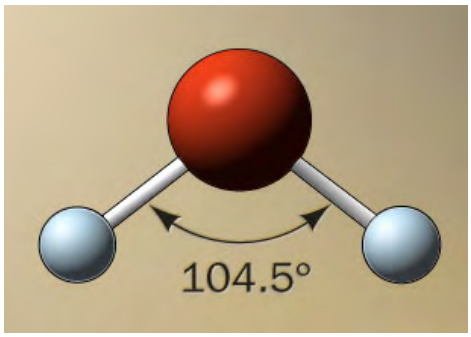
Water

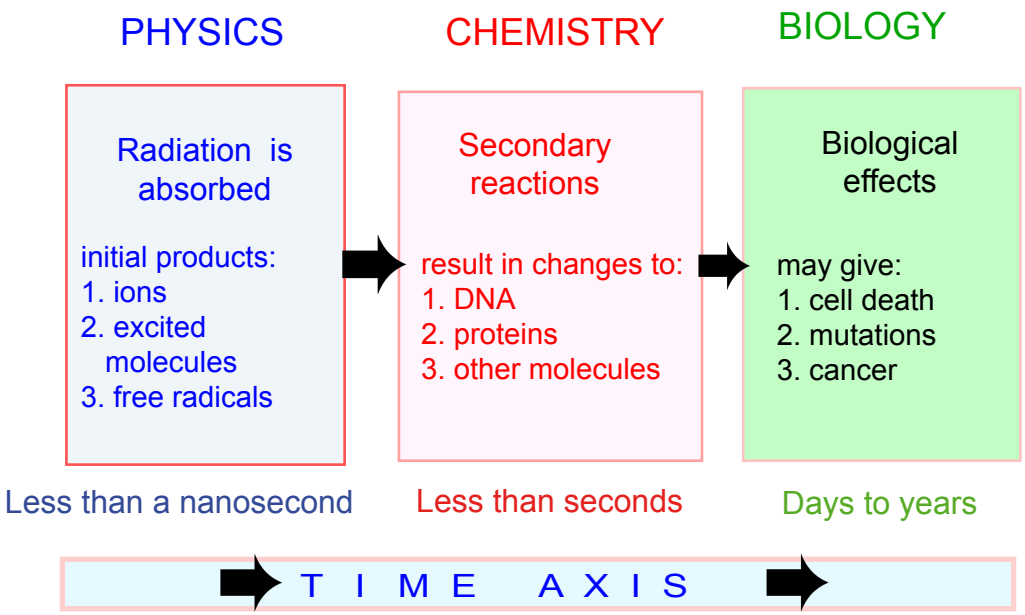


Chapter 11

Radiation Damage to Biomolecules — From water to DNA

In this chapter we shall discuss some of the basic mechanisms for the action of radiation on biological molecules – from water to DNA. In the next chapter we shall discuss the radiation effect on cells and animals and humans.

The radiation research field include physics, chemistry, biology and medicine, and it starts with the absorption of radiation. The initial processes are given in the figure below. The radiation results in a number of "***primary products***". These products (ions, excited molecules and free radicals) are very reactive with lifetimes in an ordinary cell of the order of a fraction of a second. Their reactions with molecules in the cell result in secondary processes which finally yield a macroscopic result such as cell death, cancer or genetic change.



In this figure is outlined some of the products and secondary reactions that take place when a biological system is irradiated. We intend to discuss some of the fields given above. The fields of study at the University of Oslo are mainly connected to the physical aspects and to the biological mechanisms. In the physical studies the electron spin resonance (ESR) technique has been used and for the biological mechanisms living cells cultivated at 37° C have been used.

The field of radiation biology was first promoted in the excellent book of Douglas Edward Lea from 1946 (*Actions of Radiations on Living Cells*).

Because it is not possible to cover all the interesting areas of research, the goal here is to present selected topics that will provide a sense of the scope and the progress being made.

Radiation Biophysics

The effect of any ionizing radiation starts with an interaction between radiation and the molecules in the cell. There are two different types of interactions:

- **Direct effect**

The effect is observed in the same molecule where the primary absorption occurs.

- **Indirect effect**

In this case the radiation energy is absorbed in another molecule (mainly water), resulting in the formation of reactive products that subsequently react with other molecules in the system.

In a dry system (without water), the direct effect occurs, whereas in an aqueous system the indirect effect dominates. Reactive water radicals are formed that initiate a number of subsequent processes. A living cell consists of about 70% water and 30% other materials. In such a system, the direct and indirect effects are approximately equally important.

Study of the primary radiation products is difficult because the life times of initially formed products are only milliseconds or shorter. There are two main strategies for studying these short-lived reactive products (called unstable intermediates):

1. Rapid techniques

The approach is to observe unstable intermediates in a very short time span before they disappear. In one technique, unstable intermediates are created by a short intense pulse of radiation (less than a nanosecond, 10^{-9} s). The intermediates are then detected by a very rapid measuring system, typically looking at changes in properties such as light absorption, emission, and conductivity. This approach is called pulse radiolysis.

2. Stabilizing methods

The lifetime of unstable intermediates can be increased by two different methods. Either using dry samples (remove the water) or using low temperatures. At low temperatures the secondary reactions are slowed down or even stopped. Very often this entails using temperatures below -100^{o} C. In order to attain these temperatures, liquid nitrogen with a temperature of -196^{o} C or liquid helium with a temperature of -269^{o} C is used to cool the sample. Experiments with liquid helium are very informative but difficult to do. The intermediates are first stabilized at extremely low temperatures. Then by slowly warming it is possible to observe the reactions as they unfold. This procedure makes it possible to study the secondary reactions.

For a number of years research groups have studied unstable intermediates (free radicals) formed in hormones, proteins and DNA with the method *electron paramagnetic resonance* or *electron spin resonance* (EPR or ESR). We have already mentioned this technique used for dose measurements (see page 78 – 83). Some of the main points for magnetic resonance are given on pages 204 – 207.

On the following pages we shall give the interested reader a short review of this powerful technique. We shall in particular mention the work carried out in our department at the University of Oslo and all the good colleagues we have had since the start in 1960.

EPR – ESR – the study of radicals formed by radiation

The history of magnetic resonance starts in the 1940s. The pioneers were F. Bloch and E. Purcell (NMR) and Y. Zavoisky (ESR)(pages 204 – 207). ESR concentrates on unpaired electrons (free radicals and radical ions). An irradiated sample placed in a strong magnetic field is exposed to microwaves. Under conditions which satisfy the electronic resonance of the free radical, microwaves are absorbed and this absorption yields valuable information about the molecular damage induced by radiation.

The initial products formed by radiation are mainly ***"free radicals"***. These products are characterized by having an odd number of electrons and are "paramagnetic"; i.e. they have a magnetic moment μ, which is given by the expression:

$$\mu = g\beta S$$

Here g is a characteristic constant, β is the Bohr magneton (a unit of magnetic momentum) and S is the spin of the electron.

Molecules with magnetic moments behave like small magnets in a magnetic field B. Their energy in the field is given as:

$$\mu \cdot B \text{ or } g\beta S \cdot B$$

S may have one of two values, $\pm 1/2$. Thus, there are two possible energy states. This is the key to understanding the EPR technique. Free radicals in a magnetic field are divided into two groups (the magnetic moments either oppose B or align with B) each group having a different energy.

