# Radiation pathophysiology. Free nitric and oxygen radicals.

Lecture from pathological physiology

January 13, 2005

1. Ionizing radiation
2. Units
2. Theories of the effects of ionizing radiation
3. Cellular level effects
4. Tissue level effects
5. Effects on organismic level
   - 5.1 Deterministic effects
   - 5.2 Stochastic effects
6. Radiation hygiene

## 1. Ionizing radiation

- α, β, γ and neutron
- Produce physical, chemical and biological changes in material it interacts
- May also pass through matter without interaction and damage
- Interaction and damage involve ionization and energy transfer

## Alpha radiation

- α-particles are massive, charge +2
- Relatively low speed
- Travel only short distances, (μ meters)
- Easily stopped by skin or sheet of paper
- Gives highly ionized track, hence very damaging, (internal deposition)

## Beta radiation

- β-particles more penetrating than α
- Only one-half the charge of α
- Very high velocity
- Less interaction with matter and less damaging

## Gamma, X-rays, neutrons

- γ and X-rays have nocharge or mass
  - pure energy (photons)
  - no definite range till completely absorbed
- Neutrons: heavy particles, no charge
  - travel long distances, no interactions
  - pass through most biomaterials

## 1.Units

**UNITS IN RADIOBIOLOGY**

| VARIABLE | EARLIER | CURRENTLY |
|---|---|---|
| QUANTUM OF RADIOACTIVITY | Ci | Bq $s^{-1}$<br>1Ci=3,7 * $10^{10}$ Bq<br>= 37 GBq |
| EXPOSURE | R | NO NAME<br>$\frac{COULOMB}{Kg} = 3876R$ |
| ABSORBED DOSE | RAD | Gy $\frac{J}{Kg}$<br>1Gy = 100 rad |
| DOSE EQUIVALENT | | Sv=Gy*Q<br>E.g., FOR NEUTRONS Q=10<br>DOSE EQUIVALENT WILL BE:<br>1Gy.....10Sv |

## 2. Theories of the effects of ionizing radiation

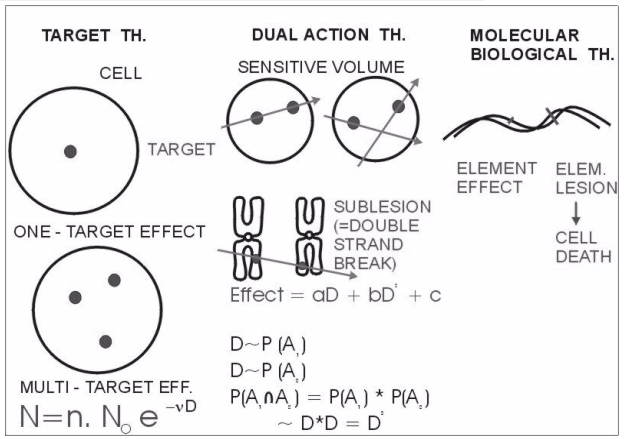
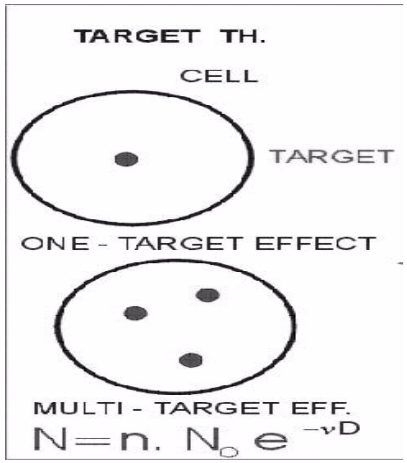



Fig. 2: Theories of the effects of ionizing radiation explain how the stabilized molecular damage is produced

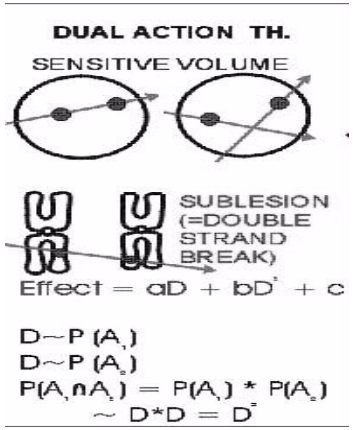
# Target theory:

- **Dose/effect curves are straight (with or without a shoulder) → there is a small sensitive target(s) in each**
- **cell with low probability to be hit, i.e., an amplifying process.**
- **Only formal theory**




