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From energy deposition to cancer

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The quantitative knowledge of radiation-induced damage and cellular response mechanisms should allow us, at least in theory, to derive risks and dose/response relationships beyond the area covered by epidemiological and experimental (animal) studies. Despite important advances in our insights into cell transformation and progression, molecular genetics and cell biology have not yet accomplished this task, and will not do so in the near future. The main reason is the enormous complexity of the genetic and epigenetic mechanisms acting day by day in the billions of stem cells present in every human organism. It is generally believed that there is a long, multistep process between primary damage in cellular components and the emergence of an overt malignancy years or even decades later. At least qualitative knowledge about many of these steps is accumulating rapidly. This contribution intends to give an overview of some of these new developments.

The health effects of ionizing radiation result from the modification and destruction of cellular components. The large energies released in a single beta- or alpha-decay amount to about 0.1 to 5 MeV (0.2 to 8×10^{-13} J). In comparison, the binding energy of typical chemical bonds in organic molecules amounts to only 300 kJ per mol, i.e. 3 eV or 5×10^{-19} J per single bond. A single beta or alpha particle in aqueous solution produces up to 150,000 ionizations and a still larger number of excitations in the short distance it travels. Biological response to such highly localized energy depositions is complex and depends on many parameters. The following basic principles of radiation biology give the main factors to consider[1]:

- About 70% of biological damage from low-LET (Linear Energy Transfer) radiation is due to indirect action of free radicals, and 30% to direct action on the target molecule. The indirect component of radiation is strongly modified by oxygen, radioprotectors and radiosensitizers.
- Cells from different tissues vary markedly in radiosensitivity. There is no systematic difference in radiosensitivity between normal and tumour cells. At doses up to about 1 Gy, low-LET radiations are relatively inefficient in killing cells. The survival curve bends, and has a steeper slope at higher doses.
- Radiosensitivity of cells varies according to their position in the cell cycle. In general, the sensitivity of mammalian cells to ionizing radiation is directly proportional to their rate of cell division, and is lower in highly differentiated cells[2]. Therefore, the cellular kinetics of tissues are important in their responses to radiation.
- The radiosensitivity of cells to low-LET radiation is greatly influenced by the presence or absence of oxygen. The response of cells to low-LET radiation can also be modified by sulfhydryl compounds which scavenge free radicals (radioprotectors) and by electron affinic compounds, such as nitroimidazoles, that substitute for oxygen and sensitize hypoxic cells (radiosensitizers).
- Repair of radiation damage, the role of oxygen, the presence of hypoxic cells and the use of radioprotectors and radiosensitizers are much less important for high-LET radiations, i.e. alpha radiation, than for low-LET radiations.

Radicals formed mainly by interaction of radiation with water molecules in the cell may react with critical structures such as the DNA of the cell nucleus, which carries the genetic code.

It is generally accepted that incorrectly repaired or unrepaired modifications of the DNA are the main cause of radiation-induced damage in cells and hence in organisms, although epigenetic effects of radiation, such as initiation of membrane lipid peroxidation[3], or loss of intercellular signaling mechanisms, such as gap junction-mediated transfer of messenger molecules[4], are also possible primary causes of radiation-induced cancer.

Four radiation effects can be distinguished at the cellular level (Table 1). Loss of proliferative capability becomes critical only when a high proportion of the stem cells of a functional unit is affected and hence displays a steep dose/effect relationship with a threshold in the range of sieverts. More subtle changes in the genome, interfering not with proliferative capabilities but with the regulation of cellular growth, may lead to the so-called late somatic effects, i.e. cancer. Loss or alteration of crucial genetic information in gonadal cells was shown in insects and rodents to result in an elevated risk of congenital diseases in future generations.

Tab. 1. Classification of cellular damage caused by ionizing radiation and the resulting effects on the organism.

Cellular Change	Threshold Existing	Dose > Gy	Effects on Organism
Cell death	yes	50	acute loss of body functions, death due to CNS (central nervous system) syndrome, vascular collapse $LD_{50/30}$ in humans
Loss of proliferative capacity	(yes)	3–5	loss of immune and barrier functions, death in weeks from break-down of immune system, intestinal linings; developmental defects during embryogenesis
Cell transformation (oncogene activation)	(no)	1	tumours, cancer after a latency period from years to decades
Cell mutation	no	0.1	changes in the DNA of germ cells increasing the potential for genetically caused defects in the offspring

Are there biopositive effects of ionizing radiation?

Before the elucidation of the genetic code, the fragile molecular structures involved, and the physical and chemical reactions initiated in these structures by ionizing particles, radiation and radioactive substances were considered to be magic drugs with many beneficial effects on biological systems. The well-known large energies involved in radioactive decay were seen as stimulants in situations of general fatigue or immune suppression, and a potential cure for many ailments of old age. In advertisements aimed at the general population, radioactive spas claim to this day that radon restores the function of hormone glands. From such a point of view, modern radiation biology and its leading proponents are seen as producing a picture which is too pessimistic and misleading, because the scientific community is concentrating its work mainly on the easily measured destructive effects of ionizing radiation on biological structures. On the other hand, and in defense against this accusation, molecular genetics and modern photobiology give no indication whatsoever that the mammalian cell may benefit in a direct way from the energy deposited by ionizing radiation.

Lately, several papers, reviews, and conferences have been devoted to the possibility of beneficial effects of low levels of ionizing radiation on organisms[5]. The hormesis (biopositive action of radiation) hypothesis may have its merits for the public debate on radiation effects by counterbalancing some extreme views on the dangers of environmental levels of ionizing radiation. However, the scientific foundations remain very weak. Much of the work cited in favour of hormesis was done at a time where the genetic code and the molecular workings of heritage and gene expression were not known. Like many other irritants, ionizing radiation does have stimulating effects. Beneficial effects of radiation doses up to the range of grays have been described, for example for the stimulation of cell proliferation. For long-lived organisms like humans the relevance of such effects found in simple biological systems is disputable. An important aspect of the problem is the definition of what is to be considered beneficial. Stimulation of cell growth may be seen as a promise of youth by some laymen but is linked to tumour progression in many experimental systems. Radiation effects on biological tissues are destructive in nature, because they unspecifically modify or destroy complex molecules which carry crucial information for the survival and proliferation of the system. Therefore, possible beneficial effects of ionizing radiation on biological systems discussed in radiation research are restricted to the induction of repair systems or their increases in efficiency, and to enhanced levels of immune surveillance or protective agents such as radical scavengers. If such protective systems would, after induction by ionizing radiation, not only lead to the error-free repair of the radiation damage but also take care of chemical and endogenous damage caused by the inherent instability of the DNA, which would have been unnoticed and unrepaired in the unirradiated organism, a net beneficial effect may be postulated. So far, no convincing evidence has been published to substantiate such models. But in view of the long chain of events between an alteration in the genome and the emergence of a visible malignancy, biopositive effects of small doses of ionizing radiation may well exist in some situations, especially in the presence of a multitude of DNA damaging agents.

