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Radiation Biology: A Handbook for Teachers and Students

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TRAINING COURSE SERIES 42

RADIATION BIOLOGY: A HANDBOOK FOR TEACHERS AND STUDENTS

TRAINING COURSE SERIES No. 42

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The Agency's Statute was approved on 23 October 1956 by the Conference on the Statute of the IAEA held at United Nations Headquarters, New York; it entered into force on 29 July 1957. The Headquarters of the Agency are situated in Vienna. Its principal objective is "to accelerate and enlarge the contribution of atomic energy to peace, health and prosperity throughout the world".

RADIATION BIOLOGY: A HANDBOOK FOR TEACHERS AND STUDENTS

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 2010

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FOREWORD

Knowledge of the radiobiology of normal tissues and tumours is a core prerequisite for the practice of radiation oncology. As such the study of radiobiology is mandatory for gaining qualification as a radiation oncologist in most countries. Teaching is done partly by qualified radiobiologists in some countries, and this is supplemented by teaching from knowledgeable radiation oncologists. In low and middle income (LMI) countries the teachers are often radiation oncologists and/or medical physicists. In Europe, a master's course on radiobiology is taught jointly by a consortium of five European Universities. This is aimed at young scientists from both Western and Eastern Europe, training in this discipline. Recently the European Society for Therapeutic Radiology and Oncology (ESTRO) initiated the launch of a radiobiology teaching course outside Europe (Beijing, 2007; Shanghai, 2009).

Radiation protection activities are governed by many regulations and recommendations. These are based on knowledge gained from epidemiological studies of health effects from low as well as from high dose radiation exposures. Organizations like the International Commission on Radiological Protection (ICRP) have put a lot of effort into reviewing and evaluating the biological basis to radiological protection practices. Personnel being trained as future radiation protection personnel should have a basic understanding of the biological and clinical basis to the exposure limitations that they are subject to and that they implement for industrial workers and the public at large. It is for these reasons that aspects of Radiobiology related to protection issues are included in this teaching syllabus.

In LMI countries, many more teachers are needed in radiobiology, and the establishment of regional training centres or special regional training courses in radiobiology, are really the only options to solve the obvious deficit in knowledge of radiobiology in such countries. Radiobiology teaching courses organized or sponsored by the IAEA are oversubscribed, and the students themselves confirm the great need for this type of teaching. Requests have been received from a number of countries in all regions asking for the IAEA to help organize radiobiology teaching. More qualified professionals are also needed for this exercise. Already there are some initiatives e.g. an IAEA project produced in 2007 a distance-learning course in the Applied Sciences of Oncology (ASO) for Radiation Oncologists *(also available on the IAEA-website since 2008*) including 10 modules in radiobiology.

This handbook for teachers and students was formulated based on the recommendations of a Consultants Meeting on International Syllabus for Radiobiology Teaching held 12-14 December 2005 in Vienna, Austria. Whilst this information is available in various books and other reports, it is summarized and collated here so that the whole document has a degree of completeness. This should be helpful in particular to those countries that do not have easy access to appropriate books and reports. Comments and suggestions on this syllabus as a teaching tool were sought from committees of the ESTRO and ASTRO (American Society for Therapeutic Radiology and Oncology).

This handbook is written in two parts:

(a) Teaching programme including a common basic radiobiology education and teaching programme for radiation oncologists, radiation therapy technologists, diagnostic radiologists, radiation biologists, medical physicists, radiation protection officers and other disciplines involved in radiation activities. This will take 1 week of teaching (30 hours), including a practical or tutorial session at the end of each day. This is followed by a further week of

advanced teaching for radiation oncologists, and a further 3 days for radiation protection personnel.

(b) Minimal Essential Syllabus for Radiobiology and two extra modules for radiation oncologists and radiation protection personnel, respectively. For each discipline, the basic module and an extra module would constitute the minimum essential syllabus and teaching requirements. It is hoped that this handbook for teachers and students will fulfil the needs of the Member States and serves the basis for regulatory requirements in these countries.

The IAEA officer responsible for this publication is J. Wondergem of the Division of Human Health.

CONTENTS

1. TEACHING PROGRAMME

A. MINIMUM ESSENTIAL MODULE FOR RADIOBIOLOGY (1 WEEK/30 HOURS)

Day 1 (including practical/tutorial 1)

Introduction

Physics and Chemistry of radiation interaction with matter

a) Interactions of electromagnetic radiations with matter, photoelectric effect, compton scatter, pair production, dependence on photon energy, dependence on Z (atomic number) of absorbing material, distribution of energy deposition (scale), half value layer

b) Interactions of particles with matter, electrons, energy dependence, alpha particles, neutrons

c) Linear energy transfer (LET)/Relative biologic effectiveness (RBE)

d) Definition of dose; gray (Gy)

e) Principles of dosimetry Ionization chambers, Themoluminescent dosimetry (TLD)

f) Radiation Chemistry of water

g) Formation and reaction of free radicals with oxygen, scavengers:

- Direct/Indirect effects of radiation on macromolecules
- Concept of chemical restitution/competition

Day 2 (including initiating practical/tutorial 2 and 3)

Molecular cellular radiobiology

a) Types of radiation lesions to deoxyribonucleic acid (DNA) and repair: base damage, single strand breaks (SSB), double strand breaks (DSB), mechanisms of repair, molecular role of e.g. protein53 (p53), ataxia teleangiectasia mutated gene (ATM)

b) Effects on chromosomes – use in biodosimetry

c) Radiobiological definition of cell death and cell survival

d) Manifestations of radiation-induced cell death (apoptosis, necrosis, mitotic catastrophe, senescence)

e) Survival curves and models, clonogenicity (main criterion), limitations of determination of cell numbers at a fixed time

f) Cell cycle: sensitivities in different phases, and cell cycle checkpoints

g) RBE – cell survival – change in slope and shoulder of survival curve, dependence of RBE on dose

h) Cellular repair: sub lethal damage repair (SLDR)/potential lethal damage repair (PLDR) cell survival, half time of repair

i) Dose rate effects: dependence on repair and proliferation

j) Chemical modifiers Oxygen effect: radiation sensitizers/protectors

k) Other cellular targets, e.g. membranes, mitochondria

l) Bystander effects at low doses

Day 3 (a.m.) (continuing of practicals/tutorials 2 and 3)

Tumour radiobiology including tumour growth and micro-environmental effects

a) Tumour growth characteristics; e.g. exponential growth

b) Dependence of tumour cure probability on dose, tumour size, fractionation, overall treatment time

c) Tumour stem cells/clonogenic tumour cell inactivation. Poisson statistics of tumour cure.

d) Time factor in radiotherapy

e) Palliative radiotherapy (tumour growth delay)

Day 4 (including practical/tutorial 4)

Normal tissue effects

a) Concept of damage manifested early versus late: underlying mechanisms e.g. oxidative stress and cell kinetics

b) Early effects:

- Clinical manifestation
- Time course and dose response, latency
- Hypoplasia due to cell killing
- Interacting factors: inflammation, cytokines
- Dose/dose-rate/time/fractionation dependence

c) Late effects:

- Clinical manifestation

- Time course and dose response, latency
- Dependence on fraction size
- Chronic inflammatory responses
- Micro vascular injury fibrosis
- Consequential late effects

d) Whole body exposure

- Radiation syndromes

Day 5 a.m.

Radiation Carcinogenesis

a) A-bomb survivors: leukaemia, solid tumours, dose dependence, dependence on age at exposure, concept of relative versus absolute risk

b) Mechanisms of multistage carcinogenesis. *In vitro* transformation, animal models, radiation-induced mutations

c) Dose response relationship, dose-rate and latency in humans, organ dependence, estimation of radiation risk

d) Definition of Sievert (Sv), organ weighting factors

Day 5 a.m.

Radiation Effects in Utero

a) Types of injury

b) Dependence on stage of pregnancy

c) Protection of the embryo

d) Dose response for mental retardation

Radiation Induced heritable damage

a) Mutations

b) Doubling dose

c) Risk estimation, single gene disorders and multi-factorial diseases

Practicals/Tutorials

a) Dosimetry with ionization chambers; shielding

b) Chromosome aberrations in irradiated lymphocytes (0-3 Gy) – dicentrics and micronuclei

c) Data analysis for cell survival curves; scoring colonies

d) Data analysis of *in vivo* fractionation studies: skin, Gastro-intestinal tract, kidney, spinal cord.

