

The sulfur atom in health and disease

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Sulfur geochemistry

- V.M. Goldschmidt's classification, based on partition of elements between three coexisting (immiscible) melts and gas phase:

Lithophile elements in silicate melt

Chalcophile elements in copper-rich sulfide melt

Siderophile elements in iron-rich metallic melt

Atmophile elements in gas phase

Biophile elements are those found at high concentrations in living organisms (absolute or relative abundance)

Sulfur is highly chalcophile, highly siderophile and highly biophile

Most of the sulfur on our planet is found in the Earth's core (probably principle 'light element' there). Helped to depress the melting temperature for metal when the Earth's core was formed; this may have been crucial for generation also of volcanic rocks with low melting temperature, rich in P and K, that functioned as sources of potassium and phosphate to the seawater when the first DNA and RNA molecules on our planet were formed, *i.e.* when life first originated - most likely close to 4.5 billion years ago (formation of MORBS reservoir and core about 4.53 billion years ago, origin of solar system 4.56 billion years ago). Formation of P-rich and K-rich oceans impossible later. Nucleotide bases may have been formed in the uppermost atmosphere by solar proton bombardment at a time when the Sun was much more active than now.

Sulfur geochemical cycle

- In igneous rocks mainly as sulfide minerals, together with chalcophile metals (e.g. iron, nickel, copper, zinc, lead and molybdenum), selenium and arsenic
- In sediments as sulfide or sulfate minerals, especially as calcium sulfate in evaporite rocks, but as iron sulfide in shales
- Average concentration in sediments much higher than in igneous rocks because much sulfur is added to atmosphere/seawater from volcanoes (as SO_2 in volcanic gases, may be also from mid-oceanic ridges)
- Very high concentration in seawater (as sulfate) compared to igneous rocks
- Transported into the atmosphere as seaspray particle sulfate and as dimethylsulfide made by living organisms
- Much of the natural sulfur content of soils on the continents comes from atmospheric deposition, *i.e.* from seawater following transport through the atmosphere
- The same is also the case with halogens except fluoride, with selenium, and to somewhat less extent also sodium and boron. Se and I transported into the atmosphere mainly because of biomethylation.
- The deposition rate of all these elements depends on the distance from the sea
- The concentration of sulfate in soils is enhanced by much evaporation in semiarid and arid zones. In deserts, desert roses (which are beautiful gypsum crystals) can thus be formed.

Sulfur biogeochemistry

- Sulfate is very soluble in the soil, when the climate is humid, and thus highly leachable
- Plants need much sulfur for their growth, compared to normal bedrock concentrations.
- If enough sulfur is not available, total protein synthesis in the plants will be limited, and the average composition of seed (*e.g.* cereal grain) protein is changed with less sulfur amino acids
- Sulfur and other plant-biophile elements are well retained and recycled by natural forest ecosystems because of active uptake in the roots
- When the forests are destroyed, it is common that the local ecosystem will become much more leaky and sulfur can be rapidly lost, with severe consequences for human health (HIV disease, tuberculosis)
- Sulfur is lost to the atmosphere together with selenium and nitrogen when plant matter is burnt (savanna fires).
- It is possible that upconcentration of sulfate in soils by evaporation (combined with less rapid leaching) may be one of the main reasons which protein concentration in plants is inversely correlated to annual rainfall, leading to corresponding geographic gradients for protein intake in the human population (much less near the southern coast of West Africa than in the northern part of the Sahelian zone, *e.g.* in Niger)
- Deficiency of sulfur amino acids in the diet is associated with **kwashiorkor**.