Microdosimetric constraints of adaptive responses in cells

Biological systems are known to adjust to many deleterious agents and environments. Even for a physical agent like ionizing radiation, a multitude of defense mechanisms can be envisaged. Primary damage to critical structures produced by reactive radicals may be reduced by their inter-

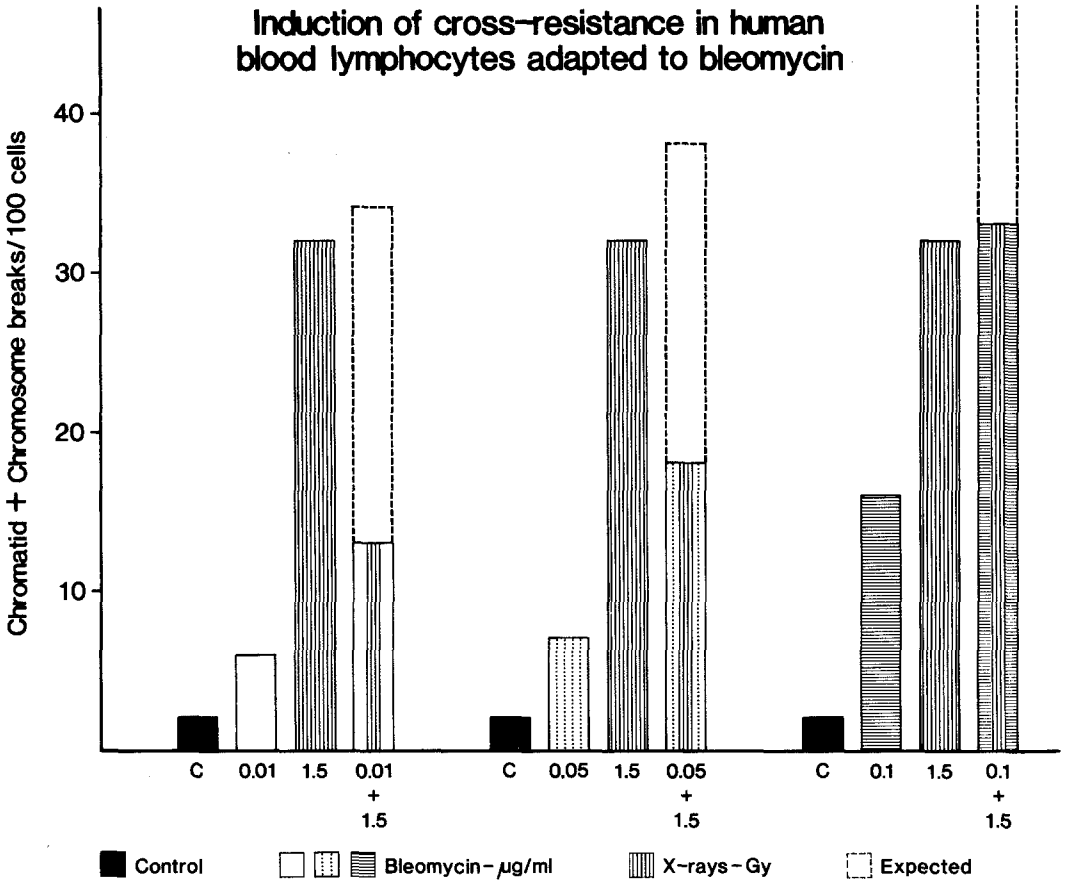

Fig. 1. Sparing effect of adaptive treatment in human lymphocytes: small concentrations of bleomycin (BLM) reduce the frequency of chromosomal aberrations induced by a subsequent challenge with 1.5 Gy X-rays[9].

ception with radical scavengers. Chemically reversible changes in the DNA may be restored more efficiently with the help of increased concentrations of hydrogen radical donors. DNA strand breaks, base modifications or losses may be repaired in a practically error-free manner by DNA repair enzyme complexes involved in cutting, synthesizing, ligating and proof-reading DNA. In bacteria, adaptive mechanisms such as the SOS response are known and the proteins involved (Lex A, Rec A, Uvr A, etc.) are well characterized[6]. Despite many earlier efforts with mammalian cells, it was only recently shown that peripheral human lymphocytes adapt to small radiation doses by becoming less sensitive to the induction of chromosome damage by subsequent high doses of ionizing radiation. This adaptive response has been shown to be triggered by X-ray doses as low as 0.01 Gy[7] or by tritiated thymidine at low concentrations[8]. In our laboratory, we also observed that a radiomimetic drug, bleomycin, elicits an adaptive response protecting against subsequent challenges by radiation or bleomycin[9]. In our view, this cross-resistance (Fig. 1) and microdosimetric considerations point to the presence of a more general stress response, which is probably of minor importance in affecting radiation risks from levels of exposure encountered in controlled occupational and non-occupational environments.

For peripheral lymphocytes, it is assumed that the adaptive response is induced independently in single cells when they encounter the passage of one or several electrons or other charged particles through their cell nucleus. With this assumption, microdosimetric considerations may define some of the criteria to be met by an adaptive dose, i.e. the minimal radiation dose to the tissue (macroscopic dose in gray or joules per kg) needed for at least one energy-deposition event in the majority of the sensitive targets. The estimation of dose and dose rate at the level of single cell nuclei for environmental radiation levels, i.e. the time period between two consecutive insults, sheds doubt on the evolutionary advantage conferred to cells which display an adaptive response solely to counter the effects of ionizing radiation. In contrast to most chemical and physical insults to biological systems, energy deposition by ionizing radiation is extremely localized in time and space. Assuming homogeneous distribution of a toxic chemical like aflatoxin B_1, even extremely small doses of 1 µg per kg (1 ppb) still mean more than 10'000 molecules in the volume of a cell nucleus.

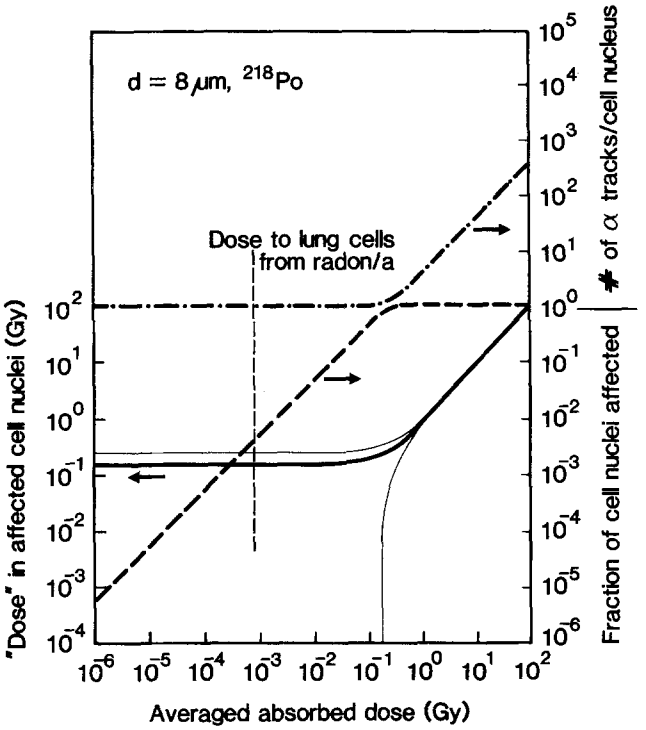

Fig. 2. Number of alpha tracks per cell nucleus of 8 µm diameter (> 200 mGy) and probability of being hit (< 200 mGy), respectively, in dependence of averaged absorbed alpha dose (based on Feinendegen[30]).

For ^{60}Co radiation, at doses similarly distant from the LD_{50} for acute effects (0.3 mGy), the probability of an electron track passing through a cell nucleus is in the range of 10%. For the same dose of high-LET alpha radiation, this probability is again orders of magnitude lower. In other words, below a dose corresponding to one energy-deposition event, i.e. one particle track per cell nucleus, only a fraction of the nuclei will experience primary radiation effects (Fig. 2). In this definition, primary effects not only include direct hits but also damage produced indirectly by free radicals derived from water. The distance radiation-induced radicals may diffuse in soft tissue before decay is very short, i.e. in the range of a few nanometers. Therefore, dose spreading by diffusion of radicals is irrelevant on the level of cellular structures larger than 1 µm. In addition, only a tiny fraction of the DNA in the cell nucleus is exposed to a single passage of an ionizing particle. A crude estimate, based on a cylindrical volume around the electron track with a radius of 50 nm, indicates that only for about 0.001% of the structures in a mammalian cell nucleus there is any probability of physico-chemical interactions from a single hit.