B. EXTRA MODULE FOR RADIATION ONCOLOGISTS (40 HOURS – INCLUDING 10 HOURS PRACTICALS)

Day 1 (including practical/tutorial)

Introduction

Physics

a) Dosimetry in radiotherapy

b) Depth doses for photons, electrons, protons and heavy particles (concept of Bragg peak), particle therapy

c) Isodose curves (fraction doses adding up, contrast with isoeffect curves, not linear), dose volume histograms

d) Boron Neutron Capture Therapy (BNCT), requirement for preferential boron uptake in tumour, concern re-vascular uptake, poor characteristics of penetration of thermal neutron beams

e) Physics of radioimmunotherapy, use of different isotopes, problems of tissue distribution, dose calculations

Molecular and cellular Biology

a) Principles of some common techniques e.g. immunoblotting, microarrays, proteomics (2-D gels)

b) Techniques to modify gene expression

c) DNA/Chromatin structure and function; (De)-methylation, (De)-Acetylation

e) Regulation of transcription, translation and post-translational modification, e.g. glycosylation, meristylation

f) Cell signalling – signalling cascades Receptor/ligand interactions; phosphorylation/dephosphorylation reactions

g) Oncogenes and Tumour suppressor genes

h) Mechanisms of action of some signal-transduction therapeutic Agents e.g. Epidermal growth factor receptor (EGFR) inhibitors, Ras inhibitors, Farnesyltransferase inhibitors (FTI).

i) Radiation effects on cell signalling, e.g. EGFR pathway

The cell cycle (and signal transduction pathways)

a) Cell cycle description

b) Methods to determine cell cycle parameters, e.g. flow cytometry – DNA staining and bromo deoxyuridine (BrdU)

c) Control of cell cycle: cyclins, cyclin dependent kinases (CDKs), cyclin dependent kinase inhibitors (CDKIs), role of p53

d) Radiation-induced cell cycle checkpoints

Day 2 (including practical/tutorial)

Cell death mechanisms

a) Radiobiological definition of cell death (loss of reproductive ability- reproductive death), abortive cell divisions after irradiation

b) Apoptosis – Developmental and stress induced, morphological and biochemical features, molecular pathways

c) Necrosis – Morphological, pathological, and biochemical features

d) Mitotic Catastrophe – Morphology

e) Cell senescence and radiation-induced differentiation

DNA damage and repair

a) Types of lesions and frequency per cell per Gy

b) Multiple damaged sites (clustered damage)

c) Types and Molecular mechanisms of DNA repair:

- Base damage
- Single strand breaks
- Double-strand breaks: homologous recombination repair (HR), non-homologous end-joining (NHEJ)
- Repair of cross-links
- Mutations affecting repair (ATM etc)
- Molecular responses to DNA damage (p53, ATM, etc)

d) Principles of assay techniques – elution, electrophoresis including comets, repair foci, plasmid-based assays

Other molecular targets

a) Membranes (Oxidative damage, lipid peroxidation, sphingomyelinase activation in endothelial cells).

b) Activation of stress response genes, radiation induced signal transduction

Cell survival curves

a) Colony formation assays versus cell viability assays

b) Dose-survival relationships

c) Linear-quadratic model; two component exponential model, definition of survival curve parameters

d) Sub-lethal and potentially lethal damage repair, half time of repair and incomplete repair, effect of unequal fraction size on repair

e) Dose rate and fractionation effects

f) Oxygen effect – level, time scale, mechanisms

g) LET versus OER and RBE; Radio-sensitizers, protectors

h) Low dose hypersensitivity, induced radio-resistance, mechanisms

i) Bystander effects, mechanisms

Day 3 (including practical/tutorial)

Tumour biology and host/tumour interactions

a) Growth kinetics of experimental tumours and cancer in patients, impact of tumour pathology, tumour progression, metastatic spread

b) Vasculature, angiogenesis and tumour microenvironment

c) Hypoxia – Oxygen measurement techniques, radiobiological-hypoxic fractions, acute/transient (perfusion-limited) versus chronic (diffusion-limited) hypoxia

d) Mechanism of reoxygenation, hypoxic cell radiosensitisers, bioreductive agents

e) Methods of correction of hypoxia-associated radioresistance in tumours: high LET radiotherapy, hypoxic cell radiosensitizers, increased oxygen concentration in breathing air, correction of anaemia

f) Tumour response assays – tumour cure 50 (TCD50), threshold dose (TD50), *in vivo/in vitro* colonies, tumour regrowth delay, (TGD), *in vitro* tumor models (e.g. spheroids), human tumour Xenografts and isogeneic/ transgenic mouse tumours

g) Differences between tumour types

h) Virally-associated cancers, molecular and biological basis to induction and radiation response of virally-associated cancers e.g. human papiloma virus (HPV) and cervix cancer, Epstein-Barr virus (EBV) and nasopharynx cancer, HBV and liver cancers, HIV and the Acquired Immuno-Deficiency Syndrome (AIDS)-defining and associated malignancies

Day 4 (including practical/tutorial)

Radiobiology of Normal Tissue damage

a) Early normal tissue damage:

- Pathogenesis in critical normal tissues (skin, G-I tract mucosa, bladder, bone marrow), kinetics/latency cell turnover and stem cell function, role of inflammation, cytokines, reactive oxygen species
- Dose response.

b) Late normal tissue damage:

Pathogenesis in critical normal tissues (Lung, heart, central nervous system (CNS), skin, kidney, liver, G-I tract, bladder, salivary gland) kinetics/latency cell turnover

- Role of inflammation, cytokines, reactive oxygen species
- Microvascular damage, fibrosis, ischaemia and atrophy
- Functional vs. structural damage
- Growth factors and stimulated regeneration (including stem cells)
- Concept of normal tissue tolerance
- Over-reacting patients - radiosensitivity syndromes
- Concept of functional subunits – parallel and serial organisation

c) Second cancers in radiotherapy patients

d) Conditioning for bone marrow transplantation

Time-Dose Fractionation

a) The 5 Rs of fractionated radiotherapy (Repair, Repopulation, Radiosensitivity, Redistribution, Reoxygenation)

b) Isoeffect curves

c) Linear-quadratic (LQ) parameters, biological effective dose (BED), linear-quadratic equivalent dose (LQED)

d) Residual injury and re-treatment

e) Accelerated repopulation in tumours and normal tissues, time factor in radiotherapy

f) Therapeutic ratio

g) Concept of tumour control probability (TCP) and normal tissue complication probability (NTCP) models

h) Modified Fractionation (Hyper-, Hypo-, Accelerated. Concomitant boost)

i) Radiobiology of resource-sparing protocols, e.g. for palliative treatments

Brachytherapy

a) Radiobiological principles

b) Half time of repair

c) Dose distribution

d) Volume specification

Volume Effects

a) Isoeffect versus iso-tolerance

b) Radiobiological interpretation of dose-volume histograms

c) Volume considerations of functional versus structural damage

d) Conformal and intensity modulated radiation therapy (IMRT) techniques

Day 5 (including practical/tutorial)

Principles of combined radiation and drug treatments

a) Spatial cooperation versus interactive effects

b) Different toxicities in tumour and normal tissues

c) Possible mechanisms of interaction

d) Principles of clinical use including concurrent and sequential treatments, role of chemotherapy in consequential late radiation toxicity, late cardiac effects

e) Tumour micro-environmental effects in chemotherapy

Biological and novel therapies

a) Biological therapies and their mechanism of action

b) Novel targets for anti-cancer drugs including vasculature and cell signal control and oncogene products

c) Bioreductive drugs, antibody-directed enzyme prodrug therapy (ADEPT)

d) Photodynamic therapy

e) Gene therapy, gene-directed enzyme prodrug therapy (GDEPT), radiation-induced gene expression including molecular switching techniques

f) Radioimmunotherapy and targeted radiotherapy

Day 6 (including practical/tutorial)

Predictive Assays

a) Rationale for normal tissues and tumours – intrinsic radiosensitivity, surviving fraction at 2Gy (SF2), cell kinetics, and hypoxia

b) Molecular, subcellular, cellular and non-invasive tests

c) Results to date

d) Future possibilities, e.g. gene expression profiling

Clinical Radiobiology of common cancers

a) Radiobiological issues in the treatment of the common cancers such as cervix, head and neck, lung, breast, prostate

b) Resistance mechanisms and clinical radiobiology

c) Cervix cancer, SF2, Hypoxia, Repopulation, Brachytherapy and external beam treatments, BED, LQED calculations

d) Head and neck cancer, optimum fractionation schedules, volume effects –Morbidity scoring scales, salivary gland sparing, role of brachytherapy

e) Lung cancer e.g. biological imaging of target volume using positron emission tomography (PET), accelerated radiotherapy. Radiochemotherapy schedules

f) Breast cancer e.g. role of hypofractionation and brachytherapy, cardiac effects, antiestrogens and radiation toxicity

g) Prostate cancer e.g. role of hypofractionation and brachytherapy, dose escalation, biochemical relapse

Practicals/Tutorials

- DNA Laboratory techniques: practical demonstrations of some of the techniques from the above lectures e.g. comet assay, micronuclei, flow cytometry (DNA analysis), gel electrophoresis

- Survival curves in practice: practical session on the shapes of survival curves, and their importance in various clinical scenarios

- Analysis of scoring of normal tissue damage: LENT/SOMA versus RTOG/EORTC scoring systems, head and neck squamous cell carcinoma (HNSCC), Cervix Ca