When the sample containing free radicals is exposed to microwaves of the correct resonance energy, transitions are induced from one energy state to another. The requirement for this is that the microwave energy is exactly equal to the energy difference between the two states. This requirement can be written:

$$h\nu = g\beta B$$

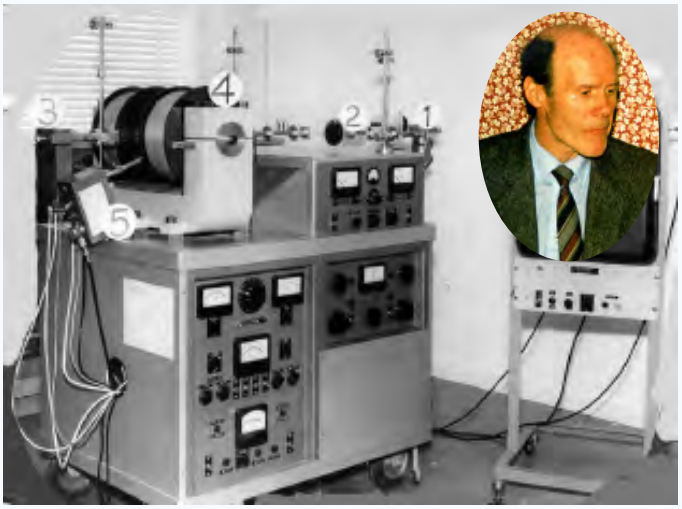
In this equation, the energy is the product of Planck's constant (h) and the microwave frequency (ν). For a magnetic field of 0.33 tesla (3,300 gauss) resonance occurs at a microwave frequency of about 10 GHz. This frequency corresponds to a wavelength of 3 cm.

An EPR-signal or spectrum is observed when the magnetic field is swept and the resonance conditions are fulfilled. Thus, the EPR spectrum of a sample exhibits the absorption of microwaves versus the magnetic field.

The *shape* of the spectrum yields information on the environment of the unpaired electron and if the electron interacts with neighboring protons and other nuclei. These interactions often make possible the identification of the initial and secondary radiation products. Since the interaction with the neighborhood is anisotropic, it is a goal to study radicals trapped in single crystals. Most of the amino acids and the DNA components (bases, nucleosides and nucleotides) can be obtained and studied in the form of single crystals.
Experiments can be carried out on irradiated samples at a very low temperature where the primary products are frozen and, thereby, stabilized. By subsequent warming the products are released and secondary reactions studied. Thus, the EPR experiments yield :

1). Identification of the free radical products formed.
2). The concentration of radicals per unit dose (chemical yield).
3). The secondary reactions spawned by the initial radicals.

EPR in Norway – a short review



The EPR in Norway startet at the Radium hospital in 1957 with a “homemade” spectrometer constructed by Otto Sørensen (left).
In 1970 the activity moved to the University of Oslo and a new JEOL spectrometer was installed (below).

Today two modern spectrometers are in operation (one is shown left). In 2013 we have excellent equipment for ESR studies.

The bottom picture is from Christmas 1979 and shows the old spectrometer with the staff; from left Arnt Inge Vistnes, Einar Sagstuen, Thormod Henriksen og Håkon Theisen.

Colleagues and coworkers

The EPR connected to radiation biophysics started in 1955 at Duke University, Durham, North Carolina.
It was the well known microwave scientist ***Walter Gordy*** that opened the field. Together with a number of students they studied radiation damage to polycrystalline compounds of amino acids and DNA-bases. They also started the single crystal studies.
Gordy`s Phd. students dominated this field for a long time. during the last half century. At the university of Oslo we have collaborated with a number of these students like Wallace Snipes, Chester Alexander, Janko Herak and for a long time Bill Nelson in Atlanta.

Walter Gordy
(1909 – 1985)

W. Snipes

C. Alexander

Janko Herak

Gordys work initiated ESR-studies in a number of other laboratories around the world. Let us mention some of them. In Europe it started with some comparative measurements to determine the absolute number of radiation-induced free radicals. The groups included was:

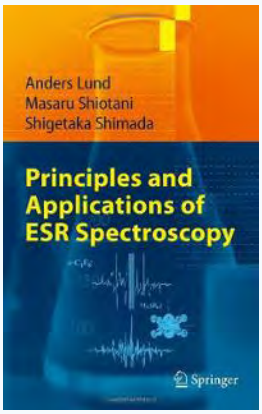
Adolf Müller

Karlsruhe Germany. Carl G. Zimmer urged Adolf Müller and W. Köhnlein to start ESR work. In this group we also can find Jürgen Hüttermann.

Stockholm, Sweden. Here Anders Ehrenberg started the ESR studies and he got coworkers like Göran Løfroth, Astrid Gräslund and Anders Lund.
Anders Lund has through all years been one of those that has developed the ESR-technique and has written books.

Anders Ehrenberg

Anders Lund
Anders is a hard working retired professor. To the right with his book "*Principles and applications of ESR spectroscopy*" from 2011.



In Netherlands the ESR group consisted of R. Braams and J. ten Bosch.

Buffalo, USA. Here we find a group headed by Halold Box.

Harold Box

EPR at the University of Oslo

The very first master thesis with ESR was in 1961. Svein Prydz studied the radicals formed in amino acids. The first dr. degree in ESR was in 1963 when Thormod Henriksen used the technique to study free radicals in radioprotective sulfur compounds.

In the period from 1960 to 2000 more than 50 master and dr. theses have been taken at UiO. A review (written in Norwegian) of all these students and their work can be found on the address:

http://www.mn.uio.no/fysikk/forskning/grupper/biofysikk/biofysikk_miljofysikk_historikk.pdf

Coworkers: A number of scientists have worked in our laboratory for longer or shorter periods. We would like to mention Bill Nelson, who was a coworker for a number of years.

We also had a long valuable contact with Bill Bernhard in Rochester.

William H. Nelson
(1943 – 2010)

Michael Sevilla and Bill Bernhard
Picture from 1995 – from the celebration of the 100 year anniversary of Roentgens discovery.