Several radiobiological inferences may be made from the inhomogeneities outlined above:

- The probability of hitting and damaging a specific DNA site, even an extremely labile one (i.e. chemically or enzymatically reactive), is very low per passage of electron or alpha-particle.
- Dose and dose rate for those cells that are hit are quite high even at environmental exposures of one to a few mGy per year.
- The enzymatic systems involved in the repair of radiation-induced DNA damage are activated only a few times during the life of an organism to handle an insult from ionizing radiation.
- Even in long-lived organisms like humans, most nuclei of stem cell lineages will never encounter the passage of an alpha-particle.
- The highly localized area of damage inside a cell nucleus resulting from a single passage probably means that only a minor fraction of the cellular and nuclear repair capacity is involved in the restoration of DNA integrity after a single hit.

To postulate a specific adaptive response to ionizing radiation, the mechanism has to confer increased protection from DNA damage at environmental radiation exposures. These exposures were quite stable over millions of years, and are in the range of 1 to 5 mGy per year for electrons resulting mostly from gamma radiation. Internal alpha decays lead to maximal doses of a few mGy in the most affected cells of the tracheo-bronchial tree. At the level of a single cell, an adaptive response that may last for a few days or cell cycles[10] will not confer any protection for the next hit, which comes months to years later. At least for ionizing radiation at environmental exposure levels, any additional energy and protein synthesis requirements of the adaptive response will be wasted. This situation is shown graphically in Fig. 3 with a logarithmic time scale, giving the time periods between two consecutive hits as multiples of an assumed adaptation period of 5 days.

In conclusion, it may be stated that due to microdosimetric constraints, adaptive mechanisms are probably not a major factor in determining the biological effects of environmental and occupational exposures to ionizing radiation. On the other hand, the low hit probabilities which form the base of this assessment, should not be used as an argument for the risk potential of ionizing radiation being low. At least for transformation events and clonal tumours, the low hit probability per single cell has to be multiplied by billions of stem cells.

Stem cell transformation and promotion

The induction of radiogenic cancer is often considered an all-or-nothing response, i.e. is assumed not to be graded in severity. This is an oversimplification because tumours may be graded histologically and clinically in severity, i.e. in mortality risk and swiftness of progression. Nevertheless, radiogenic tumours seem to be crucially dependent on initiation events whose frequency

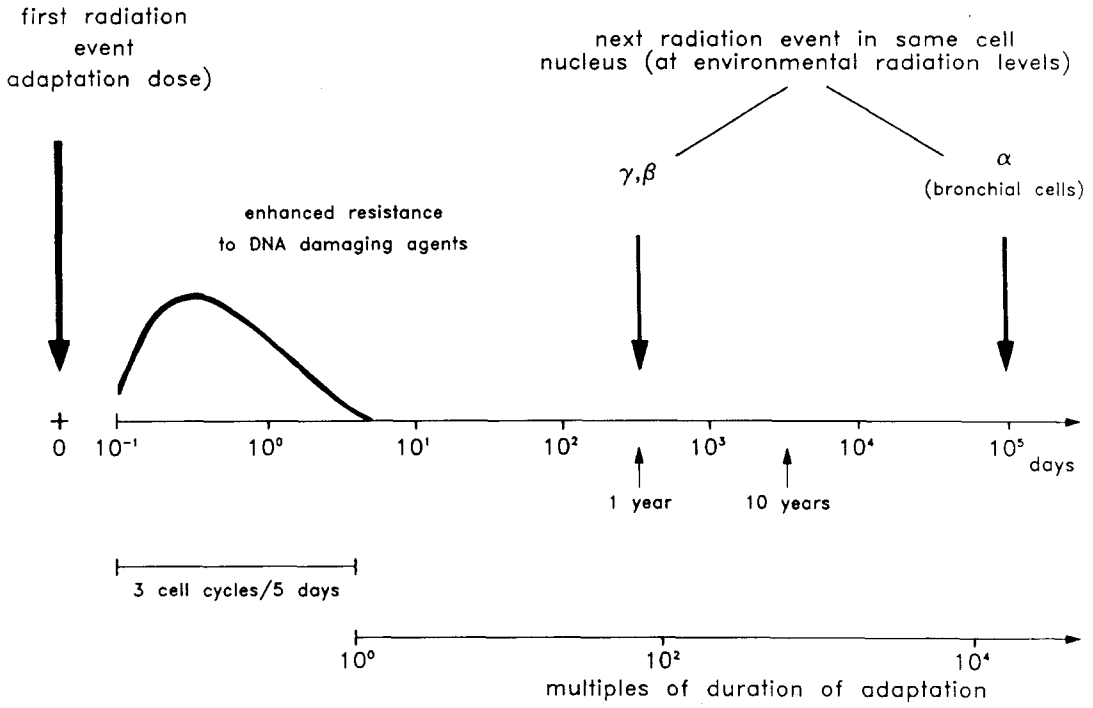

Fig. 3. Temporal relationship between the estimated duration of adaptation and the next radiation event in the same cell nucleus under environmental conditions. Annual gamma-, X-ray doses of 2 mGy and alpha-doses to the bronchial stem cells of about 1 mGy (corresponds to 2.5 mSv H_e from radon daughter exposure) are assumed [31].

is a function of dose. The seemingly probabilistic nature of the dose/effect relationship for radiogenic malignancies has led to the use of the term "stochastic" for effects of this type. Such a notion contains an intrinsic assumption that interactions of radiation with biological targets relevant for the initiation of malignancies are based on chance and may occur over large ranges of doses. The postulate of a rare cellular event being at the beginning of the long response chain between the primary radiation damage and the clinical manifestation of malignancy has important repercussions on the theoretical predictions of the shape of the dose/effect relationship. Even small increments of dose would result in a finite increased probability of cancer formation.

Hypotheses and models

In the scope of this treatise, only an overview of the wealth of concepts brought forward to explain the late somatic effects of radiation can be given. This topic is covered in depth by the scientific annexes of the UNSCEAR reports [11,12]. They contain the background information and extensive lists of references for the hypotheses developed in this chapter.

Malignant cells are defined by certain characteristic features such as unrestrained proliferative growth, angiogenesis (the ability to attract blood supply), infiltration into neighbouring and distant tissues, and the evasion of attacks by the immune system. Such characteristics could be the result of genetic modifications, i.e. a somatic mutation, or result from epigenetic changes. Epigenetic factors, which do not lead to irreversible changes in the primary structure of the genetic code, could produce de-differentiation, activation and expression of normally suppressed genes involved in the production, binding or signalling of growth factors, inactivation of regulatory genes, or the loss of growth controlling cell-cell-interactions (gap junctions) between a transformed cell and its environment. It may well be that both genetic and epigenetic factors have to act in parallel to let a cell escape the division-restraining signals of its environment [12].

Given our incomplete understanding of the origin of cancer, it is not possible to develop quantitative predictions of the effects of ionizing radiation on an organism from basic principles. However, quantitative models of cancer induction based on simplified hypotheses may help in understanding the basic issues involved [13]. Most models are based on the concept of multi-stage carcinogenesis, developed first as a model for skin cancer in mice [14,15]. The first step, initiation, is generally considered to occur in the genome of a single cell, although the involvement of a cluster of several cells cannot be excluded at this stage. Further steps towards an overt malignancy are generally assumed to be epigenetic in origin. They are often covered by the terms promotion and progression. An alternative hypothesis can be

based on two initiation events, e.g. the subsequent activation of two genes from different classes of oncogenes in the same cell, or the activation of an oncogene followed or preceded by the loss or inactivation of a tumour suppressor gene. Many cellular phenomena may also be relevant in the development of a transformed cell. At the level of the DNA, any change affecting the primary structure and hence its function may be of relevance for growth control. Point mutations, transpositions, deletions, translocations and transfections may all lead to the development of a malignancy.