- LQ model: BED, LQED, α/β ratio values:

  a) Fractionation calculations in practice

  b) Physical dose distribution and biological response distribution

  c) Combined brachy/teletherapy treatments; compensations for interruptions in treatment

  d) Importance of treating all fields per day

  e) Influence of radiation source decay with respect to repair half-time and dose effectiveness

  f) Clinical impact of errors in dose delivery

- Critical reading of relevant literature

C. EXTRA MODULE FOR RADIATION PROTECTION PERSONNEL (20 HOURS - 1 DAY ACCIDENTS, 1 DAY CARCINOGENESIS, 1 DAY REMAINDER)

Day 1 (including practical/tutorial)

Introduction

Environmental radiation exposure and radiation accidents

Dose estimation:

a) Retrospective dose estimation for past exposures: e.g. for A–bomb survivors, populations exposed by the Chernobyl accident, the Techa River pollution, the Semipalatinsk test site.

b) Radioecology: atmospheric dispersion, deposition (wet and dry), uptake in food chain, dose commitment from internal and external exposure. Relevant radioisotopes (Cs, I, Sr)

c) Biological dosimetry in accidental exposures: Stable and unstable chromosome aberrations (lymphocytes, haemoglobin and glycophorin-A (GPA) mutations)

Diagnosis and medical management of radiation syndromes

a) Lethal dose-50 (LD-50): laboratory experiments and human estimates

b) Radiation syndromes (Neurovascular, Heamatopoeietic, Cutaneous and G-I tract syndromes)

c) Diagnosis and medical management of radiation Accidents: Radiobiological rationale for therapeutic strategies such as barrier nursing, bone marrow stem cell transplantation, cytokine treatment

d) Methods of triage for treatment after a radiation accident:

- Acute symptoms (vomiting, diarrhoea, hair loss, nausea)
- Laboratory tests (Lymphocytes count and granulocyte count)

Day 2 (including practical/tutorial)

Radiation Carcinogenesis

a) Molecular mechanisms of multistage carcinogenesis:

- Initiation, promotion, progression
- Activation of oncogenes (i.e. genetic rearranged)
- Inactivation of suppressor genes (e.g. p53), loss of heterozygosity (LOH), polymorphisms
- Genomic instability, mini and microsatellites
- Genetic susceptibility to radiation-induced cancer (e.g. Retinoblastoma (Rb) gene)

b) Epidemiological evidence for radiation carcinogenesis:

- Epidemiological methods, cohort studies and case control studies
- Bomb survivor life-span studies: mortality and cancer incidence – design of study, results, dose response, latency, absolute vs. relative risk)
- Patients treated for benign diseases such as ankylosing spondylitis, mastitis, tinea capitis
- Tuberculosis patients undergoing multiple fluoroscopy
- Radon exposure of hard-rock miners or in homes, interaction with smoking
- The influence of age at exposure and gender on incidence and latency
- Dose-response relationships for radiation-induced leukaemia and cancers, particularly at low doses. Limitations of epidemiological studies
- The influence of dose rate Absolute vs. relative risk models
- Life time risk extrapolations

Heritable effects

a) Methods to determine radiation-induced rates of single gene mutations

b) Doubling dose at low dose, low dose rate irradiation

c) Critical germ cell stages for heritable radiation damage

d) Factors affecting the risk of heritable radiation damage: mutational component, potential recoverability correction factor

e) Risk estimation for single gene disorders and multifactorial diseases

Effects on the developing embryo

a) Intrauterine death, congenital malformations, and neonatal death, microcephaly, severe mental retardation, growth retardation

b) Dependence on gestational age of radiation effects on the embryo or foetus

c) Dose dependence of risk of severe mental retardation after exposure in weeks 8-15 and weeks 16-25, evidence for thresholds

d) Protection of the embryo in diagnostic radiology and from occupational exposure

Day 3 (including practical/tutorial)

Radiation protection

a) Effective and committed dose, definition of sievert (Sv), organ weighting factors, linear no-threshold (LNT) model

b) Dose limits for occupational and public exposures and their justification.

c) Dose limits for stochastic and deterministic effects

2. MINIMUM ESSENTIAL SYLLABUS FOR RADIOBIOLOGY

2.1. Introduction

This is expected to comprise 1 week of teaching of around 30 hours including discussion periods, practical sessions, tutorials, and revision using distance-learning and other texts in the student's own time.

2.2. Physics and chemistry of radiation interactions with matter

2.2.1. Sources of ionizing radiation

Ionizing radiations may be emitted in the decay process of unstable nuclei or by de-excitation of atoms and their nuclei in nuclear reactors, X ray machines, cyclotrons and other devices. During radioactive decay gamma rays are often produced alongside other types of radiation such as α or β rays. When a nucleus emits an α or β particle, the daughter nucleus is sometimes left in an excited state which, after de-excitation, returns to a lower energy level by emitting a γ ray in much the same way that an atomic electron can jump to a lower energy level by emitting visible light. Both natural background radiation from cosmic and terrestrial sources, and man-made radiations, cause ionization of atoms or molecules, which may cause injury to cells.

Living organisms are continuously exposed to ionizing radiations from natural radiation. In addition, exposures occur as a result of human activities and medical practices. Radiations are broadly categorized into natural and man-made sources (Table 2.1)**.** More than 90 % of radiation exposure to man occurs from natural sources e.g. cosmic rays, and terrestrial sources that comes from radionuclides in the earth's crust, air, food and water and the human body itself. Man-made radiation exposure to populations occurs mainly from medical uses of radiation and radioisotopes in health care, occupational sources in the generation of electricity from nuclear power reactors, industrial uses of nuclear techniques, and in the past from nuclear weapons testing. Use of ionizing radiation in medical diagnosis and therapy is widespread and constantly increasing due to useful newer health care applications. It is widely accepted that diagnostic radiation exposures can be significantly reduced by adequate safety measures and optimization of nuclear-based procedures and practices.

TABLE 2.1 AVERAGE ANNUAL EFFECTIVE DOSE OF IONIZING RADIATION TO INDIVIDUALS* (*as in year 2000)

Source	Dose (mSv)	Range (mSv)
Natural background		
External exposure		
Cosmic	0.4	0.3 – 1.0
Terrestrial	0.5	0.3 – 0.6
Internal Exposure		
Inhalation (mainly radon)	1.2	0.2 – 10.
Ingestion	0.3	0.2 – 0.8
Total	2.4	1 - 10
Man-made (artificial)		
Medical	0.4	0.04 – 1.0
Nuclear Testing		0.15 – decreasing trend
Chernobyl accident	0.002	0.04 – decreasing trend
Nuclear power production	0.0002	Decreasing trend
Total	**2.8**	**1 - 10**

2.2.2. Types of ionizing radiation

Ionizing radiation may be divided into directly and indirectly ionizing for the understanding of biological effects. Most of the particulate types of radiation are directly ionizing i.e. individual particles with adequate kinetic energy can directly disrupt the atomic structure of the absorbing medium through which they pass producing chemical and biological damage to molecules. In contrast, electromagnetic radiations, namely, X and γ rays, are indirectly ionizing because they do not produce chemical and biological damage themselves but produce secondary electrons (charged particles) after energy absorption in the material.

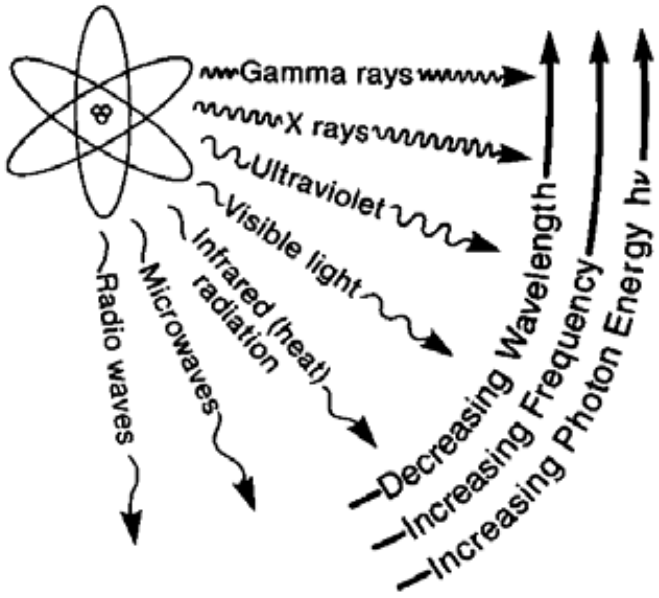

Fig. 2.1 Schematic of the electromagnetic spectrum (Hall and Giaccia, 2006).