# Dual action theory:

- **Tried to explain reciprocal chromosomal translocations. 2 sublesions (double strand breaks) in close vicinity → lesion = translocation.**
- **Dense ionizing radiation → 1 particle 1 lesion → linear term *aD***
- **Sparse ionizing radiation → 2 particles 1 lesion → quadratic term *aD2***
- **Theory not universally valid, but important accent: relative biological effectiveness (Sieverts!)**




The simplified model for DNA damage from ionizing radiation recognizes two types of damage, shown in Fig. 15.34. In type-A damage a single ionizing particle breaks both strands of the DNA, and the chromosome is broken into fragments. In type-B damage, a single particle breaks only one strand. If another particle breaks the other strand "close enough" to the first break before repair has taken place, then the chromosome suffers a complete break.

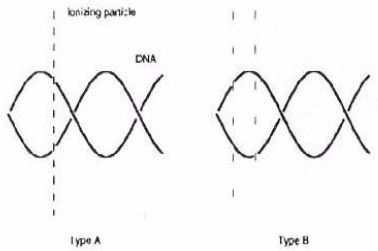



**FIGURE 15.34.** The two postulated types of DNA damage from ionizing radiation. In type-A damage a single ionizing particle breaks both strands. Two ionizing particles are required for type-B damage, one breaking each strand.

The probability of type-A damage is proportional to the dose. The average number of cells with type-A damage after dose $D$ is $m = \alpha D = D/D_0$, and the probability of no damage is the Poisson probability $P(0;m) = e^{-m} = e^{-\alpha D}$. This is the dashed line in Fig. 15.35, which is redrawn from Fig. 15.32. For radiations with higher LET the proportionality constant $\alpha$ is greater, as seen in Fig. 15.32.

In type-B damage one strand is damaged by one ionizing particle and the other by another ionizing particle. The probability of fragmenting the DNA molecule is therefore proportional to the square of the dose. The average number of molecules with type-B damage is $\beta D^2$, and the survival curve for type-B damage alone is $e^{-\beta D^2}$. This is also shown in Fig. 15.35. This leads to the *linear-quadratic* model for cell survival:

$$P_{\text{survival}} = e^{-\alpha D - \beta D^2} \qquad (15.31)$$

Ref: Hobbie

# linear-quadratic model

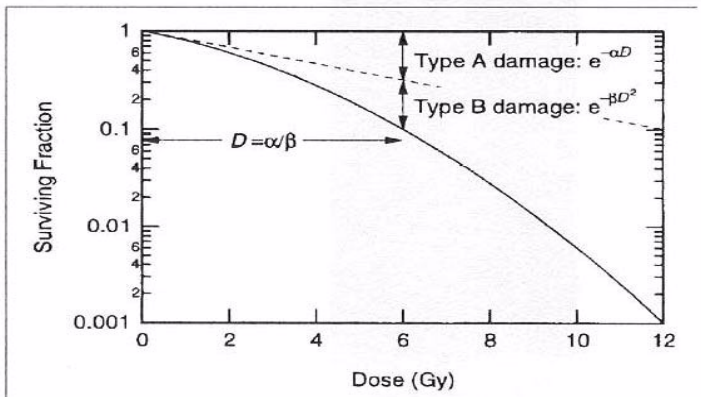



**FIGURE 15.35.** A survival curve, showing the linear exponent for type-A damage and the quadratic exponent for type-B damage.

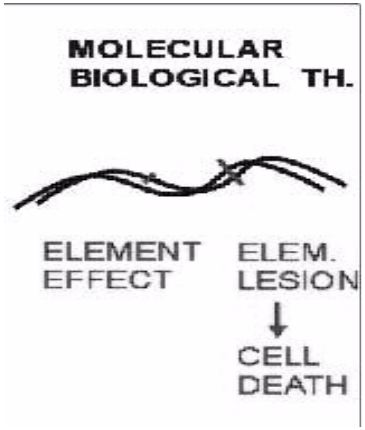
$$P_{\text{survival}} = e^{-\alpha D - \beta D^2}.$$

The dose at which mortality from each mechanism is the same is $\alpha/\beta$, as shown in Fig. 15.35.