At least in the case of oncogene activation, all these genetic changes have been shown to induce transformation. In addition to the direct and indirect induction of DNA lesions resulting from charged particles and radicals formed by ionizing radiation, misrepair both in the so-called error-free and error-prone repair pathways, or premature DNA replication before the termination of repair processes, may lead to fixation of defects in DNA structure. The dose/effect relationship of all these possibilities are probably quite different. Somatic mutations in the classical sense show induction coefficients, for low-LET radiation, in the range of 10^{-5} to 10^{-6} Gy^{-1} per locus and cell at risk. In vitro cell transformation is even more easily induced (typical induction coefficients are in the range of 10^{-2} to 10^{-4} Gy^{-1}). This is in marked contrast to the yield of malignant tumours per irradiated cell in vivo, which is many orders of magnitude lower than would be anticipated from such in vitro coefficients and the number of proliferation-component stem cells present in the different tissues of large multicellular organisms like humans. A direct link to cell transformation in vitro would also predict that larger organisms with correspondingly larger pools of stem and dividing precursor cells should be more prone to cancer than small organisms. However, rather than more cancer being found in larger mammals, annual incidence rates per unit carcinogen dose seem to be inversely related to the life span, which is generally longer for larger species. Therefore, the critical lesion may consist of a very rare event such as a "tumour-specific" chromosomal translocation. It is also conceivable that the majority of the initiated cells or clones may be lost or actively destroyed at different levels of the promotion process, which takes place during a long latency period, typically prior to the appearance of a clinically recorded tumour. Secondary influences, such as endogenous or exogenous growth factors, e.g. female steroid hormones in breast tissue, may increase the probability of an intitiated cell passing through some stages of promotion and progression, whereas other substances like the oligopeptide antipain[16] were shown to inhibit or even reverse this process in cell cultures (Fig. 4).

In view of the many changes in stem cell during the multistep development of cancer, it is not surprising to find wide variations in carcinogenicity in vivo. However, even in the presence of a multitude of promotional steps and host dependent influences at every level, the primary event of initiation may still be the rate limiting step, and therefore the decisive factor for the shape of the dose/response relationship. This contention is supported by comparative studies showing that risk coefficients for cancer induction derived from different exposure situations and tissue status were found to be quite similar (for an example see Fig. 5). Dose rates were considerably different between the single, acute exposure experienced by the survivors of Hiroshima/Nagasaki and the fluoroscopy group. The therapeutic radiation doses given to the mastitis group were delivered to an inflamed breast tissue. Despite all these confounders, the three populations show similar risk coefficients. The general shape of the dose/response relationships suggests linearity. This striking finding may imply that for some forms of malignancies, the number of stages in tumorous evolution may be low or that ionizing radiation is changing the probability of a late or even the last event in the multi-stage development of cancer.

The two best studied host factors involved in the promotion of transformed cells are endocrine status and immunological surveillance. The hormonal dependence of carcinoma of the breast, ovary and thyroid is well known. Endogenous growth stimulation can be considered as a promotional factor in multi-stage cancer induction models. On the other hand, inactivation of endocrine glands should lead to the impairment of malignant growth. The low incidence of female breast cancer in those survivors of Hiroshima/Nagasaki who had received the highest doses is thought to be a direct effect of radiation-induced impairment of hormone glands. Since functional defects occur only at elevated doses which cause pronounced cell death, any modulation of the tumorigenic response in vivo due to damage of endocrine control organs by ionizing radiation would show a high threshold in the range of one to several Gy of acute dose. The importance of immunological suppression of precancerous cells or clones formed by ionizing radiation remains to be elucidated. Studies on the influence of immunological surveillance on the induction of leukemia in mice[18] and lung cancer in rats[19] do not suggest that this host factor is decisive. On the other hand, stimulation of the immune system by exposing animals to antigens clearly increases the incidence of myeloid leukemia in preirradiated animals (Fig. 6).

For the formulation of a quantitative cancer risk model at low and moderate doses of ionizing ra-

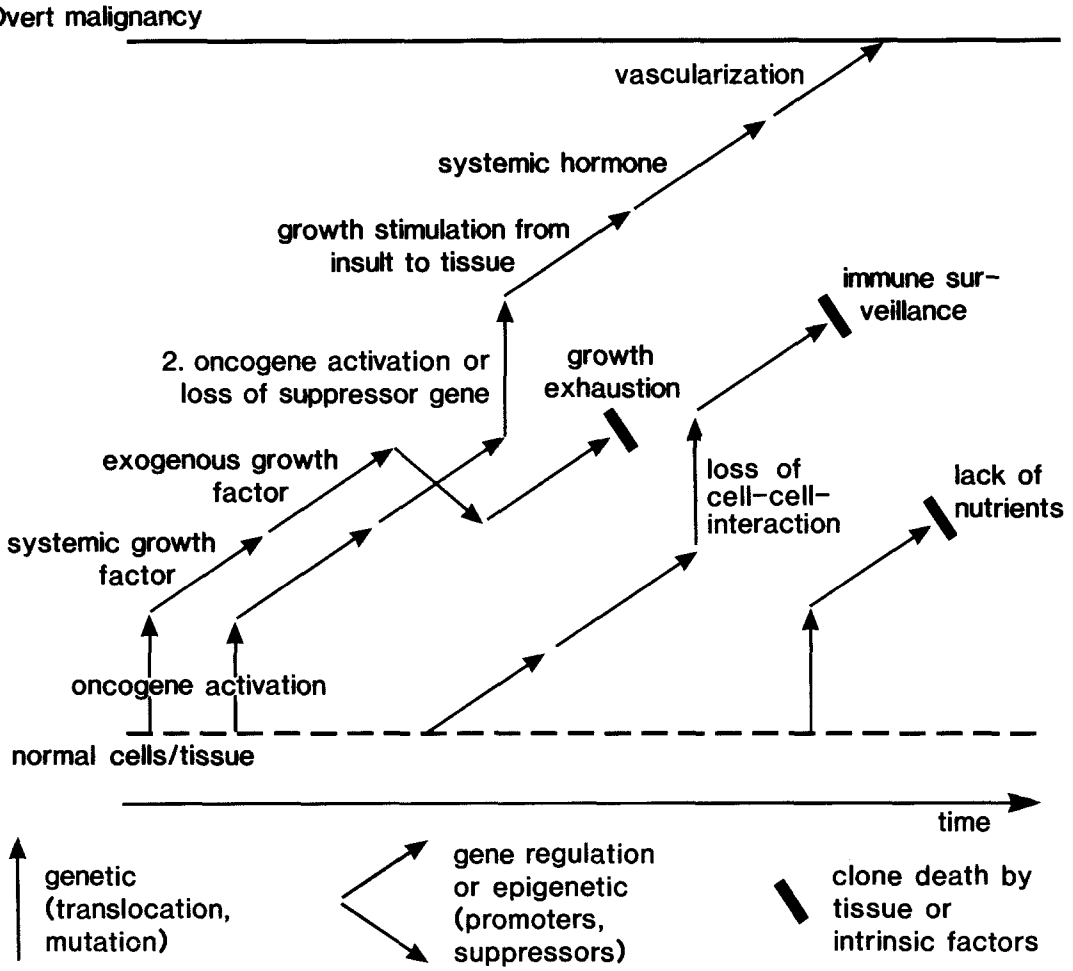

Fig. 4. A model for the multi-stage development of an overt malignancy. Some of the factors are purely hypothetical. The listing of factors is not meant to be complete[31].

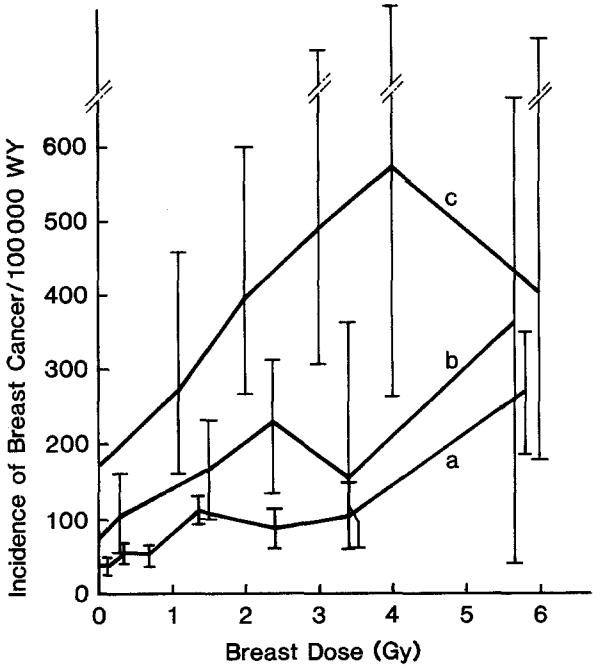

Fig. 5. Dose responses for radiation induced breast cancer in different human populations[17].
a) atomic bomb survivors (corrected by a factor of 1.5 for DS86 dose changes)
b) Massachusetts fluoroscopy
c) mastitis patients.