2.2.2.1. Electromagnetic radiation

Electromagnetic radiation includes radiowaves, microwaves, visible light, ultra violet light, X rays and γ rays (Figure 2.1). These waves are essentially characterized by their energy which varies inversely with the wavelength. They can be thought of as moving packets of energy (quanta) and in this form are called photons. The quantum of energy associated with the waves progressively increases from radiowaves with least energy to X and with highest energy, and X and γ ray photons have the ability to eject an electron from its orbit in an atom (are ionizing radiations). Ionization is the process of removing one or more electrons from atoms by the incident radiation leaving behind electrically charged particles (an electron and a positively charged ion) which may subsequently produce significant biological effects in the irradiated material (Figure 2.2). The ionized or excited atom or molecule may either fragment producing free radicals or return to the parent state. If the energy transferred by ionizing radiation to the atom is insufficient to eject orbital electrons, the electrons may be raised from lower to higher orbitals and the atom is said to be excited. Other radiations of the electromagnetic spectrum fall short of the energy required to remove an electron from an atom and they are called non-ionizing radiations. Non-ionizing radiations are generally considered harmless to biological tissues at levels below those that cause heating effects, although there remain controversies in this area and research is ongoing. Cellular phones, radar, infrared, radiowaves, microwaves, visible light, ultrasound fall into this category. Because of the longer wavelengths and, therefore, smaller energy per quanta, they are not known to cause significant chemical changes in atoms or molecules of the medium. However, the exact demarcation between ionizing and non-ionizing radiation parts of the spectrum is somewhat arbitrary because some molecules can be ionized with very little energy, and far-UV radiation can behave similarly to X and γ rays.

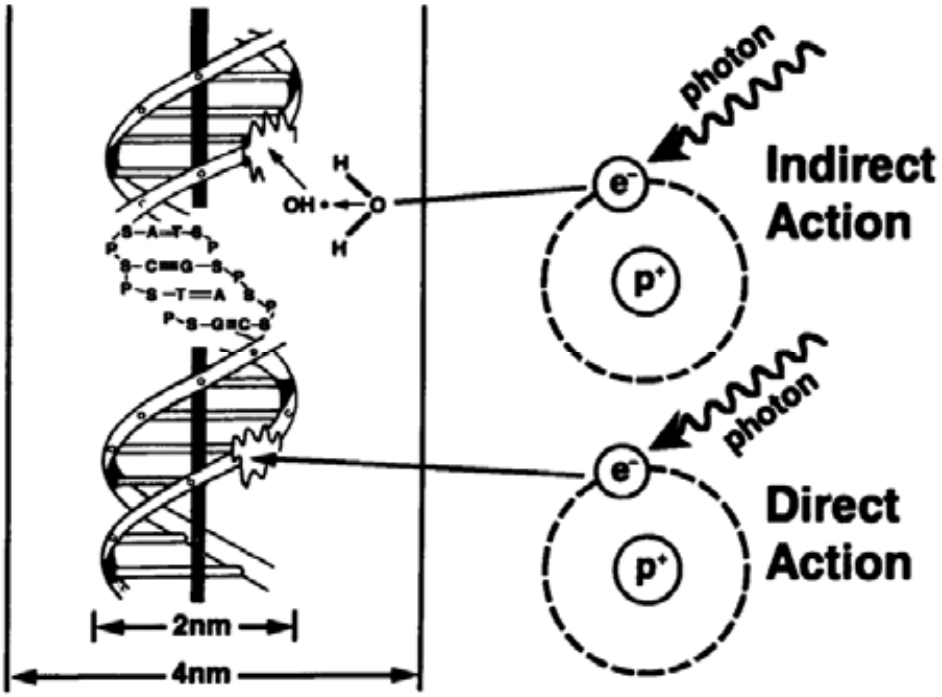

Fig. 2.2 Direct versus indirect action (Hall and Giaccia, 2006).

2.2.2.2. Interactions of electromagnetic radiation

When electromagnetic radiation travels through matter, it can be transmitted without transferring any energy or its intensity may be reduced by interaction with the traversed material. The attenuation occurs due to individual photon interactions with the atoms encountered. Biological effects arise when electromagnetic radiations, mainly X rays or γ rays, are either scattered or absorbed by the atoms of tissues/organs. Quantum theory considers electromagnetic radiation as streams of packets/bundles of energy called photons. The energy of a photon of electromagnetic radiation is given by Planck's equation, where

$$\mathbf{E = h\nu} = hc/\lambda$$

E is the energy of the photon, h is Planck's constant, and ν is the frequency of the photon. The energy of a photon is directly related to its frequency and inversely to wavelength, λ. Wave velocity is obtained by the product of frequency and wavelenth, $c = \lambda\nu$, where c is the velocity of light.

Biological effects of radiation arise when ionizing radiation interacts with an organism/tissue and leaves some energy behind. The process by which electromagnetic photons are absorbed in matter depends on their energy and the atomic number of the absorbing material.

Photons passing through matter transfer their energy through the following three main processes: photoelectric absorption, Compton scattering, and pair production (Figure 2.3).

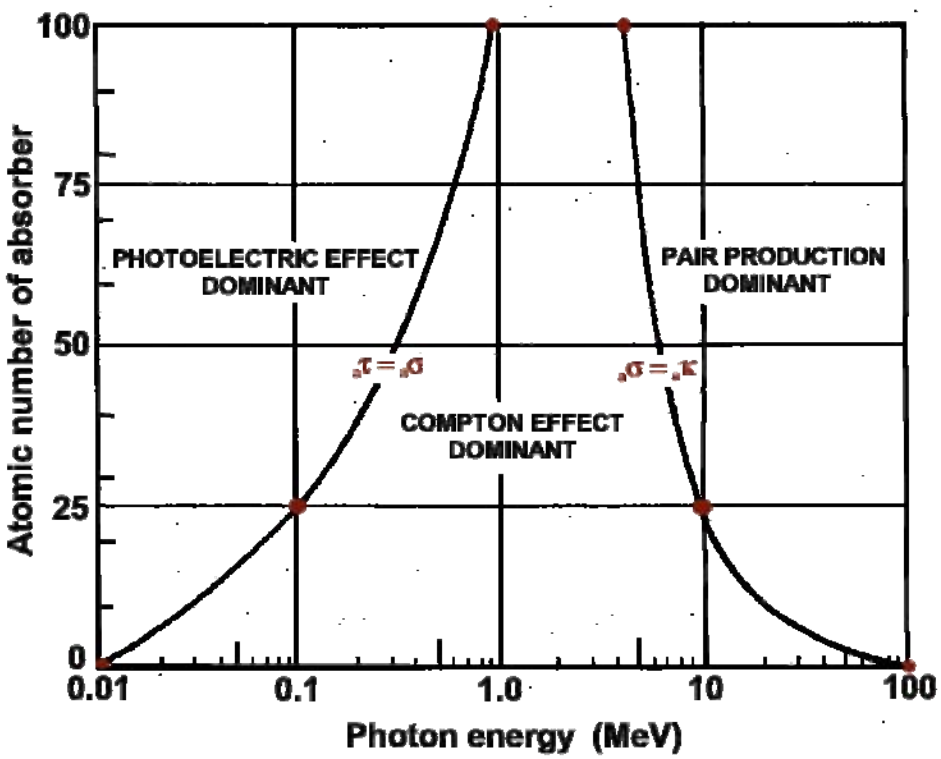

Fig. 2.3 Dominant types of interactions as a function of the atomic number Z of the absorber and the energy of the photon radiation (Podgorsak, 2005).

2.2.2.3. Photoelectric absorption

In photoelectric absorption, the photon interacts with a bound inner shell electron in the atom of the absorbing medium and transfers its entire energy to the electron ejecting it from the occupied atomic shell. The incident photon disappears and the energy transferred is used to overcome the binding energy of the electron and the remainder appears as kinetic energy of the resulting photoelectron. Thus, the kinetic energy of the ejected photoelectron equals the energy of the incident photon minus the binding energy of the electron.

$$\text{Kinetic Energy (electron)} = h\nu - E_b$$

where $h\nu$ is the energy of incident photon, and E_b is the binding energy of the electron. The ejected photoelectron travels a certain distance within the absorber and loses its energy through secondary ionizations. In this way, the entire photon energy of the incident photon is deposited in the tissue irradiated. As a result, an atom that participated in photoelectric interaction is left ionized. The vacancy created due to ejection of the electron is instantly filled by an electron from an outer orbital of the same atom, emitting the balance of energy as a photon between the respective orbits with characteristic low energy.

The photoelectric effect is the dominant energy transfer mechanism for X and γ ray photons having energies below 50 keV in biological tissues, but it is much less important at higher energies. (An electron volt is a measure of energy which is the kinetic energy gained by an electron passing through a potential difference of one volt. 1 eV = 1.602 x 10^{-19} Joules).

2.2.2.4. Compton scattering

The process of energy deposition called the Compton Effect occurs when the incident photon interacts with the outer orbital electron whose binding energy is very low compared with that of the incident photon. In this interaction, the incident photon transfers energy to an atomic electron causing its ejection from the atom. The photon is scattered with the remainder of the original energy in a different direction to that of the incident photon. Compton scatter thus causes ionization of the absorbing atom due to loss of an electron. The scattered electron (a secondary charged particle) travels some distance in matter and eventually loses energy by further ionization and excitation events to become part of the material. The probability of Compton scattering decreases with increasing photon energy. It is the principal absorption mechanism for X and γ rays in the intermediate energy range of 100 keV to 10 MeV. This range is in the therapeutic radiation range, and it also forms most of the γ radiation present in a nuclear explosion.

