- in 1st m - ECF = ICF
- from 4th m - mostly ICF (20:40)

2. Blood volume
Fetus - 125 ml/kg
Newborn - 75 – 100 ml/kg
Adults - 70 ml/kg

3. Biophysical characteristics of blood
a) Density (specific mass/gravity): – newborns - 1060 – 1080 kg/m^3
- sucklings - 1050 – 1055 kg/ m^3
- adults - 1052 – 1063 kg/ m^3

b) Viscosity – exponential dependency on hematocrit value
- rapid increase over 0.5 – 0.6
- normal hematocrit for adults 0.4, in newborns 0.5 (bigger RBC)

4. Localization of hematopoesis
see Hematopoiesis..

5. Hemoglobins
- Gower I, II, Portland
- HbF
- HbA - from 34th gestational week - newborns 60-80 % HbF
- 4th mo. 10-15 % HbF
- 1st yr. < 2%
- Adult < 0,5 %

6. RBC
- fetus - 3-5- times higher production rate, shorter life (60-90 days)
- newborns - RBC - larger: V = cca 110 fl
small "craters" (0,2 – 0,5 Gm) on their surface, but immature spleen do not destroy them
Higher consumption of glucose, ATP and higher fragility

7.WBC
Fetus - WBC count – relatively low (4 x 109/l – in 22th week)
Newborn – shortly after delivery 15 – 17 x 109/l – release of marginating leucocytes

- decrease of neutrophiles percentage – crossover with lymphocytes on 5th day. Up to 5th year of life - lymphocytes dominate, then 2nd crossover

Neutrophils: lower activity in vivo
Monocytes: after delivery increase (during first 12 hr) – later decrease
Eosinophils: mostly in premature newborns
Basophils: = as adults
Lymphocytes - increase in T-ly percentage with age

8.Platelets
Fetus - very early - from mesoblastic period
Newborn - normal - count similar to adults
Premature newborns - lower count - normal size and structure, but lower tendency to aggregate (lower concentration of thomboxanu A2)

9. Hemostasis

Embryonal blood (till 10th week) - inability to coagulate
-Since 3rd trimester – normal (adult-like) concentration of plasmatic factors of coagulation I, V, VIII.
Maternal plasmatic factors do not pass through placentar barrier-congenital defficiency of some factorcan be ascertained from blood taken from umbilical cord-

- Newborn – lower level of plasmatic factors that are dependent on vitamine K (II, VII, IX, X – only 50 % of adult concentrations)
  Vit. K defficiency ← because: low pass through placenta, low concentration in milk
  However, an ability to coagulate blood is good in newborns, owing to a big functional Reserve.

10. Fibrinolytic system
– in blood from umbilical cord- higher fibrinolytic activity from plasminogen activation in the walls of umbilical vessels

Physiology of blood in senescence

Total Body Water: decreased – mostly ECF
Blood volume: mild decrease
Blood viscosity: without changes
Plasmatic proteins: decrease in albumin and agglutinin concentrations
RBC: mild increase in volume, lower elasticity
Hemoglobin:mild decrease of concentration, lower level of 2,3 DPG (worse desaturation)
ESR: increased
WBC: mild decrease compensated by lymphocytes (T) and monocytes; Ne: older forms
Platelets: normal count, lower adhesion, higher aggregation ability
Haemocoagulation: normal
Immunity: lower T-ly related activity; propensity for infective, autoimmune, malignant

PHYSIOLOGY OF THE IMMUNE SYSTEM

Immunity – body resistance to
- pathogenic organisms
- damaged (changed own cells)

Immunity
Active:
- natural
- artificial

Passive:
- natural
- artificial

IMMUNE RESPONSES

- nonspecific (innate)
- specific (acquired)

NONSPECIFIC IMMUNE MECHANISMS:
- Physical (skin, mucosa)
- Chemical (HCl)
- Phagocytosis
- Inflammation

SPECIFIC IMMUNE MECHANISMS (lymphocytes):
- Cellular
- Humoral

LYMPHOCYTES

- T – cells (70 – 80 %)
- B- cells (20-30%)

From bone marrow

T-CELLS = thymus dependent cells – differentiation in the thymus gland

Main populations:
- Helper T-cells
- Supressor T- cells
- Cytotoxic T – cells
- Memory T-cells

Responsibility for th CELL´S IMMUNITY

B-CELLS – activation – Plasma cells – producers of antibodies – **immunoglobulins (Ig)**

Classes of Ig

IgG stimulates phagocytosis, complement reactions. It can cross via placenta.	Passive immunity in newborns Identification of microbes for phagocytosis.
IgA - in secretions (saliva, tears, breast milk, GIT...)	Agglutination of agens in secretions.
<u>IgM = IgG</u>	
IgD receptors on B-cells	Activation of B-cells
IgE receptors on mast cells and basophils	Inhibition of parasite invasion

Allergic reactions