Ref: Hobbie

## Molecular biological theory

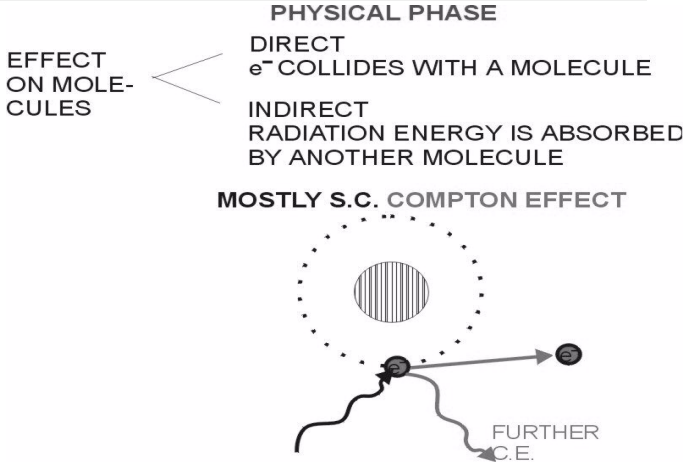
- Again: one or two particles → a combination of two primary events → elementary lesion = double strand break → difficult repair → chromosomal break → chromosomal aberration → possibly cell death
- Target = molecule, not nucleus
- The close environment of a radiation event and the repair processes taken into account

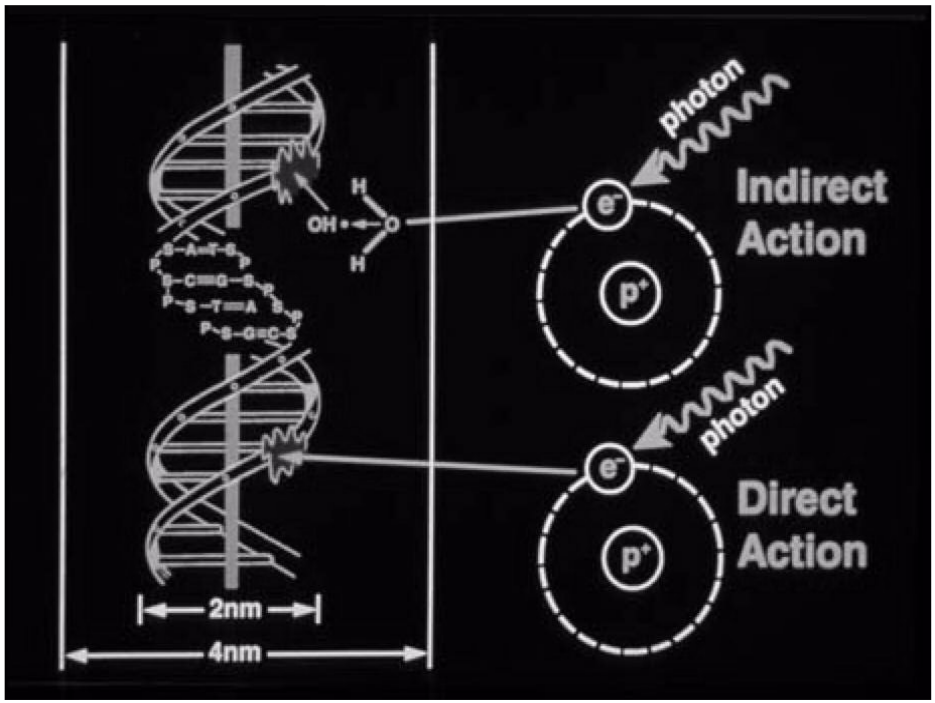



## Radical (ROS) theory

- Amplification of the effects of corpuscular radiation by production of free radicals (ROS) in water environment.
- It is compatible with the theories mentioned above and could be combined with them