Radiation products formed in water

Upon the irradiation of water, radical ions and excited molecules are formed. From these initial products, secondary free radicals are formed. There are two different pathways :

1. Excitation

$$H_2O + \text{ionizing energy} \Rightarrow H_2O^*$$

H_2O^* represents an excited molecule. The excited molecule is very unstable and loses its extra energy rapidly by a variety of pathways. One pathway is bond cleavage of the excited water molecule, resulting in the formation of **H** and **OH**. Both these products are free radicals implying that they have an unpaired electron. A known way to present this is by putting a dot to the chemical name like OH•

2. Ionization

$$H_2O + \text{ionizing energy} \Rightarrow H_2O^+ + e^-$$

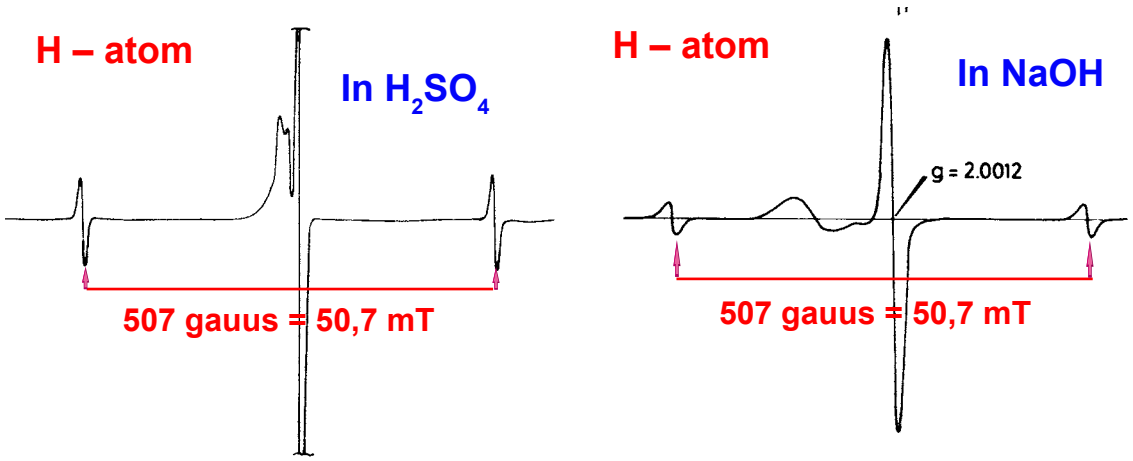
The primary products H_2O^*, H_2O^+ and e^- give rise to three reactive radicals: OH (hydroxyl radical), e^-_{aq} (aqueous or hydrated electron) and the H-atom. In a neutral solution (pH = 7), the relative amounts of these radicals formed are **2.6 : 2.6 : 0.6**, respectively.

Note that the electron ejected by an ionizing event, $\mathbf{e^-}$, is distinct from the aqueous electron, $\mathbf{e^-_{aq}}$. Thus e^-_{aq} is an electron that is enveloped by a number of water molecules and is relatively stable (for up to milliseconds). The e^- however, is a "***dry***" electron that still retains some of the excess kinetic energy acquired from the ionizing event.

Ralph Livingston

The dry electron can be solvated to form e^-_{aq}. It may also be trapped in a frozen matrix, and can thus be studied by EPR. The solvated electron may in turn react with a biomolecule such as DNA.

All the initial water radicals, H•, e^-_{aq}• and OH• have been observed with the EPR-technique.
In 1955, R. Livingston, at Oak Ridge, showed that H-atoms were formed and stabilized in frozen solutions of sulfuric acid (H_2SO_4). This experiments have been repeated and the data are given in the left figure below. H-atoms are formed in all aqueous solutions. In order to stabilize the species low temperatures are needed. At 77 K they are stabilized both in low and high pH-solutions. For pure water we must go to lower temperatures.



The EPR-spectra above exhibits the "fingerprint" of the H-atom. The two lines with a splitting of 507 gauss (50.7 mT) is the ESR-spectrum of the H-atom. The frozen samples were both irradiated and observed at 77 K. The line with the g-value of 2.0012 in the right spectrum is the evidence of a solvated electron (see more below).

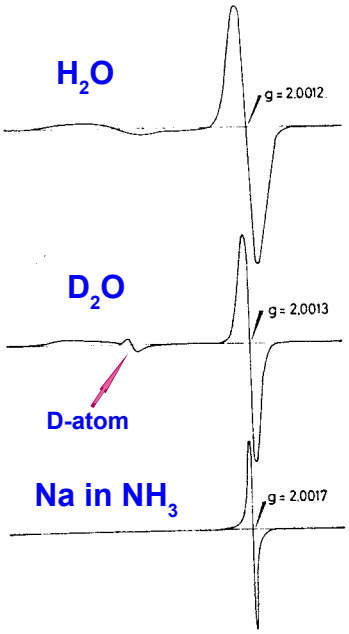
The solvated electron

Jack Boag
(1911 – 2007)
(photo from 1957)

In 1954, R. Platzmann suggested that the aqueous electron is a radiation product. This theory was confirmed by J. Boag and E. Hart in 1962. They observed the absorption spectrum of the aqueous electron in pulse radiolysis experiments.

A year later the hydrated electron was observed using EPR by T. Henriksen in experiments on frozen solutions of NaOH (see figure below).

Pellets of NaOH in both H_2O and D_2O were irradiated and observed at –196 °C. The "fingerprint" of an electron is a single line at a g-value of 2.0012. The position of the line in the magnetic field tells us that the electron is kept in a cage of water molecules. If the electron was completely free the g-value would have been 2.0023 (the socalled Lamb shift results in this deviation from 2). The cage itself has an influence on the broadness of the line. Thus, in a cage of heavy water (D_2O) the line is more narrow. If Na is dissolved in liquid ammonia, electrons are formed and trapped (bottom spectrum). In this case the cage consists of NH_3 molecules and the electron is more free. The line has a slightly higher g-value and is more narrow.



The OH-radical

The OH• radical has a far more complicated EPR-spectrum. It was identified by T. Gunter (Berkeley) in experiments on irradiated single crystals of water in 1964.

The three primary products formed in water are the starting point for a number of radiation-induced effects in biological systems. One of the goals in radiation research is to follow the processes which take place as these initial products react and yield changes to important biomolecules such as proteins and DNA.

In the case of radicals formed from proteins, DNA or the constituents of these macromolecules, the spectra are much more complicated. Through advanced techniques such as EPR and ENDOR, valuable information is being learned about the fast chemical processes initiated by ionizing radiation in biomolecules. We shall first give you a glimpse of the EPR-work on amino acids and proteins – and see the correlation between radical formation and enzyme inactivation as the biological end point.

Proteins and amino acids

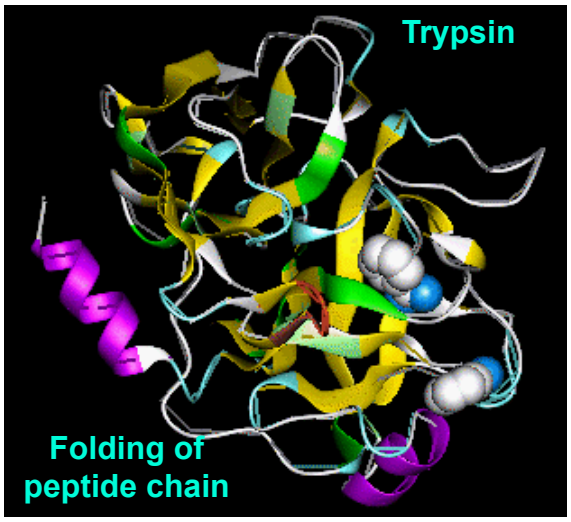
The EPR-work on amino acids and proteins have given some information that can be mentioned.