Similarities between geochemical and biochemical behaviour of S

- Most of the total sulfur content of living organisms is in the S amino acids methionine and cysteine. Humans can make cysteine from methionine, but depends on methionine from diet
- Most functions of cysteine in proteins depends on the functional properties of thiol (SH) and disulfide (-S-S-) groups
- The thiol groups bind ferrous iron and other chalcophile metals. Geochemically scarce, but toxic heavy metals, e.g. Cd and Hg, are often bound more strongly than nutritionally essential metals such as Fe and Zn.
- Also as sulfide ions (together with selenide ions) in iron-sulfur groups in proteins, *i.a.* in mitochondrial respiratory chain
- Also as sulfate, especially as part of polysaccharides. Sulfate also important for conjugation (before excretion) of xenobiotics, and some endogenous substances, such as bile acids.
- Most of this sulfate comes normally from oxidation of S amino acids (main end product of S metabolism in humans, smaller amounts *i.a.* as taurine)
- If sulfate reabsorption in the kidneys fail, this can lead to sulfate deficiency (may be common in autism)
- Sulfuric acid formed by oxidation of S amino acids depresses pH in blood plasma and urine. If not compensated for by enough bases from plant foods (especially in form of organic salts of potassium), this will lead to enhanced loss of calcium from the skeleton and risk of osteoporosis
- Imbalance between base-rich plant foods (vegetables, fruits and potatoes) and animal foods is probably one of the main reasons for the high prevalence of osteoporosis in Norway. We are not eating too much animal foods, but we eat too little plant foods to compensate for the load of sulfuric acid caused by degradation of S amino acids in the animal foods.
- Intake of S-acetylcysteine as glutathione precursor should also be compensated for by eating more base-rich foods.
- Vitamin C supplements should for similar reasons (in a country with much osteoporosis) be taken as potassium ascorbate rather than as pure ascorbic acid

Similarities and differences, between sulfur and selenium

- Selenium (Se) can be considered a "super-chalcophile" element forming stronger bonds with chalcophile metals compared to sulfur.
- All heavy metal selenides are less soluble than the corresponding sulfides.
- This can help to explain the antidote protective effect of Se at a high dosage level against mercury and cadmium. HgSe solubility product about 10^{-65} .
- Selenium is biologically active especially in form of selenocysteine and selenide ions
- Selenocysteine is incorporated in proteins during translation, codon UGA (or TGA in DNA molecule). Selenium must have been there when the universal genetic code first was formed
- Selenocysteyl-tRNA can not be formed without selenide ions as precursors (via the energy-rich compound selenophosphate)
- The cells must therefore have enzymatic pathways making selenide ions. Some of these are specific for selenium (without simultaneous formation of sulfide ions)
- Enzymes containing isolated selenol groups are more vulnerable to poisoning by heavy metals than enzymes containing isolated thiol groups, but not more vulnerable than enzymes containing two thiol groups that can bind to the same heavy metal atom
- Mammalian thioredoxin reductase contains a cysteyl group and a selenocysteyl group in neighbour positions. It can therefore form chelates with a heavy metal atom bound simultaneously both to a selenium and sulfur atom. The metal atom will thus be bound very strongly, making the enzyme extremely sensitive to poisoning by toxic heavy metals.
- Selenide and sulfide ions substitute for each other in iron-sulfur groups in enzymes, *i.e.* in the mitochondrial respiratory chain. Changes in the Se/S ratio will probably affect the functional properties of these enzymes because of a change of the standard redox potential for $\text{Fe}^{2+}/\text{Fe}^{3+}$ equilibria.

Pathways of electron transport to ribonucleotide reductase

- Ribonucleotide reductase converts ribonucleotides (precursors of RNA) into deoxyribonucleotides (precursors of DNA).
- Deoxyribonucleotides are needed not only for DNA synthesis, but also for DNA repair.
- Ribonucleotide reductase can alternatively use thioredoxin or glutaredoxin (thioltransferase) as reducing cofactors.
- Reduced thioredoxin is regenerated from oxidized thioredoxin by the selenoprotein and flavoprotein thioredoxin reductase, using NADPH as reducing cofactor.
- Reduced glutaredoxin is regenerated from oxidized glutaredoxin by reaction with reduced glutathione (GSH).
- GSH is regenerated from oxidized glutathione (GSSG) by the flavoprotein glutathione reductase, also using NADPH as reducing cofactor.
- If thioredoxin reductase is inhibited by toxic heavy metals (or Se or riboflavin deficiency) at the same time as GSH is also depleted, this can lead to simultaneous depletion of both substrates that can be used by ribonucleotide reductase for making DNA building blocks.
- Inhibition of DNA synthesis will be especially important for the most rapidly growing cell populations, such as leukocytes and enterocytes. Will therefore lead to immunodepression.
- Inhibition of DNA repair will lead to enhancement of the risk of cancer and accelerated aging (because of enhancement of the rate of accumulation of mutations in the mitochondria).

