Practice No. 3

Nitrates, nitrites and CO poisoning

Nitrates NO_3^-

- Nitrates are industrial fertilizers
- Poisoning most often due to exchange of salt and these fertilizers – appropriate storage of them is very important!
- Fertilizing can cause increased levels of nitrates in feed, especially in fodder crops and vegetables
- These agents can also contaminate drinking water
- Limits for drinking water is 50 mg/l in adults/animals

- The course of poisoning in monogastric animals is the same as in salt intoxication – nitrates are osmoticaly active substances – this type occurs only in massive intoxication by nitrates
- The signs occur within 3-7 hours after ingestion – thirst, diarrhoea, colic, excitation, convulsions
- The treatment is also similar to that described in salt intoxication – emetics, adsorbents, pure water administered gradually

- In GIT of ruminants, a big part of nitrates is converted into more toxic nitrites – reduction process mediated by bacterial microflora!

- Non-resorbed nitrates and nitrites are a source for the production of nitrosamines in guts – CARCINOGENS

Nitrites NO_2^-

- Technical and industrial chemicals, also used in food industry for preservation of meat products (antibacterial, but as a side effect also cause the rich pink colour of products)
- Quickly absorbed from GIT
- Act as poisons with effect on blood and vessels

- Limits for drinking water is 0,5 mg/l for adults/animals

- *Nitrite form of intoxication:*

- in massive intake of pure nitrites – direct effect on vessels causes the paralysis of vascular tissue, vasodilatation, decrease in blood pressure, collapse within 1 hour
- treatment: inactivation and elimination of non-resorbed substance from GIT, administration of analeptics (reinforce circulatory system), adrenalin in collapse

- *Methaemoglobin form of intoxication:*

- most common, develops for several hours, nitrites convert haemoglobin into methaemoglobin (oxidize iron from $^{2+}$ form to iron in $^{3+}$ form), which leads to insufficient oxygen transport to tissues and later to hypoxia and asphyxia

Physiological value of MetHb	**< 2,5 %**	
Mild intoxication	**10 – 15 %**	**Light cyanosis, respiration with mouth open, dyspnoea**
Medium intoxication	**15 – 20 %**	**Significant cyanosis, dyspnoea, apathy, fatigue, decreased mobility**
Severe intoxication	**20 – 30 %**	**Whole body cyanosis, dyspnoea, often convulsions of legs, immobility, abortions in pregnant animals**
Critical status, death	**50 – 80 %**	**Unconsciousness, convulsions, great hypoxia, death**

- In erythrocytes and liver, there is an enzyme called methaemoglobin-reductase, which reduces iron in haem back to Fe^{2+}. This enzyme is only of limited capacity – after depletion, signs of intoxication occur
- Fish have very high level of this enzyme, they bear higher doses of nitrites (LC 70-80 % of MetHb). Moreover, they have chloride cells on gills, where chloride and nitrite ions are absorbed and compete. In sufficient concentration of chloride ions in water, the poisoning doesn't develop – almost never found in sea water
- On the contrary, cats and young animals have a very low activity of this enzyme (LC around 50 % of MetHb) – common intoxications mainly in young cattle when they change the diet from milk to grass (higher content of nitrogen compounds in green feed)

Pathological – anatomical examination:

- congestion of organs, dilatation of vessels, gastritis, enteritis
- non-coagulable blood of brown colour, serosa and mucosa of the same colour

Diagnostics:
- clinical signs
- pathology
- examination of full blood – determination of methaemoglobin spectrofotometrically

Treatment:
- adsorbents and emetics in first, resorption stage
- antidote is methylene blue, which reduces Fe^{3+} back to Fe^{2+}, administered **i.v.** in the concentration of 1-4 mg/kg.w. in the form of solution in physiological solution

- in small animals we may also administer vitamin C, and oxygen by inhalation route or make blood transfusion
- it is necessary to keep animals absolutely calm
- in animals that will be probably slaughtered because of their bad status, we never administer methylene blue, it would cause the degradation of meat quality

Other compounds with methemoglobinisation activity

Drugs:
- benzocain, lidocain and other local anaesthetics
- paracetamol, phenacetin – analgesics
- amylnitrite – an antidote in cyanide poisoning
- sulphonamides – antibiotics

Chemicals:
- fertilizers, chlorates, aniline, toluene, benzene, carbamates, herbicides based on uric acid

Plants:
- rape seed plant, crownvetch

Carbon monoxide - CO

- A colourless and odourless gas, slightly lighter than air
- It is the product of the incomplete combustion of carbon-containing compounds, notably in internal-combustion engines
- Dangerous in spaces with bad ventilation – gas flow-heaters, gas heating, gas oven

Mechanism of action:

- Carbon monoxide in blood binds to haemoglobin and produces very stable product called carbonylhaemoglobin (or carboxyhaemoglobin)
- The affinity of CO to haemoglobin is approx. 240x higher than the affinity of oxygen, in fetal haemoglobin it is even higher
- The bond is reversible
- Moreover, CO binds also to myoglobin (can cause rabdomyolysis and renal failure); CO blocks the function of mitochondrial breathing and energy producing enzymes including cytochrome c oxidase; and also inhibit cytochrome P450 – inhibition of metabolism and detoxification

Clinical signs:

- according to stage of poisoning: weakness, vertigo, fast breathing and heart rate, headache, convulsions of skeletal muscles, slow pupillary reaction to light, cyanosis, coma, death
- also chronic intoxications described – tiredness, vertigo, problems with vision, stomach aches, parestezia
- in almost one third of affected animals, the late complications develop. We cannot predict their occurrence, they don't correlate with the degree of seriousness of the

intoxication. To such complications belong deterioration of memory, changes in behaviour, deterioration of hearing, damage of cerebellum functions, parkinsonism

Pathology:

- typical sign is cherry-red colour of blood and mucosas
- blood remains non-coagulable
- petechias in CNS
- lung oedema

Treatment:

- transfer the affected animal into fresh air, give the inhalation of pure oxygen (30-60 minutes), or shorter time in overpressure if available
- if there is a problem with heart action, administer analeptics
- at risk of lung oedema, administer hypertonic solution of glucose (40 %) or NaCl (10 %)

Practical work: Determination of nitrates and nitrites in water samples
Determination of methaemoglobin and carboxyhaemoglobin concentration in blood