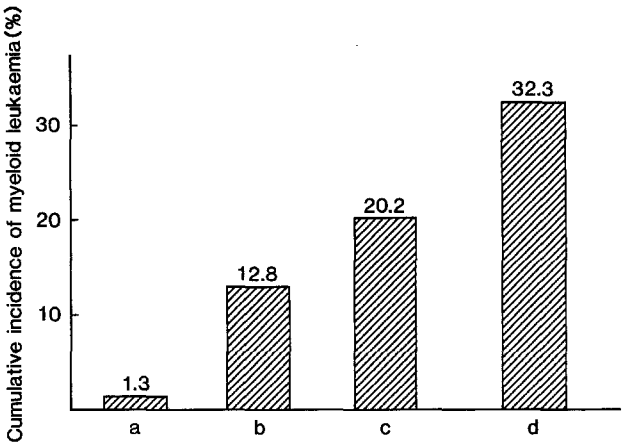

Fig. 6. Dependence of cumulative incidence of myeloid leukemia in irradiated Rf mice on the bacteriological status. Dose was 300 R (about 3 Gy) and the animals were followed up to the age of 2 years[20].
a) mice maintained permanently germ-free
b) germ-free mice which were conventionalized after irradiation
c) germ-free mice which were microbially shocked after irradiation
d) conventionally maintained mice.

diation, the following prerequisites have to be met[12,21]:

a) **Probability of malignant transformation of a defined target cell.** This will vary with the organ, dose, quality of radiation, local oxygen pressure, and temporal pattern of dose distribution. In this respect, the model should include theoretical concepts concerning the nature of the initiating event(s) and not be purely empirical. Mathematical formulations should be compatible with general knowledge of radiation and cancer biology;
b) **Probability of interaction between transformed cells or between developing clones;**
c) **Probability of cell killing, i.e. loss of proliferative capability, with dose, as non-viable cells cannot give rise to cancerous clones.** This factor depends certainly upon the tissues concerned, some of which are particularly susceptible, and upon dose, dose rate and quality of radiation and on other irradiation conditions;
d) **Effect of numerous host factors.** Among promoting factors, cell division is a good example. Enhanced proliferation can be induced by various mechanisms, including radiation-induced cell death, a phenomenon which is likely to produce a threshold-type dose response. Other factors could be the differentiation of initiated cells, immunological surveillance that suppresses development of malignant clones with antigenic characteristics foreign to the host-system, and hormonal influences;
e) **Effect of exogenous promoting agents.** These lead to more rapid appearance of tumours, to an increase in tumour yield, and perhaps also to changes in the shape of the dose/response curves, as shown by several in vitro experiments on oncogenic transformation.

Oncogene activation and loss of tumour suppressor genes

Ionizing radiation is a poor mutagen on the level of point mutations but is well known to produce double strand breaks in DNA, with a yield of about 40 per cell and Gy (low-LET) in human cells[22]. Double strand breaks may lead directly to gene rearrangements such as translocation, deletion, and probably also gene amplification. In general, cancer cells show a multitude of such chromosomal changes. The functional effects of these aberrations may be classified into two groups:

a) The induction or enhanced expression of gene products involved in growth and differentiation, as well as the modification of precursor genes, proto-oncogenes, is called oncogene activation. Such changes are often dominantly expressed.
b) The loss of genes or gene products involved in the control or suppression of proto-oncogenes is a group of modifications that has attracted interest more recently. Genetic changes of this kind are in general recessive.

Screening for malignancies and for genetic changes in malignant cells led to the postulation of a second class of genes whose expression inhibits or blocks transformation. The hypothesis of "cancer suppressor genes" was first based on indirect evidence from hybridization experiments. Malignant cells fused to normal cells appear to be benign, as though malignant behaviour is a recessive trait. The emergence of tumorigenic clones in hybrid cells is correlated with the loss of specific chromosomes of the normal parent cell, e.g. hamster chromosome 15 in v-H-*ras* expressing hamster embryo fibroblasts. Tumour suppressor genes are best defined for retinoblastoma and Wilms tumour[23].

Hypotheses on dose/effect relationships in radiation carcinogenesis

Because doses at the work place or in the indoor environment are much lower than exposures known to produce measurable risks in human populations directly, the risk estimate for low-dose ionizing radiation is derived from dose-response functions. Mathematical models in use today are always based on cumulative dose. In addition, dose rate, age at exposure, age at risk, and time since exposure may be taken into account[32]. The fact that this is a crude simplification of a complex system with many additional variables should always be remembered. Although the extent of primary damage due to ionizing radiation is less variable per unit dose than, for example, the effect of a procarcinogen which may need metabolic activation and transport through membrane barriers, the detriment to health from low doses of ionizing radiation is still dependent on many poorly known variables. The number of division-competent stem cells affected, the presence of other mitotic stimuli, and the levels of endogenous hormones which may act as promoters of transformed cells in the target tissue, are examples of variables to consider.

Radiation quality Besides the dose, various other physical factors, such as quality of radiation and dose rate are of importance for the shape of the dose/response curve. Radiation is often classified into low-LET and high-LET to describe biological effects since the quality of the radiation, i.e. the linear energy transfer, which is a measure of ionization density along the track, is a major determinant of fractionation and protraction effects. Under environmental conditions, the former class is represented by beta particles and gamma/X-rays, the latter by alpha particles and neutrons.

Low-LET radiation At the microscopic level, beta- and gamma-radiations produce similar ion-

ization patterns. Gamma photon energy is first transferred to one or several electrons which then deposit energy in the tissue by producing ionizations and excitations along their path, i.e. similarly to an electron resulting from a beta-decay. A large difference exists, however, in the macroscopic depth distribution of dose or penetration. External gamma- and X-rays reach much deeper into the body than beta-particles. This is the result of the relatively low probability of interaction for the photon in soft tissue. The ionization density along the electron path is in the range of 25 to 100 μm^{-1}. Given the fact that most of the radicals formed by the interaction of radiation with water, which is the major constituent of cells, travel only few nanometers, it is generally assumed that low to moderate doses of low-LET radiation are less efficient in producing the highly localized damage required for adjacent breaks on both strands of a DNA double helix leading to a double strand break. DNA single strand breaks are repaired efficiently and generally error-free, while DNA double strand breaks are critical for cell survival and chromosome modification because of their much slower repair and the much greater risk of misrepair, deletions or translocations. This is probably strongly influenced by the fact that correct repair of double strand breaks requires redundant information sited on the homologous chromosomes present in diploid or polyploid cells. For single strand breaks, the second undisturbed strand of the same DNA double helix provides the information directly. The mechanisms and enzyme complexes involved in the repair of double strand breaks are very complex and correspondingly slow and error-prone[6].