Collaboration of sulfur and selenium in antioxidant defence

- H_2O_2 , organic hydroperoxides and peroxynitrite are removed by several scavenging enzymes. Many of these scavenging enzymes contain selenium as a reducing cofactor, while others are indirectly dependent on selenium because they use thioredoxin as a reducing cofactor (with thioredoxin reductase being a selenoprotein)
- A number of these scavenging enzymes, but not all, use GSH as a reducing cofactor
- Selenium and glutathione are therefore both needed for normal antioxidant defence, and both can often be deficient in human populations, e.g. in Sub-Saharan Africa. Simultaneous Se and GSH deficiency is often a consequence (especially in poor countries) of the important similarities in geochemical behaviour, comparing S and Se.
- Glutathione peroxidases (Gpx1 to 4) contain Se and use GSH as reducing cofactor. Tert-uni ping pong kinetics.
- 2-Cys peroxiredoxins (peroxiredoxins 1 to 5) and selenoprotein P use thioredoxin as reducing cofactor
- 1-Cys peroxiredoxin (peroxiredoxin 6) can alternatively use GSH, ascorbate or dihydrolipoic acid as reducing cofactors.

Collaboration of GSH and selenium in oxidized protein repair

- Thioltransferase (glutaredoxin) repairs proteins that have been oxidatively damaged by formation of protein-glutathione mixed disulfides, using GSH as reducing cofactor.
- Thioredoxin removes other abnormal protein disulfide groups
- Methionyl sulfoxide reductases repair oxidized methionyl groups on protein molecules (two different stereoisomers). One of them is a selenoprotein, also called selenoprotein R. They all use thioredoxin as reducing cofactor, and are therefore indirectly dependent on the selenoprotein thioredoxin reductase.

Possible antagonism of sulfur and selenium in iron-sulfur proteins

- Sulfide and selenide ions can substitute for each other in iron-sulfur groups in enzymes. There is evidence suggesting that they normally shall contain much selenide.
- FeSe is more heavily soluble, compared to FeS.
- It may therefore be expected that a high Se/S ratio in the iron/sulfur group will stabilize Fe²⁺ compared to Fe³⁺ (enhancement of the standard redox potential)
- It is possible that this will lead to reduction of the rate of reaction between molecular oxygen and respiratory chain enzymes, leading to superoxide anion radical production in the mitochondria.
- It is also possible that it may lead to faster mitochondrial NADH oxidation, leading in turn to more rapid supply of electrons to cytochrome oxidase and faster utilization of oxygen under conditions where the oxygen supply is limited.
- It is possible that these mechanisms may help to explain anti-ischemic effects of selenium (and also substantial enhancement of the time interval before respiratory arrest and asystole during global anoxia induced by inhalation of pure nitrogen), that have been well documented through animal experiments.
- Can a high intake of S amino acids lead to depression of the Se/S ratio of iron/sulfur proteins, when Se intake is already limiting? Answer to this question not known.

Glutathione and immunological functions

- Glutathione depletion reduces the growth rate of leukocytes, perhaps because of reduction of the rate of DNA synthesis.
- Glutathione depletion is also associated with higher tendency for apoptosis (programmed cell death) at least in some types of leukocytes.
- Glutathione status also modulates the pattern of secretion of cytokines associated with the Th1 (T-helper 1) and Th2 (T-helper 2) response, with GSH depletion in dendritic cells and NK cells causing reduced secretion of Th1-associated cytokines and enhanced secretion of Th2-associated cytokines.
- Simultaneous stimulation of NK cells with IL-2 and IL-12 causes them to secrete interferon-*gamma* (Th1) if they contain enough GSH, but much IL-10 (Th2) if the GSH concentration is too low.
- The Th1 response is important for defence against viral infections, intracellular bacteria (*e.g. Mycobacterium tuberculosis*) and cancer.
- The Th2 response is important in allergy.

Sulfur amino acid deficiency affects the growth rate of pathogens

- Glutathione inhibits the growth of mycobacteria (*e.g. Mycobacterium tuberculosis*), and S-nitrosoglutathione has been found to kill them.
- Glutathione also counteracts the oxidative activation of transcription factors such as NF-*kappa*B and Sp1 that enhance the rate of HIV viral replication.
- NF-*kappa*B has also been reported to play a role in the regulation of influenza virus replication.
- The replication of HIV and several other important viruses (*i.a.* herpesvirus, human papillomavirus and Epstein-Barr virus) is inhibited by methylation of viral DNA, using S-adenosylmethionine as donor for the methyl group.
- It is thus possible that S amino acid deficiency in African diets will have a double effect by simultaneously enhancing the stimulation of virus replication (by NF-*kappa*B and Sp1) and reducing the efficiency of the brake mechanism by viral DNA methylation, especially when diets also are deficient in vitamin B₁₂ and/or folate.