2.2.2.5. Pair production

When a photon of high energy (>1.02 MeV) interacts with atoms of the medium, the incident photon can be spontaneously converted into the mass of an electron and positron pair by interaction of the Coulomb force in the vicinity of the nucleus. The oppositedly charged particles are emitted in opposite directions to each other and cause damage as secondary charge particles. A positron is the anti-matter equivalent of an electron and it has the same mass as an electron, but it has a positive charge equal in strength to the negative charge of an electron. The energy of the interacting photon in excess of the equivalent rest mass of the two particles (1.02 MeV) appears as the kinetic energy of the pair and the recoil nucleus. The positron has a very short lifetime and, at the end of its range, it combines with a free electron. The entire mass of these two particles is then converted into two γ photons each of 0.51 MeV

energy emitted in opposite directions. The secondary electrons (or positrons) produced in any of these three processes frequently have enough energy to produce many further ionizations up to the end of their range.

2.2.2.6. Dependence of absorption on atomic number

The radiation energy deposition depends on the energy of the radiation and the atomic number (Z) of the absorbing material. The mass absorption coefficient of photoelectric absorption varies directly with the third power of the atomic number of the absorber (Z^3). The effective atomic number of bone is about twice that of soft tissues, and the probability that a photon will be absorbed in bone is about six times that in an equal thickness of soft tissues. Bone is mainly comprised of calcium whereas soft tissues are comprised of low atomic number elements such as carbon, hydrogen and oxygen. On the other hand, the mass absorption coefficient for the Compton process is nearly independent of atomic number. Compton and photoelectric effects are vital for appropriate applications in X- ray diagnosis and cancer therapy. In radiotherapy, high-energy photons in the range of 1-10 MeV are preferred because absorbed dose is nearly the same in bone and soft issues whereas low energy photons are preferred in diagnosis because of the much desired large contrast in absorption of these tissues.

2.2.2.7. Half value layer

When an electromagnetic radiation like X or γ rays passes through matter, its intensity is gradually reduced or attenuated with increasing depth due to the energy deposition interactions. This results in a decrease of photons, mainly due to photoelectric absorption and Compton scattering processes. The probability for absorption in a layer of material is proportional to the mass density. For a monoenergetic beam of photons, a constant fraction decreases as the beam travels through each unit of thickness in the absorber. This results in an exponential decrease in intensity with an increase in the thickness represented by the following equation;

$$I(x) = I_0 \ e^{-\mu x}$$

where I (x) = the intensity at thickness x, I_0 = is the initial intensity on the surface of the absorber, $\mu = n \times \sigma$ is the absorption coefficient measured in cm^{-1}, n = the number of atoms per cm^3 in the material, σ = the absorption cross section in cm^2, and x = the thickness of material in cm.

The thickness of absorber that reduces the photon intensity to one half is called the half value layer (HVL). Absorption of the beam depends on the mass and thickness of the absorber and the energy of the beam. Low energy photons are much more likely to be absorbed than high energy photons, for example the first 1.5 cm of water absorbs 40 % of 50 kVp X rays. The probability that a photon will interact with an orbital electron is optimum when its energy equals the binding energy of electron in the encountered atom. The total absorption coefficient of aluminium (Atomic No. 13) for γ rays plotted against photon energy shows that mostly Compton scattering dominates. In contrast, the total absorption coefficient of lead (atomic number 82) for γ rays, plotted against photon energy shows that the photoelectric effect dominates at low energies and pair production dominates above 5 MeV. Lead is often used to protect the body from radiation exposure because of its suitable HVL properties.

2.2.3. Particulate radiations

Particulate radiations (e.g. α, β particles *(electrons),* protons, neutrons, ions), also produce their effects by causing ionization and excitation processes randomly in the atoms or molecules of the traversed material. The passage of charged particles, electrons and positively charged ions, causes intense damage (energy deposition) to molecules along the path in living tissue due to strong electrostatic interactions between the travelling particle and the electrons of the atoms of the medium.

2.2.3.1. Charged elementary particles

Protons with one unit mass and one positive charge, cause less damage than α particles (helium nuclei) because the rate of deposition of energy varies inversely in proportion to the velocity of the particle and directly in proportion to the square of the charge. At the same energy, α particles have lower velocity because of their higher mass and carry twice the charge of a proton. Radioactive materials often release α particles and because they are a highly ionizing form of particulate radiation they usually have low penetration. They quickly lose their energy and they penetrate only a few tens of microns in body tissue. They can be fully absorbed by a sheet of paper. Beta particles (β, electrons) are also emitted by radioactive nuclei, as well as being displaced from atoms and molecules by X and γ rays as discussed above. They carry a single negative charge but their path in absorbing materials such as tissue is erratic due to their light mass (approx 1/2000 that of a proton). High energy electrons ionize much less efficiently than α particles because of their lower mass (and resulting higher velocity) and lower charge. Therefore, they penetrate tissues to a greater depth than α particles**.** Generally, beta particles do not penetrate further than the skin of the human body.

2.2.3.2. Uncharged particles

Neutrons (n) are uncharged particles with a mass very similar to that of a proton and are an indirectly ionizing radiation because without a charge they cannot participate in electrostatic interactions. At the same mass and energy, neutrons are more penetrating than are charged particles. Although neutrons do not interact strongly with electrons of atoms in the traversed material and do not directly ionize atoms, they do cause a density of ionization that is, far greater than in the case of X rays. Neutrons interact with the atomic nuclei of the medium and they lose energy by different interaction processes depending on their energy (velocity) and the mass of the encountered nucleus. In soft tissues, because of the abundance of protons with mass equal to that of neutrons, fast neutrons (>1 MeV) mostly lose energy by elastic scattering through collision processes producing high energy recoil protons, which in turn deposit energy by electrostatic interactions with electrons in the tissue as described above. Neutrons begin to interact by inelastic scattering at energies above 6 MeV, and fast neutrons may interact with carbon and oxygen nuclei producing α particles, recoil protons and heavy nuclear particles.

Fast neutrons can be made into thermal neutrons via a process called moderation. In reactors, typically heavy water, light water, or graphite are used to moderate neutrons. Thermal neutrons have a much larger effective cross-section than fast neutrons, and, therefore, can be absorbed more easily by any atomic nuclei with which they collide, creating a heavier and often unstable isotope of the irradiated element. Most fission reactors use a neutron moderator to slow down, or *thermalize* the neutrons that are emitted by nuclear fission so that they are more easily captured, causing further fission. This ability of neutrons to produce radioactive

nuclei (neutron activation) which then produce ionizing radiation by their decay can be used to analyse the atomic composition of certain materials.

2.2.3.3. Ions

The nuclei of carbon, neon, silicon, argon atoms form charged ions when one or more orbital electrons have been stripped off. These can be accelerated to hundreds of MeV energies in special accelerator facilities. High energy charged ions offer special advantages in cancer radiotherapy because of the energy distribution along their track which has a high peak at its end (the Bragg peak). This allows the possibility of depositing high energy densities at depth in tissue but these facilities are as yet very limited on account of high costs and sophisticated technical requirements.

2.2.4. Linear energy transfer

When ionizing radiations traverse through matter, they lose energy gradually through various interaction processes along the length of their path. For a particular absorber, the rate of loss of energy depends on the energy and type of radiation as well as the density of the material (Table 2.2). The density of energy deposition in a material such as tissue is called the Linear Energy Transfer (LET) of the radiation. It is defined as the average energy deposited per unit length of track of radiation and the unit is keV/ μm. Note that the LET varies along the length of the track of charged particles because as the charged particle deposits energy in tissue it slows down. The rate of transferring energy (-dE/dX, loss of energy per unit distance) increases as this occurs, such that there is a peak of energy deposition at the end of the track (the Bragg peak). LET essentially indicates the quality of different types of radiation and is important because the biological effect of a radiation (its relative biological effectiveness, RBE) depends on its average LET. Charged particles generally have higher LET than X and γ rays because of their greater energy deposition along the track. Radiations are categorized into low and high LET radiations with particulate radiations usually being high LET radiations whereas X and γ rays are low LET radiations due to their sparse ionizations (Table 2.2). In general the RBE of a radiation increases with its LET up to a value of about 100 keV/μm and above this value starts to decline due to energy deposition in excess of that needed to cause the biological effect (overkill). Energy loss events are essentially randomly distributed along the track of the photon or charged particle. For low LET radiations the energy deposition events along the track of the photon are sparse relative to the dimensions of biomolecules such as DNA with the result that photons may pass through such a molecule without depositing any energy. For such radiations the amount of energy deposited in a region of the track similar in dimensions to biological molecules also various widely from a few eV up to 100s of eV. For high LET radiation the energy loss events are much more closely spaced and significant energy will be deposited along all parts of the track similar in dimension to biomolecules.

TABLE 2.2 TYPICAL LET VALUES OF IONIZING RADIATION

Radiation	Linear Energy Transfer, KeV/μm
Co- 60 γ rays	0.2
250 kVp X rays	2.0
10 MeV protons	4.7
150 MeV protons	0.5
14 MeV neutrons	12
2.5 MeV α particles	166
2 GeV Fe ions	1000

(Hall and Giaccia, 2006)

2.2.5. Radiation dose and units

The biochemical changes produced by ionizing radiations are the fundamental events leading to radiation damage in tissues. Radiation is measured either as exposure or as absorbed dose. The absorbed dose is the amount of energy absorbed in a system and generally regarded as the best way to quantify the irradiation absorption.