Fig. 3Processes leading to the stabilized molecular damage



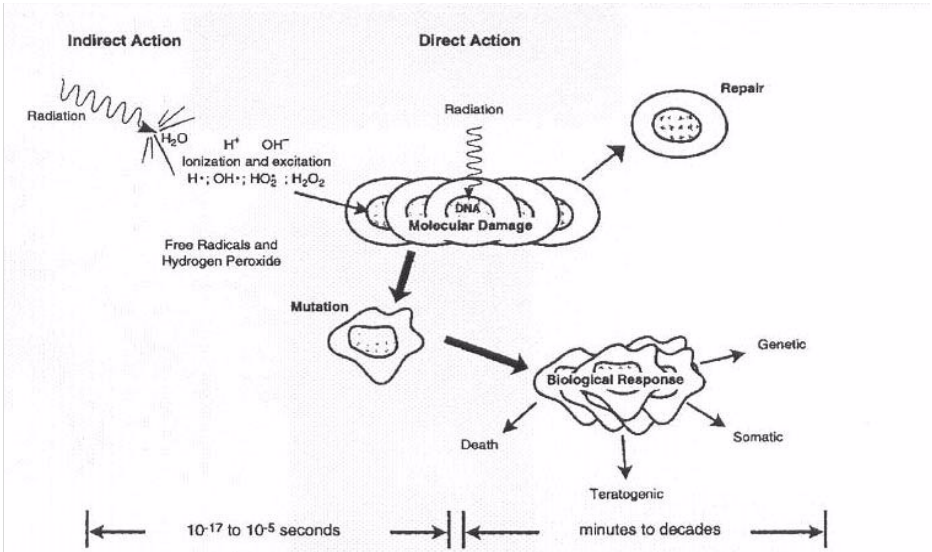
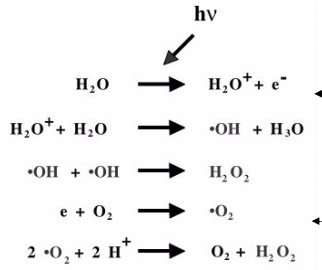



**FIGURE 25-1.** Physical and biologic responses to ionizing radiation. Ionizing radiation causes damage either directly by damaging the molecular target or indirectly by ionizing water, which in turn generates free radicals that attack molecular targets. The physical steps that lead to energy deposition and free radical formation occur within $10^{-5}$ to $10^{-6}$ seconds, whereas the biologic expression of the physical damage may occur seconds or decades later.

**Ref:** Bushberg

# Ionizing Radiation

**Causes the formation of excited and ionized molecules**.
The most important are species formed by radiolysis of water, forming products capable of causing oxidative damage.




Ionizing radiation results in the abstraction of an electron from water.

Increased radiosensitivity in the presence of oxygen.

**They produce base and sugar damage, base loss, strand breaks, and protein-DNA cross-links**.

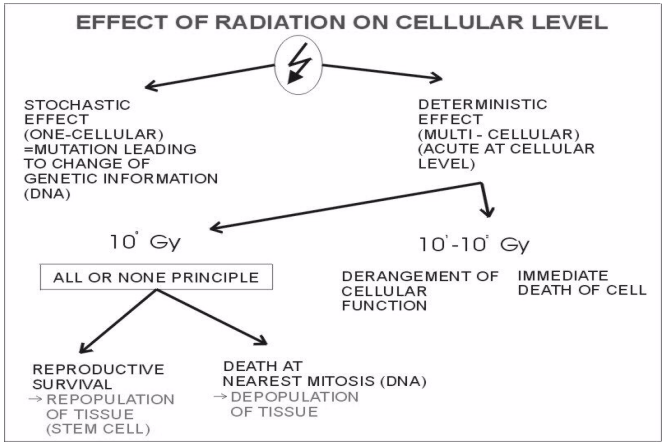
# Sequence of Events in Indirect Action

| | |
|---|---|
| | ↘ |
| $T_{1/2}$ in sec | Incident X-ray photons |
| | ↓ |
| $10^{-15}$ | Fast electrons |
| | ↓ |
| $10^{-5}$ | Ion radicals |
| | ↓ |
| $10^{-5}$ | Free radicals |
| | ↓ |
| | Macromolecular changes from breakage of chemical bonds |
| | ↓ |
| | Biological effects |
| days | - cell killing |
| generation | - mutation |
| years | - carcinogenesis |

# 3. Cellular level effects

Fig. 4

Effects of radiation on cellular level



Reproductive survival (more exactly: an ability to cycle indefinitely) – the most sensitive test of radiation damage to cells (colonies in vitro). ($D_0 \rightarrow 1/e = 0{,}37$) ≈ 1 Gy
The main mechanism of radiation damage of cells:
DNA damage, membranes - ? Radiation → DNA damage → ↑p53 → apoptosis

Mitotic delay: 1 Gy → 10% of cycle duration

Hormesis: Positive effects of very low doses of radiation (and of toxic chemicals) reported

Criticisms:

- lack of a coherent dose-response theory
- necessity of a specific (adequate) study design – difficulties inreplication
- only modest degree of stimulation – normal variation?
- lack of appreciation of the practical/commercial applications

But if real → consequences for radiation hygiene

Fig. 5Types of DNA lesions. Some of them represent a mutation, i.e. a gene which has undergone a structural change