Importance of antioxidant defence during infection

- Leukocytes use reactive oxygen species (ROS), reactive halogen species and reactive nitrogen species for killing viruses, bacteria and other enemies.
- It is also common that the mitochondrial production of ROS is enhanced in various cell types (*i.a.* muscle cells) during infection because of the effects some of the cytokines, such as TNF-*alpha*, on mitochondrial functions (which are mediated via sphingomyelinase activation and ceramide).
- It is therefore common that the oxidant stress during infections is very high. This can have several harmful effects on normal tissues, and it will also lead to enhanced degradation of several nutrient molecules that are vulnerable to oxidant attack, including various vitamins, but also sulfur amino acids in skeletal muscle (with the amino acid sulfur being converted to sulfuric acid).
- Infectious diseases such as tuberculosis and HIV disease may therefore by themselves contribute to worsening of sulfur amino acid/glutathione deficiency.
- Muscle protein degradation during infection is in large measure controlled by mechanisms depending on enhanced oxidative stress in the muscle, and can probably be counteracted by improving the antioxidative defence capacity of the muscle cells, *i.a.* by improvement of GSH status.
- The risk of death from lower respiratory infection (*e.g.* influenza) is probably strongly enhanced by poor antioxidant defence capacity in the lungs, *e.g.* because of glutathione depletion.

Antioxidant defence, ischemic pain and pain sensitivity

- Numerous experiments have demonstrated marked anti-ischemic protective effects in various organs by antioxidant nutrients such as glutathione, selenium and taurine, and also by the antioxidant hormone melatonin.
- These observations suggest large potential for harm reduction in acute medicine (e.g. brain stroke, head trauma, myocardial infarction), especially when combined with hypothermia.
- The same substances can very likely also be used for amelioration of chronic pain conditions affecting skeletal muscle, especially when combined with appropriate forms of physiotherapy and training (e.g. Alexander technique, walking on uneven ground in the forest).
- The sensitivity of C-fibres (unmyelinated nerve fibres important for pain sensation, especially in form of chronic pain) is enhanced by protein kinase A (*i.a.* following stimulation of the C-fibres by prostaglandins) and protein kinase C (PKC). Various PKC isozymes found in the C-fibres are activated by oxidative stress. Poor GSH status in the C-fibres must be expected to lead to more PKC activation and therefore enhanced sensibility of the C-fibres to painful stimuli, as e.g. too much lactic acid accumulation because of ischemia (lack of oxygen).

Glutathione, selenium and prostaglandin synthesis

- NSAIDs (non-steroid antiinflammatory drugs) act in large measure by inhibiting the synthesis of prostaglandins (which sensitize the C-fibers, and also are important for transmission of pain signals inside the central nervous system).
- Prostaglandins are made by enzymes (COX-1 and COX-2) that must be oxidized for activation. Oxidative stress can, moreover, also cause activation of phospholipase A₂, which liberates prostaglandin precursor fatty acids from membrane phospholipids. And the expression of COX-2 in leukocytes is enhanced by oxidatively activated transcription factors, such as NF-*kappa*B.
- Depletion of glutathione or other important antioxidant nutrients, such as selenium, must therefore be expected to lead to enhancement of the rate of prostaglandin synthesis in disease situations. This will not only lead to more pain, but also enhancement of neurogenic inflammation caused by secretion of pro-inflammatory peptides (such as substance P) from C-fibers after activation.
- A synergistic interaction must be expected between GSH or Se depletion and overabundance of arachidonic acid in the diet. Arachidonic acid is the precursor of 2-series prostaglandins, and it is oxidized more rapidly by COX-1 and COX-2 compared to eicosapentaenoic acid (EPA) which is precursor of the 3-series prostaglandins.
- It is common that poultry and swine meat contain too much arachidonic acid and too little EPA and DHA because of imbalance between linoleic acid (LA) and *alpha*-linolenic acid (ALA) in the animal feed, with much lower ALA/LA ratio than is natural for the animal species concerned. Could easily have been corrected.