2.2.5.1. Exposure

The radiation exposure is a measure of radiation based on its ability to produce ionization in air under standard temperature and pressure, and is the quantity indicated by many radiation detectors such as ionization (eg Geiger-Muller) chambers. The (S.I.) unit for exposure is Coulombs/kg in air (or Roentgen R in old units: 1 R = 2.58 x 10^{-4} C/kg air). The unit of exposure is only defined for air and cannot be used to describe dose to tissue. Nevertheless ionization chambers are widely used to calibrate medical radiation devices and conversion factors to calculate absorbed dose from exposure have been carefully documented for different radiation energies and tissues.

2.2.5.2. Absorbed dose

The amount of energy absorbed per mass is known as radiation dose. Radiation dose is the energy (Joules) absorbed per unit mass of tissue and has the (S.I.) units of gray (1 Gy = 1 J/ kg). In the past the rad (radiation absorbed dose) was used, where 100 rad = 1 Gy (1 rad = 1 cGy). Various types of radiation dose units are used in radiobiology and Table 2.3 presents some of the frequently used dose units for measuring these radiation quantities.

2.2.5.3. Equivalent dose

As discussed above the biological effectiveness (RBE) of each type of radiation varies greatly depending largely on LET. For radiation protection and occupational exposure purposes the term ‘equivalent dose’ is used to compare the biological effectiveness of different types of radiation to tissues. The (S.I.) dose equivalent (H_T) in sievert (Sv) is the product of the

absorbed dose (D_T) in the tissue multiplied by a radiation weighting factor (W_R), often called the quality factor.

TABLE 2.3 SUMMARY OF RADIATION DOSES AND UNITS

Dose	SI Unit	Old unit	Conversion factor
Exposure	C/kg air	Roentgen	1 R = 2.58 x 10^{-4} C/kg air
Absorbed dose	gray (Gy)	rad	100 rad = 1 Gy
Equivalent dose	sievert (Sv)	rem	100 rem = 1 Sv

Equivalent dose is expressed as a summation to include the effects of irradiation of tissue by more than one type of radiation. In the past the unit rem (radiation equivalent man) was used to compare doses received by different types of radiations (100 rem = 1 Sv). The quality factor for low LET radiations is 1 so that for low LET radiations 1 Sv = 1 Gy.

$$H_T = \sum W_R \times D_T$$

2.2.5.4. Effective dose

Effective Dose is used to estimate the risk of radiation in humans. It is sum of the products of equivalent doses to each organ/tissue (H_T) and the tissue weighting factor (W_T) (Tabel 6).

$$E = \sum W_T \times H_T$$

The unit of effective dose is the Sievert (Sv).

2.2.5.5. Collective dose

Collective dose is defined as the dose received per person in Sv multiplied by the number of persons exposed per year i.e. man-sievert per year. This unit is generally used for protection purposes and in population response calculations.

2.2.6. Principles of radiation dosimetry

Absorption of radiation in material produces many changes, which form the basis to dose measurements based on physical, chemical and biological effects. Different detectors have been used to develop dosimeters for ionizing radiation and some of them are used to measure relative dose distributions for therapeutic electron and photon beams. A few of them are used for measurements of absolute or reference absorbed dose called primary standards. Detectors can be divided broadly into three categories: those that measure directly the quantity of energy absorbed, detectors that measure ionization and those that quantify free radicals formed in the absorbing medium.

Secondary chemical dosimeters are widely used commercially and have proved beneficial to clinical and scientific communities for both research and applications in photon radiation dosimetry. Among the most popular dosimeters are the Fricke chemical dosimeter, thermo-luminescence dosimeters (TLD) and ion chambers or diode dosimeters. These dosimeters are each characterized by their own merits and are useful in particular conditions of operation. The fundamental requirement for a suitable dosimeter is the linearity of response as a function of radiation dose within a wide dosage range.

2.2.6.1. Chemical dosimeters

The Fricke chemical dosimeter is based on chemical change by absorption of radiation and used to measure, X, γ and electron doses. The principle consists of the chemical change of ferrous ions (Fe^{+2}) into ferric ions (Fe^{+3}) by absorption of radiation energy. Measurement is accomplished by optical absorption of ferric ions, which has a high extinction coefficient allowing determination of concentration changes. The major drawback is the unreliability in the presence of undesirable impurities. The method is highly unstable in air especially after irradiation but is relatively cost effective. The measurements are highly linear with increasing dose up to more than 150 Gy.

2.2.6.2. Thermoluminescence dosimeters (TLD)

Thermoluminescence is based on generation of trapped electrons by exposure of lithium fluoride to radiation. The measurement of dose consists of measuring the luminescence induced by thermal treatment after radiation exposure. The light emitted is proportional to radiation dose. Lithium fluoride chips provide good spatial information but require careful calibration and rather laborious read-out. In addition, TLD are oxygen sensitive which imposes a limitation. The method is not as cost effective as the Fricke dosimeter, it lacks ease of preparation and the measurements become nonlinear at absorbed doses above 10 Gy. Optically stimulated thermoluminescence (OSL) is used in another device based on aluminum oxide and this requires no processing. It was originally developed for radiation therapy but is now also used for diagnostic purposes.

2.2.6.3. Ionization chambers

Ionization chambers consist of an air-filled chamber containing two electrodes to which a voltage is applied. They measure the current flow which occurs due to the ionization of the air molecules exposed to radiation. They are capable of giving instant readings with good accuracy. The chambers are easy to use but are poor in providing spatial information. Diode dosimeters are based on the principle of ion collection formed by radiation incident in the chamber. Measurement consists of collection of ions on the cathode, formed by exposure to radiation, but this technique requires intricate circuitry and is not cost effective. Ion chamber performance depends on the voltage applied for charge collection.

2.2.6.4. Film dosimetry

Special radiographic films have been developed for verification of dose in radiotherapy practice. This has proved useful for measuring dose profiles but the method has limited accuracy and dose range for determination of absolute radiation doses.

2.2.7. Direct and indirect effects

The physical interactions of ionizing radiation leads to loss of energy of radiation and production of ionization and excitation of atoms and molecules which may convert into free radicals in pico to femto seconds after physical interaction with atoms ($10^{-13 \text{ to } -15}$ s). These radicals react with neighbouring molecules and produce secondary DNA or lipid radicals by reaction with another neighbouring molecule. Chain reactions may also occur, particularly in lipids, and may play a role in damage to cell membranes. Free radicals are fragments of molecules having unpaired electrons, which have high reactivity with cellular molecules and, therefore, have a short life. They can be detected by fast measuring techniques like pulse radiolysis and flow electron spin resonance (ESR).

Free radicals are generated in great number by ionizing radiation due to the process of energy absorption and breakage of chemical bonds in molecules. These are known to play a major role in radiation effects on biological tissues and organisms. These radicals are highly reactive and found in a number of biological processes, metabolism, oxidation, reduction, and pathological diseases and cancer induction. Both electromagnetic and particulate radiations act on cells to cause free radicals and subsequent molecular damage through direct as well as indirect actions. When ionizing radiation energy is deposited in a macromolecule that is important for the biological effect observed (often DNA for cell killing), it is called a direct effect of radiation. Alternatively, photons may be absorbed in the water of an organism causing excitation and ionization in the water molecules. The radicals formed after passage of radiation and water radiolysis, namely the hydrated electron (e_{aq}^-), the hydrogen atom ($H^\cdot$) and the hydroxyl radical ($^\cdot OH$) contribute in causing damage to biological systems.

A compound with a high rate constant of reaction can scavenge primary free radicals of water radiolysis. Free radicals of biomolecules can be restituted by hydrogen donating compounds, such as thiols and cysteine. Alternatively, they can be fixed by reaction with oxygen or oxygen mimicking compounds, which makes them permanently damaged. This is called 'the oxygen effect', which forms the basis of increasing molecular and cellular damage in the presence of oxygen. These chemical reactions form the basis of searching for compounds which can sensitize cell/tissue damage or protect them against radiation, and which are of direct relevance to radioprotection and cancer radiotherapy.

2.2.7.1. Direct effects

Ionizing radiation (IR) can act on biological molecules (RH, representative of hydrocarbons) causing ionization and excitation. One or more chemical bonds may be broken giving atoms or molecules with unpaired electrons, which are very reactive and have a short life. The formation of these radicals occurs in the picosecond time range after the passage of the photons. The bond may be repaired or cross-linking may occur due to radical-radical reactions. These free radicals may also react with oxygen, and in the case of lipids may initiate chain reactions (see below).

$$IR + RH \rightarrow R^\bullet + H^\bullet$$

Both $H^\cdot$ and $R^\cdot$ radicals can react with another molecule e.g. DNA, lipids, proteins.

$$R^\bullet + R'H \rightarrow R'^\cdot + RH$$

Radicals can produce cross linking reactions.

$$R^\bullet + R^\cdot \rightarrow R^\bullet - R^\cdot$$

It is estimated that about one third of biological damage by γ radiation is caused by direct effects. This process becomes more dominant with high LET radiation, such as neutrons or α particles.