Figure 7 shows the spatial relationship between the critical structure of DNA and the ionization density at the beginning of the electron path from a ^{14}carbon decay[24]. Recoil and transmutation, i.e. conversion of the carbon atom into a nitrogen atom at the place of radioactive decay, only produces one single strand break or a base loss, leading to a temporary strand break during repair. Therefore, in view of the large average distances between consecutive ionizations, the probability of a beta-particle producing a double strand break along the path or even at the beginning of the track is quite small. Only the last few ionizations of an electron are clustered more densely, leading to a higher potential for complex local damage. This has considerable implications for the kinetics of repair and the effects of fractionation and protraction. Earlier hypotheses assumed that low-LET radiation mainly produces the critical double strand breaks by the interaction of two independent particles. Since the probability of two electron tracks interacting with the same DNA segment is proportional to the square of the dose, quadratic dose/effect relationships would result from such a model. Given the fast repair of single-strand breaks in mammalian cells, a quadratic dose rate/effect relationship should be seen. These notions were not confirmed by microdosimetric considerations which indicate very low probabilities that a target with a radius of only few nanometers will be hit by two independent electrons. Below 1 to 2 Gy the chance of such an event is practically zero. However the hypothesis cannot be refuted totally because it is possible that lesions quite far apart interfere with each others' repair, thus creating the possibility of an important quadratic contribution in the dose/effect equation for low-LET radiation.

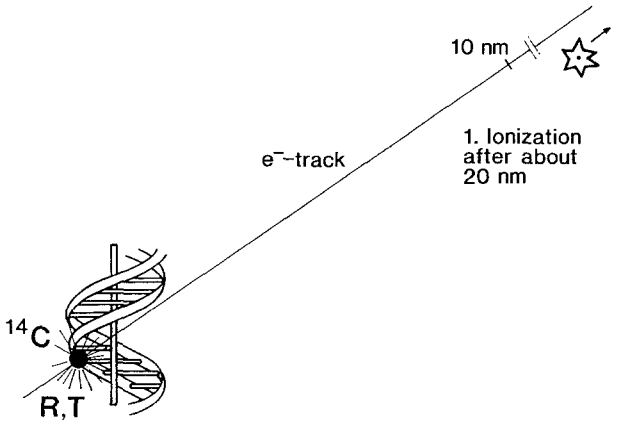

Fig. 7. Dimensions of the DNA double helix in comparison with the interaction density at the beginning of the electron track resulting from a ^{14}C decay ($E_{Average}$ about 50 keV). The local effects due to transmutation, T (i.e. conversion of a carbon into a nitrogen atom), and recoil, R, lead to one DNA single strand break[24].

An upward concave dose/response relationship is observed for sparsely-ionizing radiation in many animal experiments. In addition, a sparing effect of dose fractionation and protraction is generally seen for low-LET radiation. For lung adenocarcinomas in BALB/c female mice and for lung adenomas in RF mice, a quadratic term was only found at quite high dose rates, far above the environmental exposure rates[25]. New epidemiological findings based on the reassessment of the dosimetry, and – more important – a longer follow-up of the survivors in Hiroshima and Nagasaki, suggest that at least for female breast and lung cancer the quadratic term in the dose/effect equation is less important than was originally thought.

High-LET alpha-radiation Alpha-radiation with a typical path length in soft tissue of about 50 µm for an initial particle energy of 5 MeV clearly belongs to the high LET-radiation. The corresponding ionization density along the track is in the range of 2,000 μm^{-1} or 2 nm^{-1} (Fig. 8). If these values are compared with the dimensions of critical structures in the cell nucleus such as the

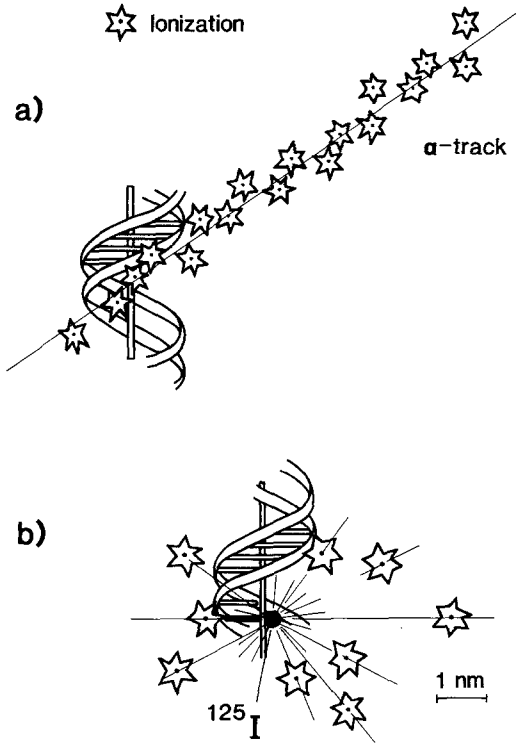

Fig. 8. Dimensions of the DNA double helix in comparison with the ionization densities along an alpha track (a) and at the site of a ^{125}I decay producing an Auger cascade with about 20 low energy electrons (b)[24].

DNA double helix, it becomes clear that a single alpha-particle may cause damage at almost any molecular endpoint which can be considered as the starting point of transformation. This means that the formation of double strand breaks, or even of two or three adjacent double strand breaks which may be needed for chromosome rearrangements, e.g. translocation of an oncogene to an active part of the genome, will follow single track kinetics, giving a linear dose/effect relationship. In addition, the energy deposition patterns would not predict any sparing effects from the lowering of the dose rate, from fractionation or protraction, because dose and dose rate at the level of the cell nucleus are barely affected by this shift. If a cell nucleus is hit by an alpha particle, the energy deposited in a fraction of a second amounts to about 0.4 Gy (or 8 Sv) averaged over the nucleus. Alpha-doses well below the value of 0.4 Gy lead to an extremely inhomogeneous distribution of dose at the level of the cell nuclei. A high-LET dose of 0.4 mGy (0.0004 Gy) per year to an organ means that only a tiny fraction of less than 0.001 of the cell nuclei are hit once per year with a maximal dose of 0.4 Gy and a dose rate of many kilograys per second. For low-LET radiation, the maximal energy for a cell nucleus hit once is considerably lower, i.e. in the range of 3 mGy or 3 mSv.

The pediction of linearity for high-LET effects based on microdosimetric considerations is also supported by results from animal experiments. In contrast to gamma-, X- and beta-radiation, the incidence of tumours shows little dependence on dose protraction and fractionation for high-LET radiation[23]. Contrary to the sparing effect found in general with low-LET radiation, low alpha or neutron dose rates or fractionation lead in some test systems to an enhancement of effects as compared to a single high dose-rate exposure. In C3H 10T1/2 cells, Hill and coworkers found an increase in the transformation frequency of up to a factor of 9 by reducing the neutron dose rate from 0.38 Gy · min^{-1} to 0.86 mGy · min^{-1}[26]. Cell survival was not significantly affected by the shift in the dose rate. No convincing explanation of this phenomenon has yet been offered. Hypotheses brought forward include the induction of error-prone repair or the facilitation of the expression of "sub-effective transformation damage"[26].

Most of the epidemiological data from miners exposed to high-LET radiation from ^{222}radon and its decay products suggest a linear or even downward concave dose/effect relationship for low and intermediate doses[27,28]. On the other hand, an increase in the latency period with lower dose would result in an upward concave relationship especially for exposures received late in life.