Oxidatively activated transcription and inflammatory diseases

- The immune response during infection is associated with enhanced oxidative stress.
- This leads to oxidative activation of transcription factors, such as NF-*kappa*B and AP-1, that participate in the regulation of a very large number of genes associated with inflammation.
- While activation of these genes is necessary for a normal immune response, over-activation can often be harmful to the host organism.
- Allergic diseases are also associated with enhanced oxidative stress, especially because of eosinophil cell activation.
- NF-*kappa* B enhances the expression of proteins that allow eosinophil cells to accumulate at sites of allergic inflammation and also enhance their survival.
- A vicious circle may thus arise, since the eosinophils when activated will also make much ROS activating NF-*kappa*B.
- Poor GSH or Se status would be expected to lead to aggravation of this vicious circle because of impaired scavenging of oxidants.

Roles of taurine

- Intracellular osmolyte
- Antioxidant. Mechanism poorly understood, most likely by formation of mixed complexes with iron, thus preventing prooxidant catalytic effects of iron, especially when bound to organic phosphate compounds (*e.g.* iron-ATP and iron-DNA complexes). Effect complementary to that of ascorbate?
- Allosteric regulator of membrane calcium transport, *e.g.* calcium uptake in mitochondria. Net effect on cytosol calcium concentrations similar as for magnesium, even though mechanisms are completely different
- Binding of extracellular taurine to GABA and glycine receptors may cause inhibition of leukocytes (*e.g.* Kupffer cells) and peripheral nerve fibres (antinociceptive effect). Perhaps mainly important as retrograde inhibitory transmitter in CNS, might be important for protection of postsynaptic cells against damage caused by overstimulation of NMDA receptors
- Reaction with reactive halogen species leads to formation of taurine chloramine and taurine bromamine that following transport into cytosol inhibit activation of the proinflammatory transcription factor NF-*kappa*B.
- Bromide good substrate for eosinophil peroxidase, but not chloride. Formation of taurine bromamide during allergic diseases must be expected to depend on the Br⁻ concentration in blood plasma, which in turn depends on the dietary intake.
- Ordinary table salt has much lower Br⁻/Cl⁻ ratio than in seawater and forest plants, perhaps one among several reasons for exacerbation of allergic diseases.

Roles of S-adenosylmethionine

- Methyl donor in numerous transmethylation reactions, *e.g.* degradation of catecholamines and catecholestrogens by COMT
- Important for protection against ROS production by redox cycling of catecholestrogens
- S-adenosylhomocysteine competitive inhibitor of transmethylation reactions with SAMe as methyl group donor
- Ratio SAMe/S-adenosylhomocysteine depends on folate, vitamin B₁₂ and vitamin B₆ status
- Antioxidant effect reported for SAMe, perhaps because of iron complex formation

Regulation of synthesis of brain dopamine and serotonin

- **Amino acid precursor uptake via blood-brain barrier**
- **Phosphorylation/dephosphorylation of rate-controlling enzyme**
- **Formation of complex with regulatory protein**
- **Oxidative and/or nitrative stress inhibition of rate-controlling enzyme (*S*-glutathionylation, tyrosyl nitration)**
- **Presence of enough enzyme cofactors, such as iron**

Precursor amino acid uptake through blood-brain barrier

- Common uptake system for tryptophan, phenylalanine, tyrosine, leucine, isoleucine and valine.
- All compete with each other; uptake rate for one depends on concentration ratio in blood plasma between this one and the sum of the others
- Leucine, isoleucine and valine are consumed as fuel in skeletal muscle, especially during physical activity.
- Physical activity will therefore enhance the uptake rate for tryptophan and tyrosine in the brain
- This is more important for serotonin than for dopamine synthesis because of end product inhibition for tryptophan hydroxylase, but not for tyrosine hydroxylase

Oxidative and nitrative stress in the brain

Basal ROS generation because of electron leakage from mitochondrial respiratory chain even in resting cells.