2.2.7.2. Indirect Effects - Water Radiolysis

The absorption of energy depends on the abundance of material in the path of the radiation. Water is the most predominant molecule in living organisms (about 80 % of the mass of a living cell is water). Therefore, a major proportion of radiation energy deposited will be absorbed in cellular water. A complex series of chemical changes occurs in water after

exposure to ionizing radiation. This process is called water radiolysis. The understanding of chemical changes in water is essential in studies of radiation effects on living cells.

Interaction of radiation with water causes ionization and excitation process producing short-lived H_2O^+ radical-cations, fast electrons, and electronically-excited water molecules (H_2O^+). H_2O^+ ions and excited water molecules are unstable and decompose within 10^{-13} s to form $OH^\bullet$ and $H^\bullet$ radicals

$$IR + H_2O \rightarrow \quad H_2O^+ + e^-$$

$$H_2O + H_2O^+ \rightarrow H_3O^+ + OH^\bullet$$

$$IR + H_2O \rightarrow \quad H_2O^* \rightarrow H_2O + \text{photon emitted}$$

or

$$H_2O^* \rightarrow OH^\bullet + H^\bullet$$

The hydroxyl radical has an unpaired electron and is a highly reactive oxidizing agent. It can diffuse a short distance and react with critical target molecules producing another radical. This can react with water forming an anion which rapidly dissociates to give a hydrogen atom ($H^\cdot$). The ejected secondary electrons may interact with a water molecule to form hydroxyl ions and a hydrogen atom (a hydrogen radical), or they may lose energy by a sequence of interactions with the medium until they attain thermal energies after about 10^{-11} s. The thermalized electrons are then solvated by dielectric interactions with neighbouring water molecules to form e^-_{aq} i.e. e^-_{aq} is a free electron in a solvent cavity surrounded by a sheath of orientated water dipoles. It reacts with a proton to give a hydrogen atom ($H^\cdot$):

$$e^- \quad + \quad H_2O \rightarrow H_2O^- \rightarrow \quad OH^- + H^\bullet$$

$$e^-_{aq} + H^+ \rightarrow H^\bullet$$

e^-_{aq} is the strongest known reducing species at pH 7.0. In oxygenated solutions, e^-_{aq} is converted to O_2^-, which is a strong oxidizing agent and the precursor of hydrogen peroxide:

$$e^-_{aq} + O_2 \rightarrow O_2^-$$

These primary water radicals (e_{aq}, OH, $H^\bullet$) have high reactivity towards molecules of cells, DNA, lipids and other subcellular constituents. In oxygenated solutions, hydrogen atoms can react with oxygen to give hydroperoxyl free radicals ($HO_2^\bullet$):

$$H^\bullet \quad + O_2 \rightarrow \quad HO_2^\bullet$$

The relative yields of the water radiolysis products depend on the pH and LET of the radiation. The concentration of these radicals are expressed in terms of a G value which is defined as the number of radicals or molecules produced per 100 eV of energy absorbed in the medium. Typical G-values are $G_{e\text{-}aq} = 2.6$, $G_{OH\cdot} = 2.6$, $G_{H\cdot} = 0.6$.

2.2.7.3 Free radical scavengers

Certain compounds with a high rate constant of reaction may scavenge the primary radicals of water radiolysis (e.g. dimethylsulphoxide). Hydroxyl radicals can also be scavenged by a

number of –SH containing compounds as a moiety in their chemical structure. The hydrated electron can be efficiently scavenged by oxygen producing a number of oxygen-centered radicals. Scavenging of hydroxyl radicals forms one basis for development of radioprotectors. Amifostine (WR 2721), an aminothiol, is one of the well-known protectors which has potential application in radiotherapy. Thiol compounds may also donate hydrogen atoms to radical sites on other biological molecules such as DNA but scavengers act primarily against the indirect effect induced by water radicals. Hence they have reduced efficacy for high LET radiation for which the direct effect plays a more prominent role in biological damage such as cell killing.

2.3. Molecular and cellular radiobiology

2.3.1. Radiation lesions in DNA

Radiation causes a wide range of lesions in DNA such as single strand breaks in the phosphodiester linkage, double strand breaks on opposing sites or displaced, base damage, protein-DNA crosslinks and protein-protein crosslinks involving nuclear proteins such as histones and non-histone proteins. The presence of histones and DNA in a 1:1 weight ratio makes histones prime candidates for crosslinks. The number of DNA lesions generated by irradiation is large, but the number giving rise to cell kill is extremely small. The numbers of lesions induced in the DNA of a cell by a dose of 1-2 Gy are approximately: base damages > 1000; single strand breaks (ssb) ~1000; double strand breaks (dsb) ~40. Dsb play a critical role in cell killing, and there are experimental data showing initially-produced dsb correlate with radiosensitivity and survival at low dose, and unrepaired or mis-repaired dsb to correlate with survival after higher doses. Increasing evidence suggests the importance of complex dsb lesions after high LET irradiation. Knowledge of radiation track structure has been used to explain the wide variation and wide distribution of lesions in DNA. The importance of clusters of energy deposition events (ionizations and excitations) at track termini of secondary electrons resulting in multiple closely-spaced lesions (multiply damaged sites) within a range of 20 nm, has been recognised as important for cell killing and in regard to the ability of cells to repair such lesions.

2.3.2. Major types of DNA repair

There are multiple enzymatic mechanisms of DNA repair in cells that act on different types of lesions. For double strand breaks there are two primary repair pathways, non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ repair operates on blunt ended DNA fragments resulting from broken phosphodiester linkages. There is a requirement for Ku70/Ku80 repair proteins to recognize the lesion termini, binding of the Ku-heterodimer to DNA-PK (protein kinase), and activation of the XRCC4 ligase enzyme by this complex for final religation of the fragments after enzymatic “cleaning up” of the broken ends of the DNA molecule, by a variety of other recruited proteins, so that ligation can occur. Repair by NHEJ operates throughout the cell cycle but dominates in G1/S-phases. The process is error prone because it does not rely on sequence homology. Dsb repair by homologous recombination (HR) utilizes sequence homology with an undamaged copy of the broken region and hence can only operate in late S- or G2- phases of the cell cycle. It starts by nucleolytic resection of blunt ends, binding of NBS/MRE11/rad50 protein complex to the DNA termini, followed by strand exchange facilitated by attachment of rad51/XRCC2 protein. Then there is DNA synthesis of the missing nucleotides on the undamaged templates and ligation. This creates a complex strand crossover between the damaged and undamaged strands known as a Holliday junction, which is finally resolved before the repair process is complete. Other DNA repair mechanisms such as base excision repair (BER), mismatch repair (MR) and nucleotide

excision repair (NER) respond to damage such a base oxidation, alkylation, and strand intercalation.

2.3.3. Damage recognition and signalling

A first step in recognition of radiation damage (strand breaks) to DNA is ATM binding to DNA termini. This induces kinase activity in ATM which phosphorylates and activates the CHK kinases, which in turn phosphorylate p53. As a result p53 is released from MDM-2 and is stabilized to induce p21, which inhibits the cyclin-dependent kinase cyclinE-CDC-2 controlling the G1-S transition in the cell cycle. The resultant G1 arrest (G1 block) after irradiation ensures that the damaged DNA is not replicated before repair. Tumours showing mutant p53 or p53 null status, as the result of p53 destruction by viral protein E6, fail to initiate a G1 arrest and may not restitute damaged DNA before replication. But even p53 mutant cells display a G2 arrest and may exercise repair options (or induce apoptosis) and thus prevent mitotic propagation of defective DNA in M phase. Repair signalling starting at ATM proceeds via downstream activation of BRCA1, c-Abl, NBS1 and RAD 51 to initiate DNA repair. An alternative response to DNA damage is induction of apoptosis initiated by p53, although this occurs extensively after irradiation only in a few specific cell types, such as cells of hematopoietic lineages, endothelial cells, germ cells and oligodendrocytes. C-Abl, BID and the proapoptotic factor BAX (in the Bcl-2 family of proteins) respond to sequential phosphorylation cascades starting with ATM.

2.3.4. Consequences of unrepaired DNA damage: Chromosome damage

Mutations from low dose exposure influence base pairing, coding, transcription and gene expression. Chromosome analysis in mitotic spreads (karyotyping), micronucleus formation and fluorescent in situ hybridisation (FISH) can detect unrepaired DNA damage in chromatids by a variety of DNA damaging agents including radiation. Aberrant chromosomes arise when broken ends rejoin with other broken ends to generate rings, dicentrics, translocations and other chromosome aberrations. Dicentric chromosome aberrations arise post replication from the joining of 2 broken chromatids in different chromosomes and can be use as a marker for radiation exposure. Acentric fragments and dicentrics are unstable aberrations and may not survive past the next mitosis, implicating loss of genetic material which may signal death in diploid cells. In polyploidy cells such losses may be of lesser consequence Micronuclei contain acentric fragments and may be detected by stimulating lymphocytes (or certain other cell types) into division followed by treatment with cytochasin B, which allows nuclear division but stops cellular division. The micronucleus assay, although somewhat less sensitive, is a simple and effective alternative to chromosome analysis. The use of the micronucleus assay has been studied for the purpose of radiosensitivity testing of patients using lymphocytes, but limitations exist due to assay variability.