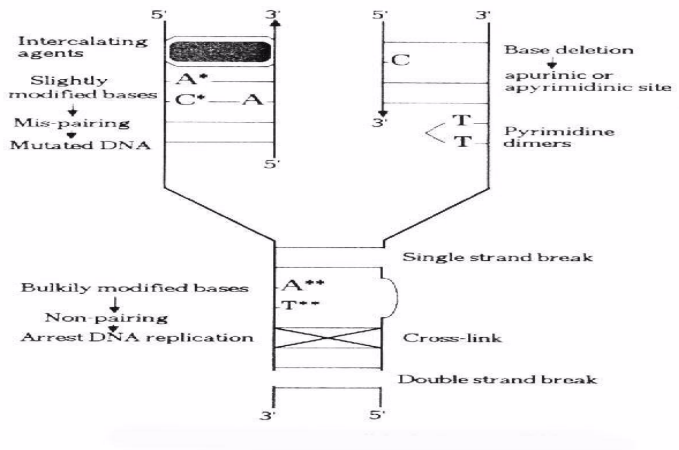
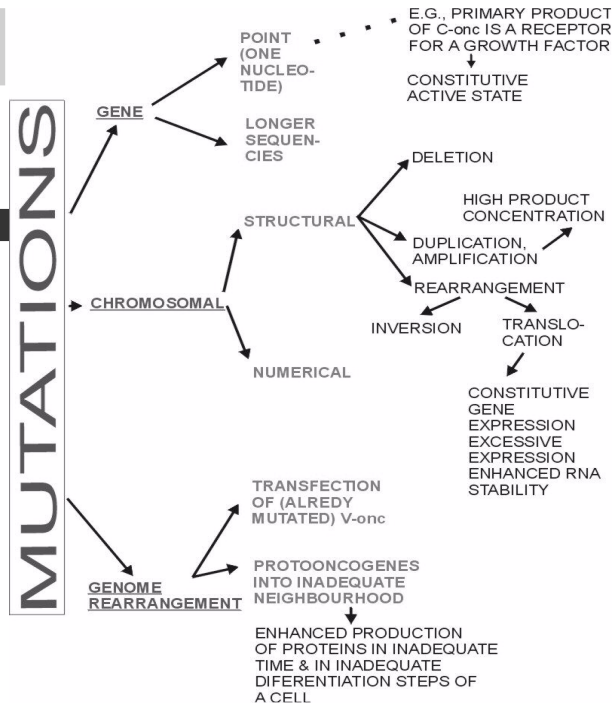





Fig. 6Types of mutations. Consequences independent on the mechanism of origin or on the „age“ of the mutation

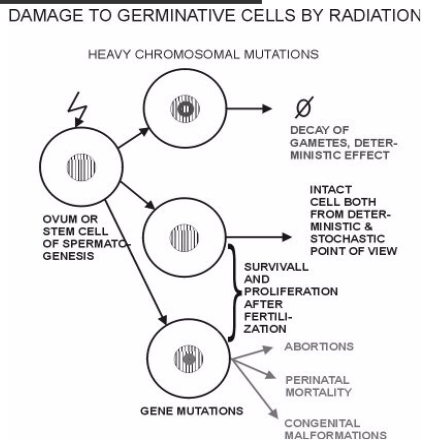


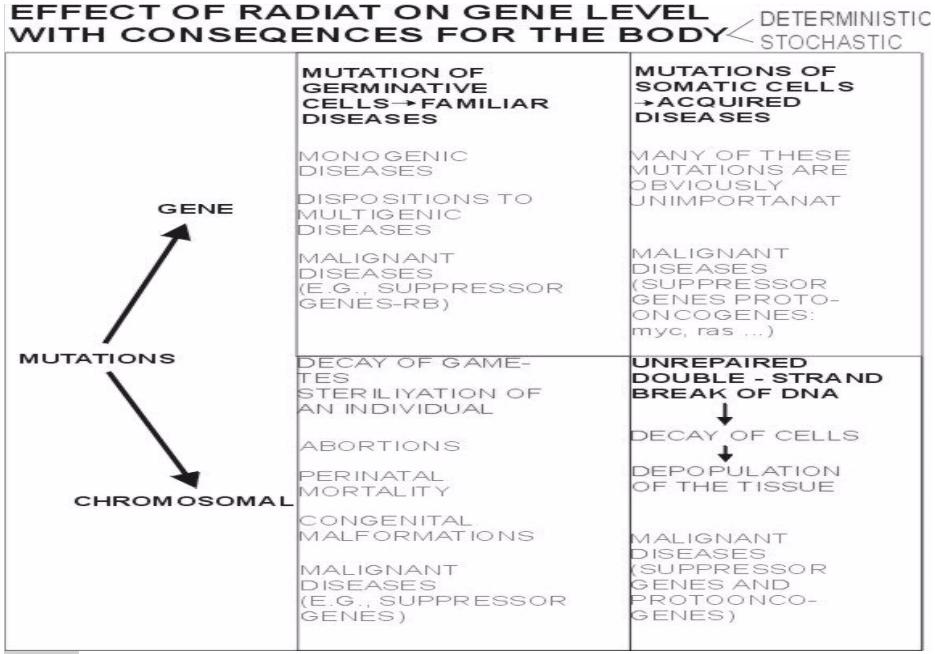
# Damage to the germ line cells by ionizing radiation