Enhancement of NO and superoxide anion radical production following NMDA receptor activation. ROS and/or reactive nitrogen species probably essential for learning/memory

Antioxidant defence enhanced and NO synthase activity decreased by melatonin during sleep

Diurnal wakefulness/sleep cycle might be regarded as cyclic alternation between oxidative/nitrative stress dominance and repair dominance in the brain

Oxidative stress regulation of protein kinases and phosphatases

- Several protein kinases and phosphatases are regulated (activated or inhibited) by oxidative stress
- At least one of these (MAPK subfamily), being activated by oxidative stress, is a positive regulator of tyrosine hydroxylase
- Tyrosine hydroxylase and tryptophan hydroxylase are also regulated by other protein kinases

Oxidative/nitrative stress inhibition of neurotransmitter synthesis

- Oxidative and/or nitrative stress inhibit:
- Tyrosine hydroxylase (dopamine synthesis)
- Tryptophan hydroxylase (serotonin synthesis)
- Glutamate decarboxylase (GABA synthesis)
- Inhibition by S-glutathionylation reversed by thioltransferase + GSH
- Inhibition by tyrosyl nitration not reversible
- It is possible that oxidative stress first will lead to enhancement of dopamine synthesis because of MAPK activation and next to reduction of dopamine synthesis by direct inhibition of tyrosyl hydroxylase. This mechanism might perhaps explain why xenobiotics making ROS in the brain (*e.g.* alcohol when degraded by CYP2E1) first may cause euphoria, but afterwards will have the opposite effect

Protective roles of glutathione

- Reducing cofactor for glutathione peroxidases (scavenging of H_2O_2 , organic hydroperoxides and peroxynitrite), tert-uni ping pong kinetics
- Scavenging of NO by formation of S-nitrosogluthathione reduces formation of peroxynitrite
- S-nitrosogluthathione has itself antioxidant effect in the brain
- One of the reducing cofactors (together with ascorbate and dihydrolipoate) for 1-Cys peroxiredoxin (peroxiredoxin-6)
- Reducing cofactor for thioltransferase (glutaredoxin) when reversing S-glutathionylation of enzymes
- Electron transport (via glutaredoxin) to ribonucleotide reductase, making deoxyribonucleotides needed for DNA synthesis and repair
- Detoxification of xenobiotics, including some common drugs, by glutathione-S-transferases or spontaneous reaction with reactive metabolites (following intake of paracetamol). Probably important for protection of mitochondrial DNA against premature aging

Glutathione transport and cellular uptake

- Cysteine/iron has strong prooxidant effect
- GSH/iron has much less prooxidant effect
- GSH is therefore preferred over cysteine itself for transport of cysteine via the blood
- GSH can be taken up by many cells types through high-affinity or low-affinity uptake systems, without previous degradation
- Low-affinity uptake system has been found in non-human species for GSH in the blood-brain barrier
- The uptake rate for GSH in the brain is probably directly regulated by the blood plasma GSH concentration
- Reason for this unknown, most likely phylogenetically ancient signal function of GSH concentration in the blood, e.g. in regulation of satiety/food intake

Control of GSH concentration in blood plasma

- GSH can be taken up without degradation from the intestinal lumen
- GSH is also synthesized from precursor amino acids in the liver and next exported as GSH to the blood and as GSSG to the bile
- Glutathione synthesis in the liver depends on availability of all three precursor amino acids, but especially S amino acids; glutamine also important as precursor
- Oxidative stress (e.g. from alcohol degradation) stimulates glutathione synthesis in the liver, but enhances also GSSG excretion to the bile.
- Perhaps biphasic dose-response curve for alcohol on net rate of GSH secretion from liver to blood plasma, with moderate doses of alcohol leading to enhanced GSH secretion because of stimulation of glutathione production, while higher doses have the opposite effect because of too much GSSG secretion to the bile
- Liver GSH synthesis is reported to be stimulated by insulin, corticosteroids, TNF-*alpha* and IL-6
- Liver GSH synthesis is inhibited by catecholamines and glucagon
- The name melancholia for a disease is perhaps not only a result of ancient superstition or speculative philosophy

Role of melatonin in brain antioxidant protection

- Melatonin itself is a good antioxidant (scavenger of several harmful species)
- As a hormone, melatonin enhances the expression of several antioxidant enzymes in the brain, including glutathione peroxidase
- Melatonin binds to calmodulin, counteracting activation of neuronal NO synthase by calcium/calmodulin
- Melatonin is synthesized not only in the pineal gland, but also in other organs including the intestine
- Melatonin synthesis has been reported to depend on tryptophan status
- Reduction of diurnal melatonin production because of too much artificial light in modern urban societies?
- Clinical experience from use of high-dose melatonin as adjunctive therapy for cancer patients (Lissoni et al.) suggests high doses can be used safely with little risk of adverse side effects
- Unknown what effect this kind of therapy can have on the expression of antioxidative enzymes in the brain of human patients