1. Secondary radical reactions can be studied, – both in frozen aqueous solution as well as in crystalline compounds.

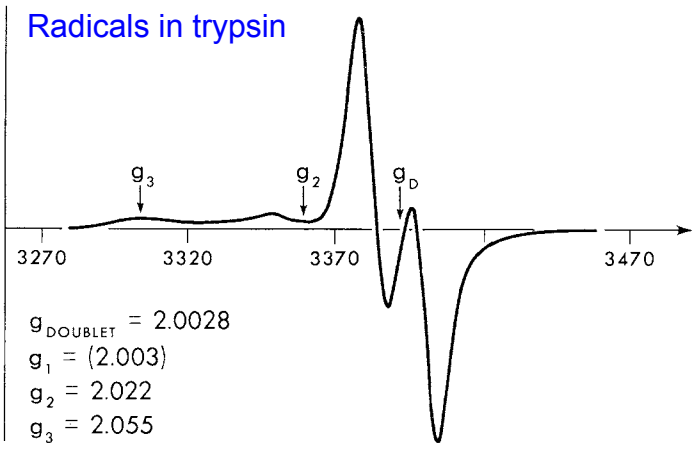
2. ***The radicals formed are independent of LET.*** That is; the same radical species are formed whether we use x-rays or heavy ion beams. However, the number of radicals stabilized vary with LET. We give one example of such experiments.

3. The formation of radicals in enzymes can be correlated to a biological end point such as inactivation.

Trypsin – radicals and inactivation



Trypsin consists of a single chain polypeptide of 223 amino acid residues. The amino acid sequence of trypsin is crosslinked by 6 disulfide bridges. Trypsin is a member of the serine protease family. The active site of trypsin include histidine and serine. Trypsin will cleave peptides on the C-terminal side of lysine and arginine amino acid residues.



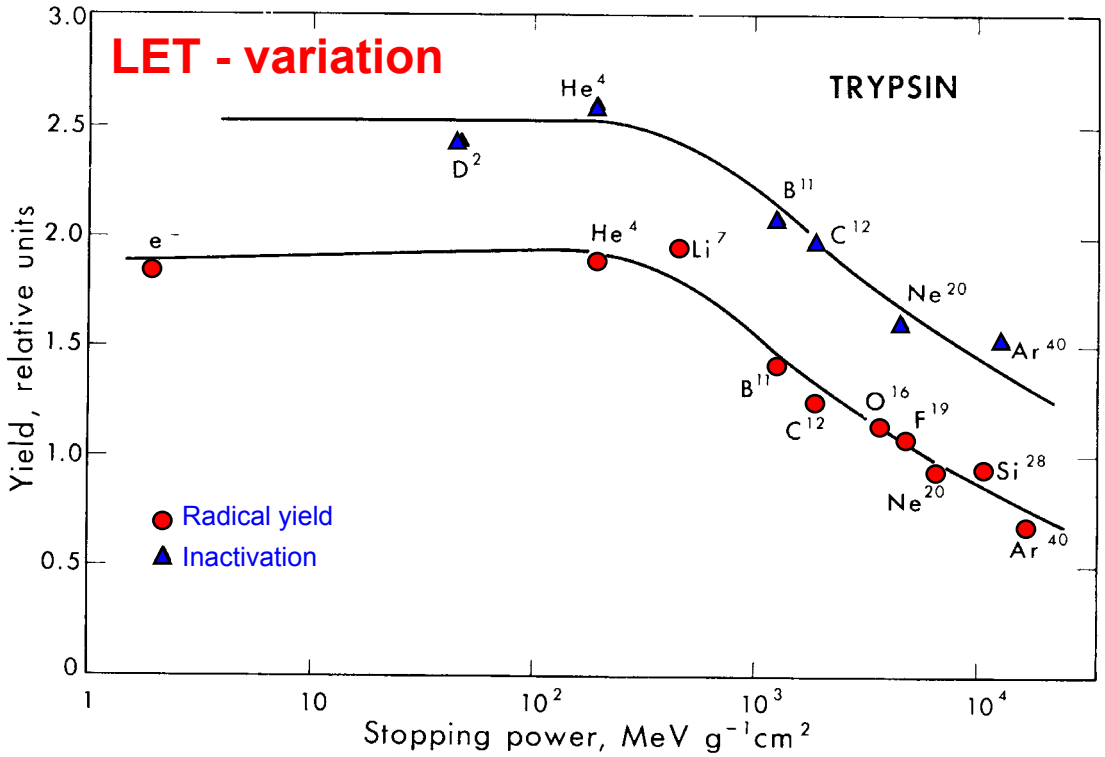
When trysin is irradiated the ionizations and excitations are evenly distributed in the molecule (determined by the electron distribution). ***Secondary reactions*** occur and we end up only with two types of radicals as shown in the EPR-spectrum to the left. The doublet is due to a backbone radical, whereas the resonance with the three g-values (2.003, 2.022 and 2.055) is due to a sulfur radical – probably located to the disulfide bonds that are important for the integrity of the molecule.

Correlation between radicals and a biological end point

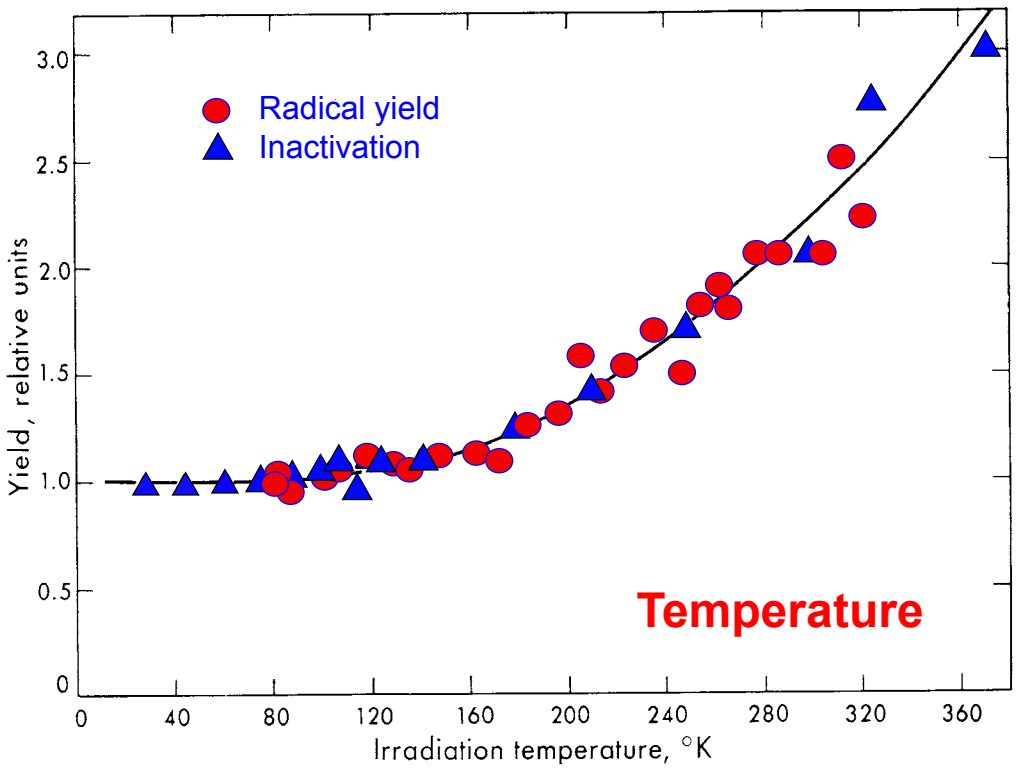
The biological effect of radiation is the results of the mechanisms initiated – that is the ioniizations and the excitation of the molecules. The free radiacals formed are "intermediate products". We assume that the secondary reactions yield the biological effects. Below we present some old experiments which demonstrate the connection between the free radiacals formed in enzymes (here trypsin) and the inactivation of the enzyme (in the solid state). The experiments are carried out with the Berkeley heavy ion linear accelerator (HILAC) in the beginning of the 1960-ties by Tor Brustad (the inactivation expeiments) and Thormod Henriksen (the ESR-measurements).