As stressed before, the most important aspect of LET in the assessment of low level effects is the temporal and spatial distribution of radiation events in critical tissues. Based on microdosimetric data, a low dose of 10 mGy (1 rad) received over a time period of 10 years will translate into only a few events per cell nucleus. For low-LET radiation, 10 mGy produce about 7 electron tracks per cell nucleus; the average time period between two events in the same cell would be more than one year. For high-LET radiation, a macroscopic dose of 10 mGy does not even translate into one alpha track per cell nucleus in the affected tissue. Only a small probability of being hit, in the range of 4% for an 8 µm diameter volume, results. Dose rates at the level of the cell nucleus for low exposures of any LET always remain extremely high (above a mGy per second). Such microdosimetric considerations suggest linearity for genetic changes occurring in single cells. Since alpha tracks passing through a cell nucleus induce cell death (loss of proliferative capacity) with a high probability, localized stimulation of stem cells may occur even at low doses. Correlations of such epigenetic effects with dose or dose rate are not well known. Figures 9 and 10 give computer-simulated numbers of hits for six cell nuclei encountering environmental low-LET radiation, and for six stem cells below/in the bronchial epithelium of an uranium miner, respectively. From the indoor exposure, for the life periods before and after the mining experience at the limit

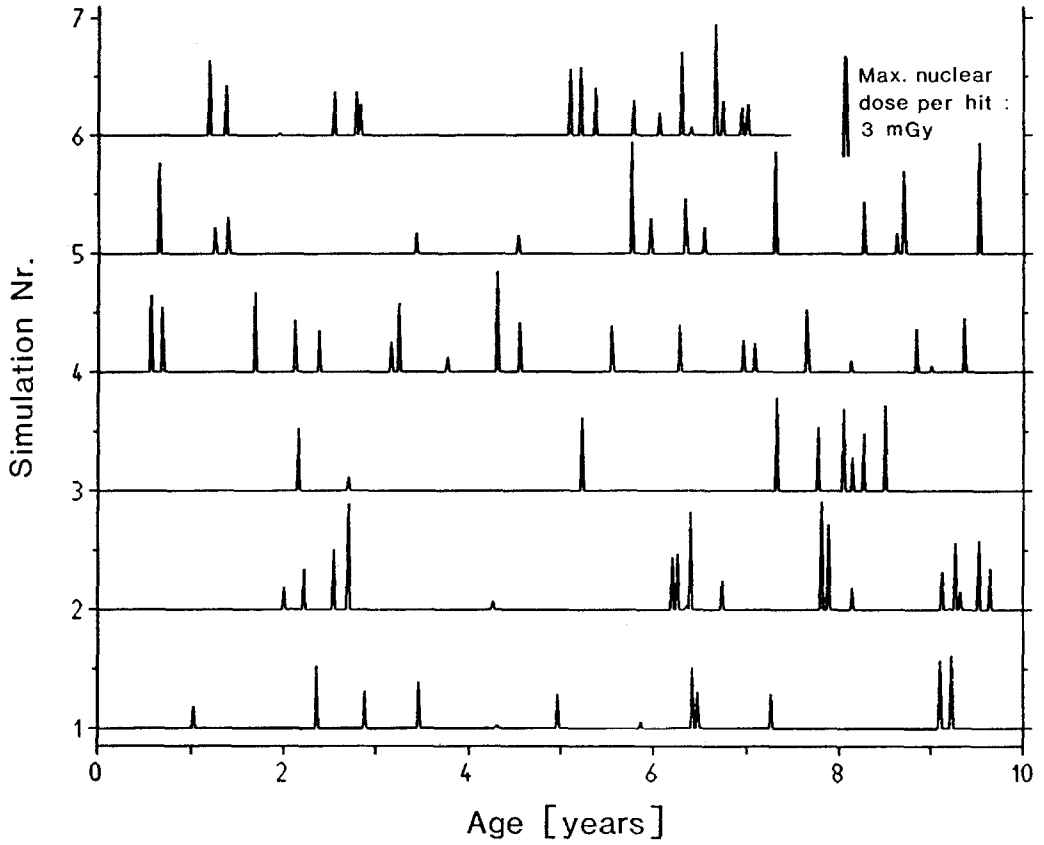

Fig. 9. Computer simulated hits for 6 cell nuclei over a period of 10 years from environmental beta/gamma irradiation. Macroscopic annual doses of 2 mGy and maximal nuclear doses of 3 mGy per hit are assumed.

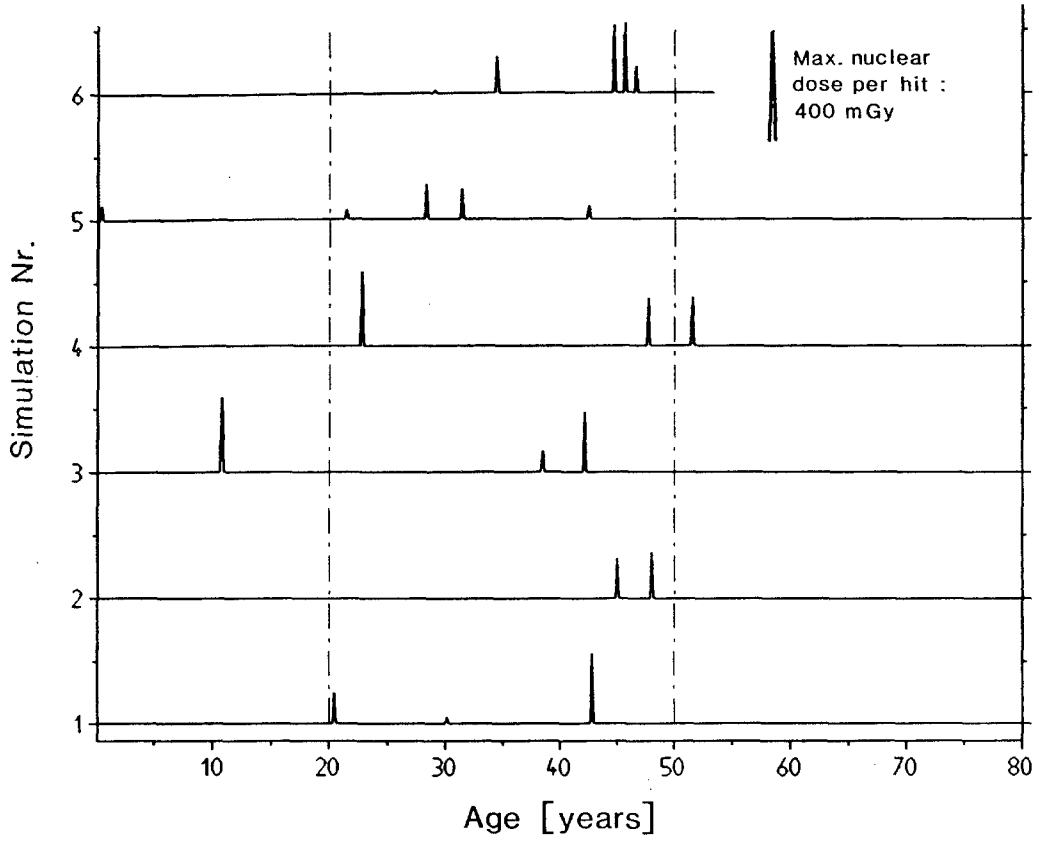

Fig. 10. Computer simulated hits for 6 bronchial cell nuclei over a lifetime from environmental and occupational radiation exposure. Model assumptions: 1 mGy environmental radon exposure to the lung per year (corresponding to about 2.5 mSv H_e), 20 mGy per year (50 mSv H_e) during occupational exposure between the age of 20 to 50, maximal nuclear dose of 400 mGy per hit.

for occupational exposure, it is seen that the majority of the stem cells in the most critically exposed parts of the lung will not even encounter one alpha track from indoor radon during a human lifetime of 80 years. The probability of being hit resulting from a radon daughter exposure of 160 mSv H_e (2 mSv per annum) is less than 50 percent [29].

Microdosimetric concepts do not only help in the quantification of doses and dose rates in critical compartments, they may also help in the understanding of epigenetic effects. Since the local cellular effects from alpha tracks are acute and non-stochastic, cell death and effects on cell proliferation may occur even at low doses. Figure 10 shows that bronchial stem cell nuclei exposed at the occupational limit encounter less than one hit per year, but typically several during an employment period of more than one decade. Under the assumption that many cells hit by an alpha track lose their capability for division, an exposure at the occupational limit already leads to profound changes in the repopulation kinetics of stem cells. This is an epigenetic aspect of alpha radiation which may have its influence on the dose effect relationship at low exposures, i.e. may lead to a deviation from linearity.

Summary

Recent progress in molecular biology, genetics and microdosimetry has considerably increased our knowledge of the mechanisms of radiation-induced carcinogenesis. However, as a result of the complexities involved in the many genetic and epigenetic changes in cells leading to the expression of malignancy only years or even decades after radiation exposure, risk coefficients for the quantification of health detriment still have to be derived largely from epidemiological data and animal studies. On the other hand, improved understanding of molecular and cellular mechanisms is increasingly important in testing and refuting hypotheses about the relative carcinogenic potential of different radiation qualities and dose rates, and of low-level exposures.