Role of manganese in antioxidant protection

- Mn is a necessary cofactor in mitochondrial superoxide dismutase
- Cu and Zn are necessary cofactors in cytosolic superoxide dismutase
- Reduction of superoxide dismutase activity because of cofactor deficiency will lead to enhancement of the rate of peroxynitrite production, other factors being equal
- Peroxynitrite is mutagenic and can cause damage to mitochondrial DNA (NB existence of mitochondrial NO synthase)
- Reduced activity of mitochondrial superoxide dismutase must therefore be expected to contribute to enhancement of the rate of mitochondrial DNA aging
- Strong competition between ferrous iron and manganese for uptake into intestinal epithelium; iron is therefore a Mn antagonist
- Natural ratio between Mn and Fe intakes from plant foods about 1/2.
- No iron pills are sold, at least not in Norway, with this Mn/Fe ratio.

Roles of carnosine and homocarnosine

- pH buffers.
- Anti-carbonyl protection, *e.g.* against toxic aldehydes formed as by-products during glycolysis
- Antioxidant protection, probably by combination of different mechanisms
- Share plasma membrane transport system with histidine
- Homocarnosine (formed from GABA as precursor) dominant in most of the brain, but carnosine found in cells processing olfactory stimuli
- Higher concentration in astroglia than in nerve cells in the brain, probably reflecting partition of glucose metabolism between astroglia and nerve cells with much glycolysis in astroglia followed by lactate transport to the nerve cells where lactate is oxidized
- Possible because of membrane transport mechanism that brain carnosine and homocarnosine concentrations might be influenced by carnosine concentrations in blood plasma.
- Important in skeletomuscle diseases associated with ischemic pain?

We should use diet rather than drugs for reduction of ordinary pain!

- All drugs that are now commonly used for treatment of common pain conditions have side effects.
- Many years ago, it was common to use acetylsalicylic acid (aspirin) as first choice e.g. for treatment of headache, but this was abandoned in favour of acetaminophen (paracetamol) because of more frequent lethal side effects (especially in form of gastric hemorrhage) than with paracetamol.
- It was not then known that paracetamol is mutagenic.
- Different groups have later reported about mutagenic effects of paracetamol. It is converted into a reactive metabolite by the enzyme CYP2E1, with the reactive metabolite being next detoxified by reaction with glutathione, but the CYP2E1 reaction is most likely also attended by ROS production, with the ROS also being mutagenic
- CYP2E1 has been found in the mitochondria in brain cells in a non-human species. As the distance between the inner mitochondrial membrane (where this enzyme is located) and mitochondrial DNA is much shorter than between the endoplasmic reticulum (where CYP2E1 also is found) and nuclear DNA, it may be expected that the mutagenic effect will be much stronger on mitochondrial than on nuclear DNA.
- The pharmacological effect of paracetamol depends on uptake of the drug by the central nervous system.
- Thus, it is possible that paracetamol may function as a very precisely targeted 'smart bomb' to cause premature mitochondrial aging of brain cells.

We need much better control with all mutagenic drugs

- Humans are now exposed to a large total burden of mutagens both in form of man-made xenobiotics (e.g. synthetic pesticides and drugs) and substances that are formed naturally, but that we are exposed to at completely unnatural dosage levels (e.g. from tobacco smoke and alcohol).
- Also large mutagenic burden from diet in many countries, e.g. suboptimal intake of nutrients needed for normal DNA repair (such as niacin, folate and vitamin B₁₂), or for protecting DNA molecules against peroxy-nitrite, ROS and mutagenic products of lipid peroxidation (such as selenium, sulfur amino acids and manganese).
- *Nil nocere!* Medical doctors and scientists have a prime responsibility, not themselves to contribute to deterioration of the genetic health of future generations because of overutilisation of mutagenic drugs.
- No effort should therefore be spared to try to find treatment alternatives that will spare future generations from genetic damage, for diseases where it is common to use mutagenic drugs today.
- Mutagenic substances should not be used as contraceptives.
- Protection of the human genome should be regarded as the most important priority for all prophylactic medicine.