Inactivation. The inactivation curve for trypsin is exponential with regard to the radiation dose. The parameter used for inactivation (the yield of inactivation) is therefore the reciprocal of the D_{37} dose. The variation of the inactivation with increasing LET is given in the figure on the next page.

Temperature. Another type of experiments is also given in the lower figure (next page). The dry enzyme was irradiated with carbon ions at various temperatures. All measurements were carried out at room temperture. Again there is a striking correlation between the radical formation (the secondary radicals – mainly the sulfur radiacals) and the inactivation.



In this figure the inactivation of trypsin (in the dry state) is given av a function of LET. In the lower curve the formation of secondary radicals (mainly sulfur radicals) are given. Both the inactivation yield and radiacal formation yield is in the figure given in relative units. It appears that the data exhibits the same curve shape.



In this figure the inactivation of trypsin is compared to the formation of secondary radicals as a function of the irradiation temperature. The radiation is carbon ions with energy 10.4 MeV/nucleon. The same radiation equipment was used in the two types of experiments. Furthermore, all observations are carried out at room temperature. Thus it is a correlation between the secondary radicals formed and the inactivation.

Conclusion

The old experiments presented above suggest that the inactivation of trypsin is due to the formation of secondary radicals – ***probably a radical with the spin density localized to the disulfide bonds.*** A rupture of disulfide bonds may unfold the peptide chains and thus inactivate the molecule.

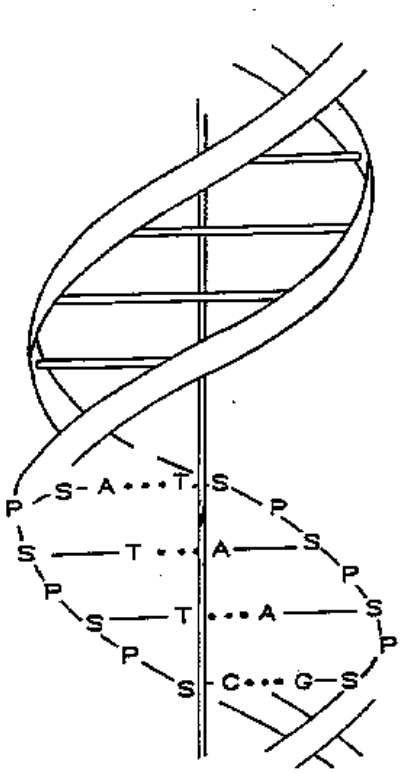
A large number of EPR-experiments have been carried out on amino acids and proteins. We encourage the reader to consult the publications from the biophysics group.

Although proteins are damaged by radiation, the consequences are generally **not** significant. This is because proteins exist in multiple copies and, if needed, new copies can be generated using the information stored by the DNA (deoxyribonucleic acid). ***But what if the DNA is damaged?***

Radiation damage to DNA

The effects of radiation on chromosomes, genes and DNA have been a major focus of research. The reason for listing these three names together is that genetic information is encoded by DNA molecules that in turn are packed as chromosomes. Scientists have studied the effects of radiation, chemicals, ultrasound and UV that alter the molecular structure of DNA. Of considerable importance is the correlation between the DNA damage and the subsequent biological effects. A correlation is anticipated because the information carried by DNA is essential for cellular replication and differentiation. Since DNA is extremely important for the last part of this book and for biology we shall give a short history of the experiments that resulted in the Watson – Crick model.

History and structure of DNA



In order to discuss radiation damage to DNA, we shall first give a short review about the history and some details about the DNA structure .

The research done to understand the structure and significance of the *hereditary molecule* is exciting and a number of Nobel prizes have been awarded to individuals working in this area.

In the years 1951 to 1953, work on determining the molecular structure of DNA reached a milestone of epic importance. A biologist, James D. Watson, and a physicist, Francis Crick, working at the Cavendish laboratory in Cambridge, England formulated the double helix model for the three dimensional structure of DNA. Publication of their paper "*Molecular Structure of Nucleic Acids*" in *Nature,* in 1953, brought them instant acclaim. The structure provided immediate insight into the relationship between DNA's structure and its function.

Watson and Cricks model work was based on X-ray diffraction studies by Furberg and Franklin and chemical work by Chargaff. The most important work was carried out Rosalind Franklin. Unfortunately, Rosalind died in 1958, only 37 years old, and she never earned the Nobel prize she deserved.

Watson and Crick presented the DNA model in the English Journal, *Nature* on April 25, 1953. The model was relatively simple and provided insight into how genes work and how hereditary information is transmitted.

It all started with Friedrich Miescher who discovered DNA more than 100 years ago. It was early realized that chromosomes contained genetic information, but it was first through the work of Griffith and Avery on bacteria that DNA was found to be the key molecule.

Friedrich Miescher
(1844-1895)

DNA was isolated, analysed and recognized as a unique macromolcule in1869 by Friedrich Miescher, Switzerland.
He studied leucocytes and was able to isolate undamaged nuclei free of cytoplasm. Miescher found that his new substance, which he called nuclein, contained 14 percent nitrogen, 3 percent phosphorus, and 2 percent sulfur. He concluded that the substance was not a protein.

Fredrick Griffith
(1879 – 1941)

In 1928, Fred. Griffith demonstrated that one type of Streptococcus pneumonia bacterium (called R) could inherit the properties of another type (called S) by attaining an extract from dead S-bacteria.
This "*transforming principle*" suggested that the extract contained the heredity molecule – and it was of great importance to identify it. This important work was carried out by Oswald Avery, Maclyn McCarty and Colin MacLeod in 1944.

Oswald T. Avery
(1877 – 1951)

Colin MacLeod
(1909 – 1972)

Maclyn McCarty
(1911 – 2005)

Oswald Avery (already emeritus, 67 years) worked with MacLeod and McCarty at Rockefeller Institute. They showed that the "effective" substance in Griffiths experiment was the DNA-molecule and that DNA is the carrier of genes in the cell.
We also have to mention that Alfred Hershey and Martha Chase in 1952 confirmed the conclusion in experiments with radioactive tracers (see page 194).

Today it is well known that DNA is the important molecule that it seems difficult to appreciate the magnitude of these scientific achievements. The above mentioned research paved the way for the intense experiments from 1951 – 1953 which resulted in the double helix model.

On the road towards the double helix we have to mention a couple of other scientists that made significant contribution.