→ decay of gametes, sterility

→ reproductive survival, but mutations→ abortions, perinatal mortality, congenital malformations (evolution: „hopeful monsters“)

- One point mutation will be sufficient to do that




Chromosomal mutations as a measure of the absorbed dose

# Repair of radiation effects:

DNA repair systems: physical continuity preferred to information content → mutations

Reparative regeneration – on the level of tissues (see later)

Fractionation of dose or ↓dose rate → ↓biological effect, esp. in tissues with slow turnover (compared with tumors!)

Radiosensitivity of cells depends on:
- presence of oxygen
- effectivity of DNA repair systems
- phase of the cell cycle

The specific function of cells is relatively radioresistant

# 4. Tissue level effects

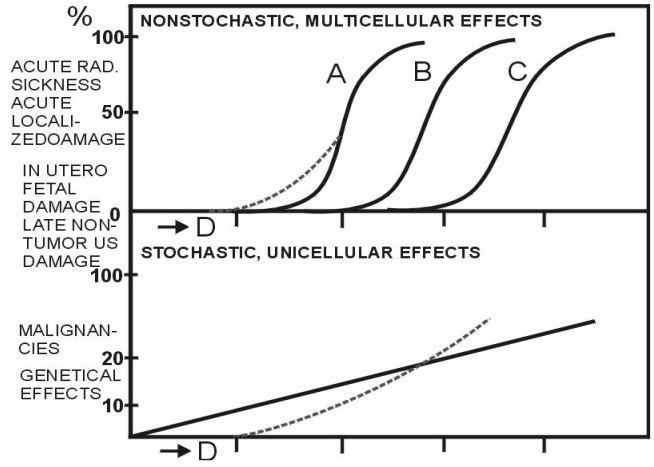
Example: Radiation damage of the blood forming organs (see practicals)

Cytokinetic parameters of a tissue determine its reaction to irradiation (radiosensitivity of the cells, dynamics of depopulation and recovery); mitotic fraction of a given tissue → time needed to the manifestation of tissue damage. Non-dividing cells are not sensitive in the LD50/30 region. Stem cells – a key position in the recovery of self-renewal cellular systems

Disturbance of the function of a tissue has a delay – the mature compartment is intact, it takes some time for the failure of proliferative compartments to „arrive“ to the periphery; life span of cells → the slope of the decline in the periphery (RBC 120 days, granulocytes and platelets 10 days)

# 5. Effects on organismic level

Fig 9 Deterministic (non-stochastic) and stochastic effects of the ionizing radiation






# 5.1 Deterministic effects

- acute radiation sickness
- acute localized damage
- damage to the embryo/foetus inutero → loss of „formative mass“ (microcephalia, microphtalmia etc.)
- late non-tumorous damage: cataract, chronic radiation dermatitis, pulmonary fibrosis etc.

Loss of large numbers of cells, large doses, S-shaped dose /effect curves (Fig. 9, upper part): dose threshold and plateau. Typical clinical presentations. Tissue recovery

Acute radiation sickness:

Radiosensitivity of tissues → the dose needed for depopulation (bone marrow, gut, brain)

Transit time → delay (timing) of effects (brain, gut, bone marrow)

Acute localized damage:

- Radiation dermatitis
- Germinative epithelium → sterility, premature menopause

# 5.2 Stochastic effects

- malignant tumors (esp. lungs, mamma, thyreoid, bones)
- genetic damage (germinative cells → progeny: abortus, perinatal mortality, congenital malformations); quantitatively less important than tumor induction