Résumé

De la déposition d'énergie au cancer

Les récents progrès en biologie moléculaire, en génétique ainsi que dans la microdosimétrie ont considérablement accru nos connaissances des mécanismes d'induction de gènes cancéreux dus au rayonnement. Cependant, le coefficient de risque pour quantifier les dommages à la santé, résultat de nombreux changements génétiques et épigénétiques très complexes dans les cellules, aboutissant seulement des années ou décennies après l'exposition aux radiations à des tumeurs malignes, doit toujours être déterminé largement à l'aide d'études épidémiologiques ou d'expérimentations animales. D'autre part, une meilleure compréhension des mécanismes moléculaires et cellulaires est de plus en plus importante pour tester ou réfuter des hypothèses concernant le potentiel cancérigène relatif des différentes qualités des radiations et des quantités de dose, ainsi que d'expositions à faible dose.

Zusammenfassung

Von der Energiedeposition zum Krebs

Die Fortschritte der letzten Jahre in Molekularbiologie, Genetik und Mikrodosimetrie haben unsere Kenntnisse der Mechanismen, die zum strahleninduzierten Krebs führen, stark erweitert. Trotzdem führt die Komplexität der vielen genetischen und epigenetischen zellulären Veränderungen, die erst Jahre oder Jahrzehnte nach der Exposition zu klinisch fassbaren Tumoren führen, dazu, dass Risikokoeffizienten für die Quantifizierung von Gesundheitsschäden heute immer noch weitgehend aus epidemiologischen oder tierexperimentellen Studien gewonnen werden müssen. Andererseits ermöglicht das verbesserte Verständnis molekularer und zellulärer Vorgänge in vermehrtem Masse, Hypothesen über das relative karzinogene Potential verschiedener Strahlenqualitäten, Dosisraten und von Niedrigststrahlung kritisch zu testen oder zu widerlegen.

References

1 *Chen DJ, Strniste GF, Tokita N.* The genotoxicity of alpha particles in human embryonic skin fibroblasts. Radiat. Res. 1984; *100*: 321.

2 *Bergonie J, Tribondeau L.* Interprétation de quelques résultats de la radiothérapie et essai de fixation d'une technique rationelle. C. R. Acad. Sci. (Paris) 1906; *143*: 983–985.

3 *Petkau A.* Protection and repair of irradiated membranes. In: Free radicals, aging, and degenerative diseases. Alan R. Liss, New York 1986.

4 *Löwenstein WR.* Junctional intercellular communication and the control of growth. Biochimica et Biophysica Acta 1979; *560*: 1–65.

5 *Luckey T.* Hormesis with Ionizing Radiation. CRC Press, Boca Raton, FL 1980.

6 *Howard-Flanders P.* Inducible repair of DNA. Scientific Am. 1981; *245*: 56–64.

7 *Wolff S.* Pre-exposure of human lymphocytes to 1 cGy (1 rad) of X-rays halves the amount of chromosome damage induced by subsequent high dose exposures. In: Proc. 8th Int. Congress of Radiation Research. (Fielden EM et al., eds.), Taylor & Francis, London, 1987; 212.

8 *Olivieri G, Bodycote J, Wolff S.* Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. Science 1984; *223*: 594–597.

9 *Vijayalaxmi, Burkart W.* Resistance and cross-resistance to chromosome damage in human blood lymphocytes adapted to bleomycin. Mutation Res. 1989; *211*: 1–5.

10 *Wolff S, Afzal V, Wiencke JK, Olivieri G, Michael A.* Human lymphocytes exposed to low doses of ionizing radia-

tion become refractory to high doses of radiation as well as to chemical mutagens that induce double strand breaks in DNA. Int. J. Radiat. Biol. 1988; *53*: 39–48.
11 UNSCEAR 82, United nations scientific committee on the effect of atomic radiation, ionizing radiation: Sources and biological effects. United Nations, New York 1982.
12 UNSCEAR 86, United nations scientific committee on the effect of atomic radiation, ionizing radiation: Sources and biological effects. United Nations, New York 1986.
13 *Whittemore AS.* Quantitative theories of oncogenesis. Adv. Cancer Res. 1978; *17*: 55–88.
14 *Farber E, Cameron R.* The sequential analysis of cancer development. Adv. Cancer Res. 1980; *31*: 125–226.
15 *Fry RJM, Ley RD, Grube D.* Studies on the multistage nature of radiation carcinogenesis. In: Hecker et al., eds. Carcinogenesis. Vol 7, Raven Press, New York 1982: 155–165.
16 *Little JB.* Influence of noncarcinogenic secondary factors on radiation carcinogenesis. Radiat. Res. 1981; *87*: 240–250.
17 *Boice JD, Land CE, Shore RE, Norman JE, Tokunaga M.* Risk of breast cancer following low-dose radiation exposure. Radiology 1979; *131*: 589–597.
18 *Mole RH.* Dose-response relationship. In: Boice JD, Fraumeni JR, eds. Radiation Carcinogenesis: epidemiology and biological implications. Raven Press, New York 1984; 403–420.
19 *Nolibé D, Masse R, Lafuma J.* The effect of neonatal thymectomy on lung cancers induced in rats by plutonium dioxide. Radiat. Res. 1981; *87*: 90–99.
20 *Walburg HE, Cosgrove GE, Upton AC.* Influence of microbial environment on development of myeloid leukemia in X-irradiated RFM mice. Int. J. Cancer 1968; *3*: 150–154.
21 *Trosko JE, Chang CC.* Environmental carcinogenesis: an integrative model. Quart. Rev. Biol. 1978; *53*: 115–141.
22 *Saenke AS, Gotlib VJ.* DNA Repair and radiosensitivity of cells. Studia Biophysica 1981; *86*: 73–74.
23 *Burck KB, Edison TL, Larrick JW.* Oncogenes: An introduction to the concept of cancer genes. Springer-Verlag, New York 1988.
24 *Burkart W.* Zur Gefährlichkeit der in das genetische Material des Zellkerns eingebauten Radionuklide ^{14}C, ^{3}H und ^{125}I: Effekte durch Rückstoss, Transmutation und Auger-Elektronen im Vergleich mit der Wirkung der beta- und gamma-Strahlung. EIR-Bericht Nr. 590, Würenlingen 1986.
25 *Ullrich RL, Storer JB.* Influence of gamma-ray irradiation on the development of neoplastic disease. III. dose rate effects. Radiat. Res. 1979; *80*: 325–342.
26 *Han A, Hill CK, Elkind MM.* Repair processes and radiation quality in neoplastic transformation of mammalian cells. Radiat. Res. 1984; *99*: 249–261.
27 International Commission on Radiological Protection, Lung cancer risk from indoor exposures to radon daughters. ICRP-Publ 50, Pergamon Press, Oxford 1987.
28 *Hornung RW, Meinhardt TJ.* Quantitative risk assessment of lung cancer in U.S. uranium miners. Health Phys. 1987; *52*: 417–430.
29 *Burkart W, Heusser P, Vijayalaxmi.* Microdosimetric constraints on specific adaptation mechanisms to reduce DNA damage caused by ionizing radiation. Rad. Prot. Dos. 1990; *31*: 269–274.
30 *Feinendegen LE, Hug O, Jacobi W, Oberhausen E.* Zur Toxizität inhalierter heisser Partikel, insbesondere Plutonium. In: Empfehlungen der Strahlenschutzkommission zu speziellen Fragen des Strahlenschutzes in den Jahren 1974–1984, G. Fischer Verlag, Stuttgart 1976.
31 *Burkart W.* Radiation biology of the lung. The science of the total environment 1989; *89*: 1–230.
32 Committee on the biological effects of ionizing radiations. Health risks of radon and other internally deposited alpha-emitters, BEIR IV. Washington: National Academy Press, 1988.

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