In 1950 Erwin Chargaff found that a peculiar regularity in the ratios of nucleotide bases.
In the DNA of each species he studied, the number of adenines approximately equaled the number of thymine, and the number of guanines approximately equaled the number of cytosine. This suggests that the bases exists in two base pairs (Chargaffs rules):

A – T and G – C

Erwin Chargaff
(1905 – 2002)

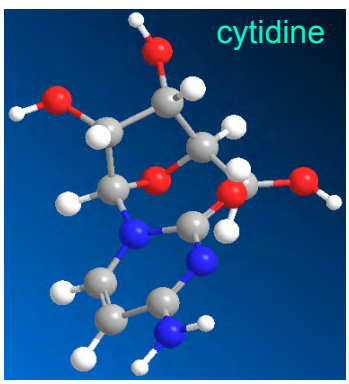
The physical method used was x-ray crystallography. In order to attain information of the structure of the molecule the sample should be in the crystalline form – preferably a single crystal.
In the way towards the DNA-structure we would mention two significant crystallographers and their work; namely Sven Furberg and Rosalind Franklin.

Sven Furberg worked on the structure of DNA components. In the work on cytidine (see the model of the molecule at the bottom of the page) he found that the base plane (the plane of cytosine) was perpendicular to the sugar molecule. Crick mentioned this observation in the following way; *"a remarkable achivement for the time"*.

Sven Furberg
(1920 – 1983)

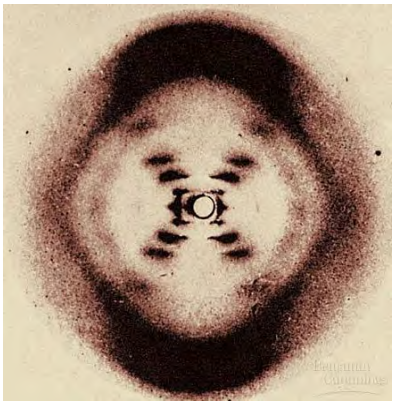
Based on the structure of cytidine, Furberg suggested a model of DNA consisting of a single stranded helix. In his model DNA was a long chain of sugar molecules (marked S) and phosphate groups (marked P) ; -S-P-S-P-S-P-. The base planes were perpendicular to the axis of the helix and the distance between the base planes was 3.4 A (1 Angstrom = 10^{-10} meter). This model was correct in most aspects but lacked the important idea of a double stranded helix.

Rosalind Franklin
(1920 – 1958)



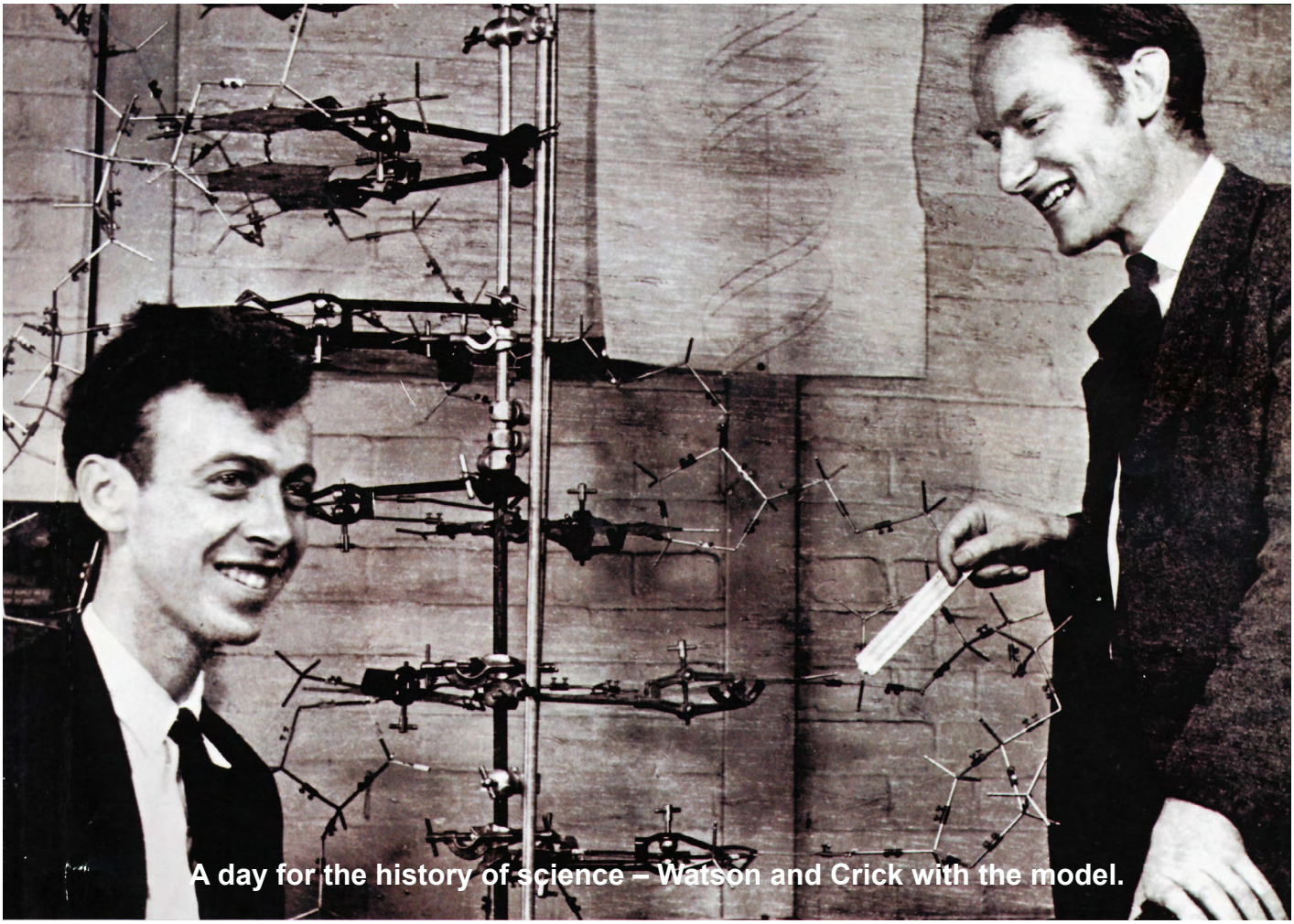
Rosalind Franklin was a brilliant crystallographer. She worked with DNA and was able to distinguish between two types of DNA depending on the hydration. Both types have a helical structure.

To the right is the famous picture (Photo 51) taken by Rosalind Franklin in 1952.



The diffraction image of DNA, observed by Franklin provided the key missing piece of information for Watson and Crick's discovery of the structure of the DNA molecule. The DNA-model they suggested is based on the work carried out by Chargaff, Furberg and foremost Franklin and the "Photo 51".

The last step towards the structure of DNA was carried out in the famous Cavendish Laboratory in Cambridge. This laboratory is connected to a number of signifacant discoveries and was headed by James Clerk Maxwell, Lord Rayleigh, J.J. Thompson, Erenst Rutherford and from 1938 Lawrence Bragg. He was an excellent X-ray crystallographer and the laboratory studied the structures of biological molecules – like myoglobin and hemoglobin (Kendrew and Perutz). In this laboratory Francis Crick already worked when James Watson came from the United States and they started to build models of the DNA molecule. In the spring of 1953 they arrived at the model shown in the famous picture below.



A day for the history of science – Watson and Crick with the model.

They published the model in the paper "*Molecular Structure of Nucleic Acids*" in *Nature,* in 1953, The paper was only a single page and few, if any papers have had such an impact. Watson was only 25 years old and Crick was 37.