One cell (mutation, malignant transformation), low doses, dose/effect curves as in Fig. 9 (bottom): uncertainity about the effect of low doses (threshold? linear? hormesis?). Doses are additive

Stochasticity: causal connection with radiation cannot be proved in individual cases; no dependency of the disease intensity on the dose

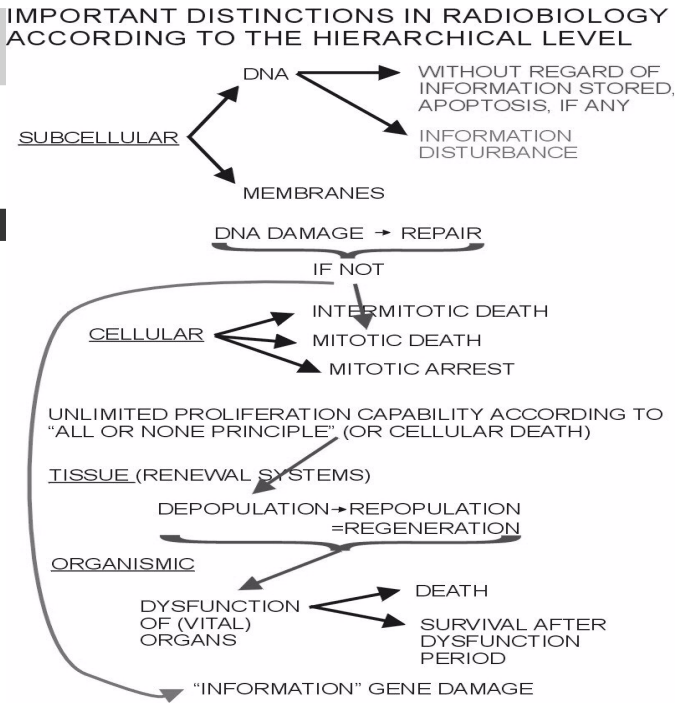



**Fig. 10**
**A hierarchy of radiation effects**



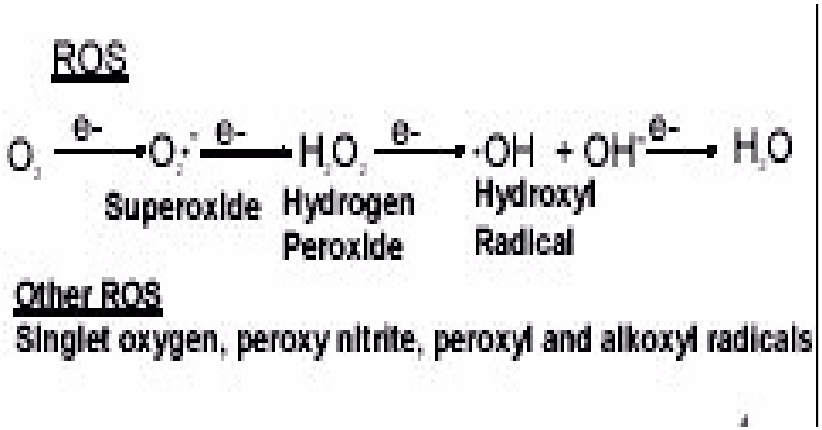
## 6. Radiation hygiene

**In practice: linear character of the dose/response curve in the region of small doses is presupposed → ALARA principle: As low as reasonably possible**
**Natural radiation background + medical radiation sources → the vast majority of exposures**

## Reactive oxygen species (ROS)

1. **Chemistry of reactive oxygen species (ROS)**
2. **Sources, defense mechanisms and pathological consequences**
3. **A survey of pathological conditions connected with ROS**
4. **Antioxidants in aging and disease**

# 1. Chemistry of reactive oxygen species (ROS)




**Superoxide radical O.- is produced by a single electron transfer onto molecular O2, which is thus reduced. Its main effect is an electron transfer onto further molecules, e.g., Fe3, Cu2, ferricytochom c etc. Superoxide radical is a source for hydrogen peroxide and hydroxyl radical.**

**Hydrogen peroxide H2O2 is produced spontaneously or under the catalytic influence of superoxid dismutase from the superoxide radical. No radical, mildly reactive. However, it may penetrate freely through membranes and is a source of potent oxidants as e.g. of hydroxyl radical and oxidative iron compounds**