The important difference between this model and the model suggested by Furberg is that it is a ***double helix.*** This fact is important for replication. Watson and Crick expressed it as follows; "*It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material".*

DNA structure

DNA is a long molecule (a polymer) which has 6 different building blocks, the 4 bases; cytosine (C), thymine (T), guanine (G) and adenine (A), as well as a sugar molecule (S) and a phosphate group (P). Phosphate, sugar and a base form a nucleotide and DNA is made up of two long chains of nucleotides.

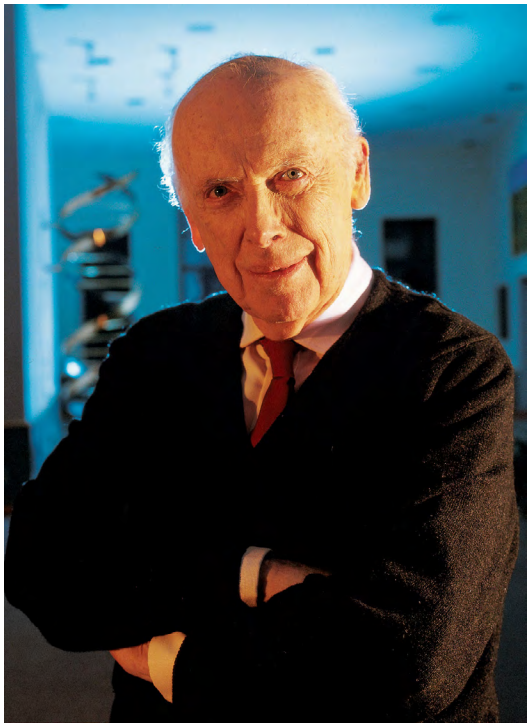
If all the DNA in a single human cell was tied together and stretched out, it would be approximately 2 meters long. One strand of DNA binds to the other strand through hydrogen bonds that extend between base pairs. Thus, C binds specifically with G and A binds with T forming the C – G and A – T base pairs.

The human double helix contains about 6 billion base pairs. Three adjacent bases (a triplet or codon) on one strand code for a certain amino acid in a protein. If a protein consists of 200 amino acids, the DNA that codes for this protein consists of at least 600 bases, *i.e.*, 200 triplet codons. ***This is a gene.***

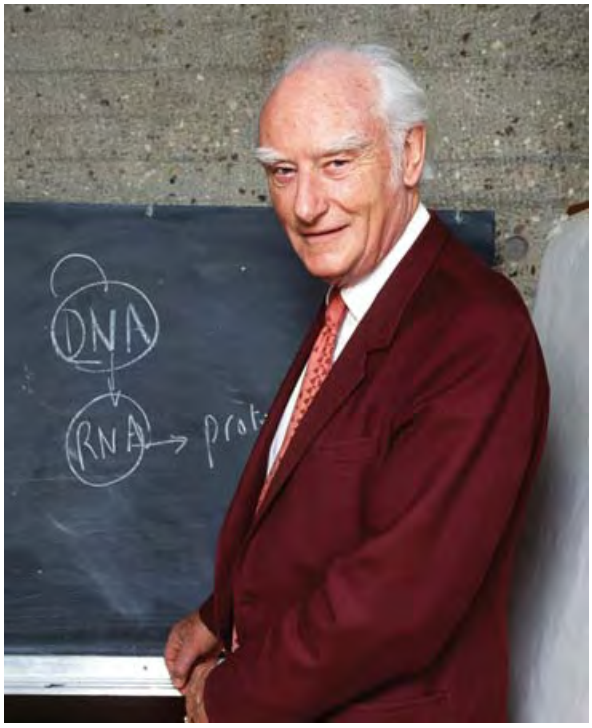
If an error arises in one of the bases, a "wrong" amino acid may be inserted into the protein. This may change the properties of the protein. An example of an error of this type can cause "sickle cell anemia", a dreaded sickness mainly found in Africa.

Human beings vary because of differences in the DNA base sequence. This can be demonstrated in so-called DNA-tests. Such tests are now used for positive identification of people (for example in criminal court cases).

Scientists around the world are engaged in research on how external agents such as radiation and chemicals induce changes in DNA. Some changes kill the cell, others change the cell and cause cancer, while other changes are without observable effects.



James Watson
(1928)



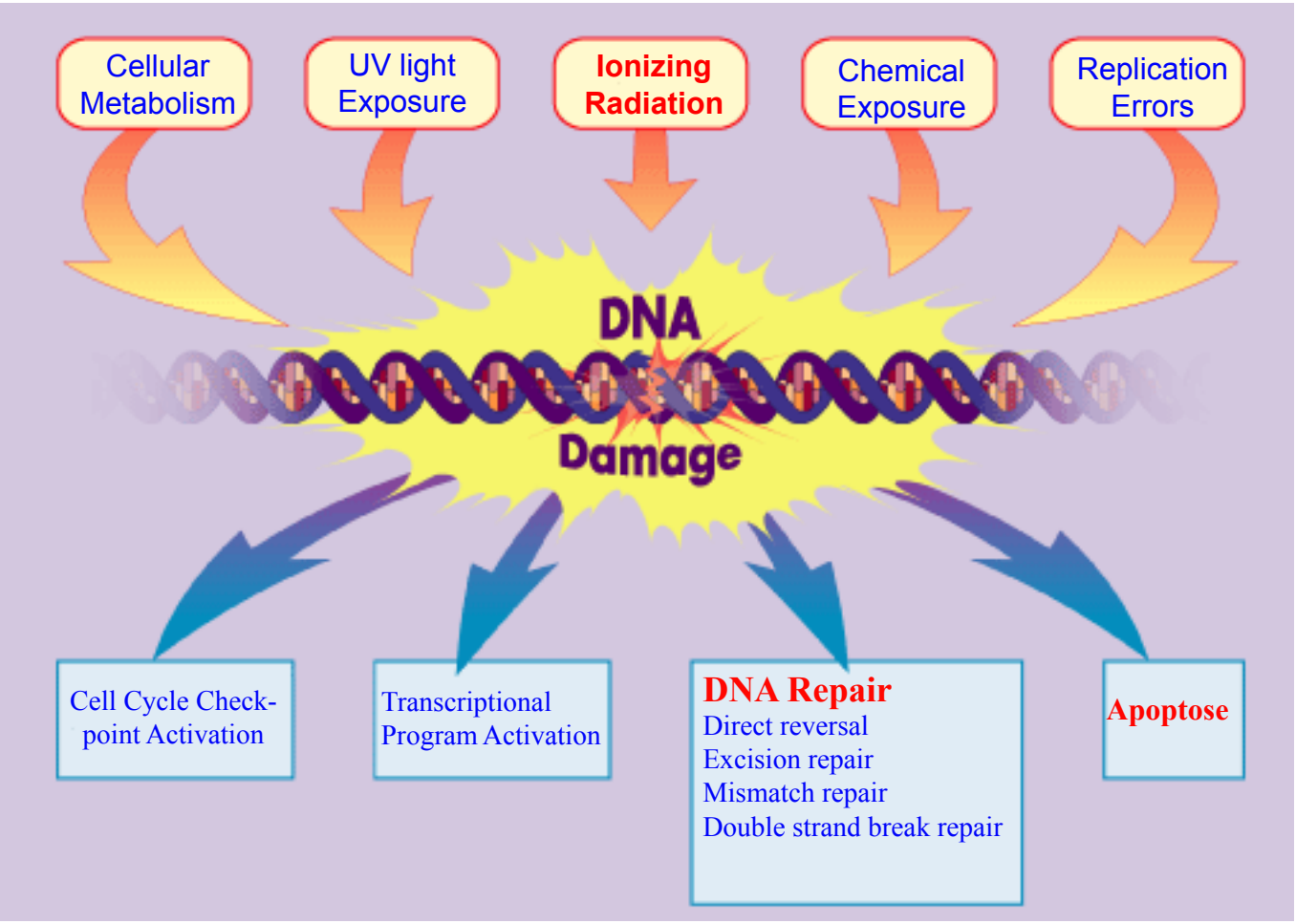
Francis Crick
(1916 – 2004)