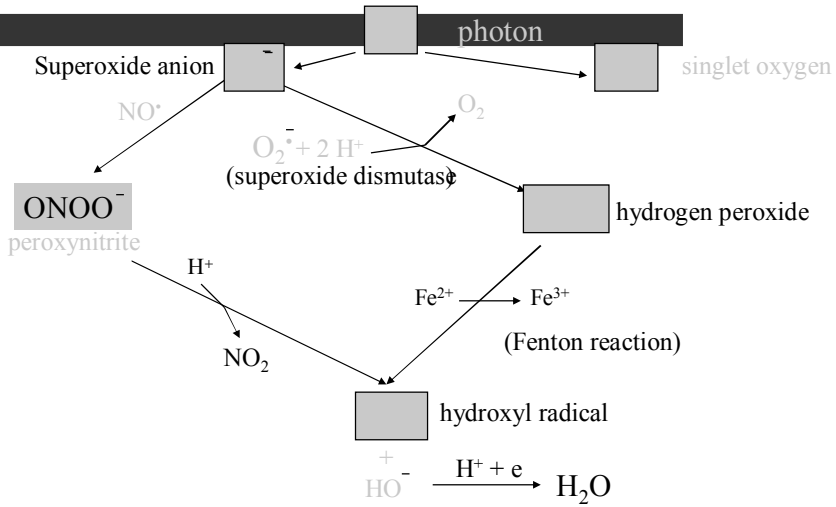
*Most potent form ROS and highly reactive is* **hydroxyl radical (HO·).**

**It may be produced**

- **indirectly by decomposition of hydrogen peroxide by means of Fenton reaction catalyzed by iron or cooper**
- **by interaction of nitric oxide NO with O·**
- **by radiation hydrolysis of water**

**A „normal" form of O2 is s.c. triplet oxygen 3O2. Activation energy transfer to the triplet oxygen → spin inversion → producing singlet oxygen 1O2 or O2*. It is a potent oxidant with extremely short life span. In biological systems formed via photosensitized reactions, chemiexcitation processes and enzymatic reaction. It may produce serious tissue damage.**

## Interconversion of Reactive Oxygen Species



## 2. Sources, protective mechanisms and pathological consequences

Fig. 2 Schematic overview of the main sources of ROS and their biological effects: oxidation of bioconstituents, stimulation of „repair" mechanisms, and induction of protein synthesis

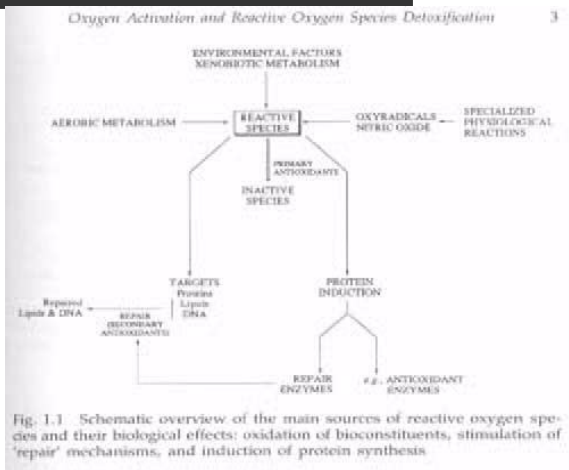


Fig. 1.1 Schematic overview of the main sources of reactive oxygen species and their biological effects: oxidation of bioconstituents, stimulation of 'repair' mechanisms, and induction of protein synthesis

## Sources

- **Superoxide radical and hydrogen peroxide are produced by biological sources. Modestly reactive, but via different redox reactions → highly reactive species (e.g. hydroxyl radical)**

***1. Aerobic metabolism:***

- **electron-transfer chain in mitochondria → hydrogen peroxide**
- **heart mitochondria → superoxide radical**
- **activity of various oxidases, e.g. xanthin oxidase (ischemia – reperfusion)**
- **microsomal electron transfer chain**

***2. Environmental factors:*** **airborn polluutants, photochemic smog, industrial chemicals, ionizing radiation, metabolism of xenobiotics. Further *ischemia and reperfusion,* traumas and infection**

***3. Specialized physiological reactions:*** **NADP (H) oxidases in phagocytes, releasing of EDRF, autooxidation of hemoglobin in erythrocytes etc.**

## Protective mechanisms

**Three types:**

1. **Primary antioxidants: some vitamins, micronutrients and enzymes**
2. **Secondary antioxidants: „repair" enzymes scavenging non-functional cellular components (proteolytic and lipolytic enzymes) or really repairing the macromolecules (DNA-repair systems)**
3. **Proteins inducible by oxidative stress**

# To be alive is dangerous