In a human cell, the DNA-thread is packed into 46 units (the chromosomes). With the use of particular methods it is possible to study the chromosomes under the microscope when the cells are in division (in mitosis).

Growth takes place by division of cells. Each cell goes through a cycle and, before division, the content of DNA must double. The goal is to make the new DNA identical to the old DNA in the *replication process*. It is in fact, fantastic to realize that very few significant errors normally arise. Some errors actually arise routinely but they are immediately recognized and repaired. We shall return to the repair mechanisms and other defense mechanisms in the next chapter.
If an error arises that is not repaired or if it is misrepaired and the cell still divides, we have a mutation. If the mutation occurs in an ordinary cell in the body it is called a *somatic mutation*. If the cell is capable of reproduction, it may lead to cancer.

A mutation in a sex cell is called a *genetic mutation*. Such mutations can take place spontaneously and people have for a long time speculated about the mechanisms. One way of producing genetic mutations is by radiation.

The evolution of species requires mutations. Thus, a slow development of the species is based on accidental mutations. Research work seems to indicate that the frequency of the spontaneous mutations is increased by radiation and it has been a long range goal to determine the dose which doubles the mutation rate for animals and humans. This is called the *doubling dose (see chapter 13)*.



An illustration of how different causes can give damage to the DNA-molecule. The largest number of damages comes from the life processes going on (cellular metabolism). This damage is called endogeneous damage. Damage from UV, ionizing radiation and chemical substances are called exogeneous damage. In the following we shall concentrate on the damage caused by ionizing radiation.

Radiation damage to DNA

The most important types of damage to the DNA molecule, induced by radiation, are outlined in the figure below. There are four common types of damage:

1. Single strand breaks

A single strand break is simply a break in one of the sugar-phosphate chains. This damage is usually simple to repair and, in experiments, it has been shown that approximately 90% of the single strand breaks are repaired in the course of one hour at 37° C.

2. Double strand breaks

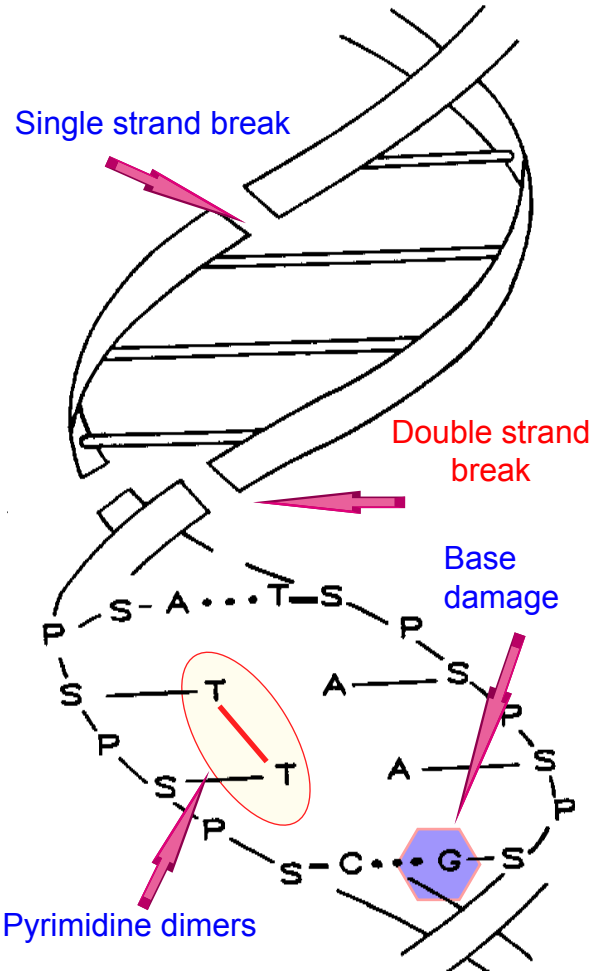
This type of damage involves both strands of the DNA helix, which are broken opposite to each other or within a distance of a few base pairs. This type of damage (also called a clustered damage) would kill the cell and in experiments with bacteria a correlation is found between double strand breaks and cell death (David Freifelder in the 1960-ties).

Double strand breaks are more difficult to repair correctly. However, they actually are. The DNA-molecule is packed together with proteins supporting the structure and preventing the pieces from falling apart, even when breaks occur on both strands of the helix. There are in fact a number of mechanisms that complex organisms (such as humans) have evolved for repairing double strand breaks. But as one might guess, this type of break is more difficult to repair and does correlate with cell death and observable damage to chromosomes.

3. Base damage

Experiments indicate that the radiation sensitivity varies from one base to another. After an initial ionization, rapid electronic reorganizations take place with the result that the damage is transported to certain regions of the macromolecule. The base guanine is particularly sensitive.

Damage to a base is one of the starting points for a mutation. If a base is changed, information may be lost or changed. As the result of a misrepair or no repair, the altered triplet codon is likely to lead to insertion of the incorrect amino acid in the protein. In turn, the changed protein might not function properly.



4. Pyrimidine dimers

Pyrimidine dimers are also examples of clustered damage. In this example, two adjacent bases, T and T, on the same strand have been chemically altered. This is but one out of a myriad of possibilities. All the possibilities have the common feature that two or more damaged sites lie in close proximity to one another.

We shall return to repair mechanisms, but would like to mention the problems posed by clustered damage. One of the strands is needed to replicate the adjacent strand. When both strands are damaged at the same site there is no template to work from. This is in contrast to damage such as a single base alteration or a single strand break.

EPR work at UiO

Einar Sagstuen

Eli Olaug Hole

The EPR-group at the University of Oslo has been engaged in studies on the damage to the DNA and its components, i.e. bases, nucleosides, nucleotides and larger fragments. The studies have included work at temperatures from that of liquid helium and up to room temperature.

A large number of students working for their master degree and dr. degree have been guided by Einar Sagstuen and Eli O. Hole. You can read about the work and the results gained on the homepage of the ESR laboratory. We shall give you a couple of examples.

So far you can see the work carried out by students for their master thesis and PhD. thesis in the historical overview – written in Norwegian. The address is:

http://www.mn.uio.no/fysikk/forskning/grupper/biofysikk/biofysikk_miljofysikk_historikk.pdf