2.3.5. Radiobiological definition of cell death

Cells are generally regarded as having been “killed” by radiation if they have lost reproductive integrity, not by whether they physically survive in the population. Loss of reproductive integrity can occur by apoptosis, necrosis, mitotic catastrophe or by induced senescence. Although all but the last of these mechanisms ultimately results in physical loss of the cell this may take a significant time to occur, e.g mitotic catastrophe may not happen until several divisions have taken place. Apoptosis or programmed cell death is a strong feature in embryological development and in lymphocyte turnover. Previously, this early form of cell death was called interphase cell death. Apoptosis can be identified by microscopy and

typical shrinkage of cellular morphology, condensation of chromatin, nucleosome laddering indicating chromatin degradation, cell membrane blebbing, activation of caspases and release of cytochrome c. Exposed phosphatidyl serine in the cell wall permits binding of annexin V and assessment of apoptosis by flow cytometry. The characteristics of apoptosis (which is non-inflammatory) are in contrast to those of necrosis, typified by cell edema, poor staining of nuclei, increase of membrane permeability, shut down of cell metabolism, and an accompanying inflammatory response. Senescence or replicative senesence (RS) is observed when cells stop dividing, and this differs from the behaviour of stem cells and tumour cells which do not show these limitations. Senescent cells are somewhat edematous and show poor cell-cell contact, increased polyploidy, decreased ability to express heat shock proteins, and shortening of telomeres. Apoptosis occurs in particular cell types after low doses of irradiation e.g. lymphocytes, serous salivary gland cells, and certain cells in the stem cell zone in testis and intestinal crypts. Reproductive cell death is a result of mitotic catastrophe which can occur in the first few cell divisions after irradiation, and it occurs with increasing frequency after increasing doses. Cells that fail to divide successfully after irradiation can also undergo apoptosis at that stage. Cellular necrosis generally occurs after high radiation doses. A rapid fall of cell numbers after irradiation is likely to be due to apoptosis but may also occur by mitotic catastrophe in rapidly proliferating populations. Whether apoptosis reflects overall cell killing in tumour cell inactivation by radiation is currently unresolved and may only be the case for certain types of tumour cells.

2.3.6. Suvival curves and models

The accepted gold standard for measuring the radiosensitivity of a cell population is the retention of reproductive integrity or mitotic intactness i.e. the ability of a cell to undergo more than 5-6 cell divisions (and produce a viable colony containing at least 50 cells). This is referred to as cell survival and percent survival after irradiation is calculated by correcting for the 'plating efficiency' of unirradiated cells, this often being less than 100% due to the true proportion of colony-forming cells in those plated being low, or potential influences of the media, pH, temperature and cell specific factors. Measurements of apoptosis or MTT or SRB vital dye staining growth assays are often used instead of a colony assay for measuring radiosensitivity for reasons of simplicity, shorter assay time, and operation in multiwells permitting large number of parameters to be tested e.g. a range of growth inhibiting drugs. Major disadvantages of these approaches are the narrow range of doses and survivals that can be used, greater assay variability, and, particularly, that the assays do not test mitotic viability. Hence these assays rely on the (often unfounded) assumption that there is a clear relationship between apoptosis or cellular growth and cell survival over a wide range of doses and survival levels.

Survival curves are best shown as a semilog plot of survival against irradiation dose, generally in the dose range of 1 – 10 Gy for single cells. The most common model used today is the linear-quadratic model, fitted using a second-order polynomial, with the constants α and β describing the decline of survival (S) with increasing dose (D).

$$S = e^{-(\alpha D + \beta D2)}$$

Equal cell kill of linear and quadratic components is achieved when dose D = α/β. For high LET irradiation the quadratic component is small or non-existent.

An older model is the single hit/ single target model described by

$$S = e^{-D/Do}.$$

Do is effectively the reciprocal of α (above) and represents the dose which reduces survival to e^{-1} or 37 %. The linear relationship is consistent with data from some bacteria but it does not apply in eukaryotic cells (except at high LET), which show shouldered survival curves that can be accommodated by a single-hit multitarget model described by:

$$S = 1- [1 - e^{- (D/Do)}]^n.$$

This is reliable at high dose but not at low dose, because it does not describe accurately the 'shoulder' region at low doses, even if another single-hit term is added.

For practical purposes, there are merits of using survival at 2 Gy (SF2), because this is a dose fraction using commonly in radiotherapy.

2.3.7. Cell cycle effects

Renewing cells in a growing population (e.g. skin, gut, bone marrow, tumour cells or cells in culture), but not when resting in Go phase, participate in the cell cycle. Replication of the genome occurs in S-phase and mitotic propagation to daughter generations occurs in G2/M phases. Typical cell generation times are 10 – 40 hours with the G1 phase taking about 30 %, S-phase 50 %, G2 phase 15 % and M-phase 5 % of the cell cycle time, although G1 phase time may vary and be much longer in slowing proliferating populations. In interphase the majority of cells are in G1 or Go. There are checkpoints at the G1/S and G2/M boundaries that monitor the fidelity of genomic processing. Binding of cyclins to cyclin dependent kinases activates the kinase complex to negotiate the checkpoints: cyclin B1/ p34 CDC-2 for G2/M transition, cyclin D1/cdk-4 for M/G1 transition, cyclin E/cdk-2 for G1/S and cyclinA/cdc-2 for S/G2 transition. Drugs that abrogate cell cycle blocks e.g. caffeine and pentoxifylline, are radiosensitizing by rapidly re-establishing the B1/p34 CDC-2 pair, promoting early mitotic progression before complete recovery and directly inhibiting HR repair in G2. In p53 mutants (i.e. in most tumours) and in cells of p53 null status arising from p53 destruction after viral infection (by the HPV E6 protein), p21 induction is abolished and p21 controlled inhibition of G1/S transition cannot occur. In the absence of the G1 block, cells enter a block at G2/M. Most tumour cells being p53 mutant hence would display altered checkpoint expression and limited repair routes with opportunities for therapeutic intervention. Tumour cell heterogeneity and multiple ploidy are complicating factors.

Radiosensitivity differs throughout the cell cycle with, in general, late S-phase being most radioresistant, G2/M being most radiosensitive and G1 phase taking an intermediate position. The greater proportion of repair by HR than by NHEJ in late S phase may explain the resistance of late S phase cells. The open structure of DNA helps explain radioresistance in G1. Chromatin compaction and poor repair competence (reduced enzyme access) could explain the high radiosensitivity in G2/M. Attempts at cell synchronization in tumours by irradiation to increase overall sensitivity and to harness this scenario clinically have not been successful.

2.3.8. Relative biological effectiveness (RBE)

When effects of equal doses of different types of radiation are compared, they produce unequal biological effects. Comparison of effects of different types of radiation is expressed as relative biological effectiveness (RBE). Historically the effect of 250 kV X rays was taken as the standard, but more usually now it is > 1 MeV photons (from Co-60). RBE is defined as the ratio of doses of γ rays (Dγ-ray) and the test radiation (Dr) is required to produce an equal

amount of a particular biological effect i.e. Dγ / Dr. For determination of RBE in mammalian cells, a surviving fraction of, say 0.1 or 0.01, can be used. In animal experiments, the biological effect measured for some particular functional endpoint can be used. A dose of 1 Gy of α particle produces a much larger amount of a chosen biological effect than 1 Gy of γ rays. Survival curves of human kidney cells using different radiation modalities e.g. 2.5 – 26 MeV α-particles and 250 kVp X rays (Barendsen 1968) illustrate the wide range of radiobiological effectiveness of a given irradiation dose. Perfect linearity was shown for 4 MeV α-particles and a wide shoulder on the cell inactivation curve for 250 kVp X rays.

RBE varies with cell system, endpoint and dose. RBE is higher at lower doses because of the lesser efficacy of the reference radiation per unit dose at low versus high doses i.e. the wider shoulder for lower LET radiations. RBE increases with increasing LET of a particular radiation and peaks at about 100 keV/ μm. It declines with further increases in LET in many mammalian cells, which is usually explained by 'overkill' effects of 'wasted' ionizations at these very high ionization densities. RBE is higher with low dose rates of the low-LET reference radiation, because in general there is a dose-rate effect with low LET radiations but not for high-LET radiations. RBE is lower for high-dose single fractions and larger for multiple small fractions (e.g. Joiner, 1987). Also, there are RBE differences between tissues and tumours. RBE values tend to be higher for some late-responding normal tissues, which is consistent with the concept, discussed in the next section, that late responding tissues have greater repair capacity than early responding tissues.

2.3.9. Cellular repair exemplified in survival curves

There is an increase in cell survival when the same dose is given as 2 fractions separated by 2 or more hours, compared to a single fraction. Greater survival when the dose is split in this way is attributed to sublethal damage repair (SLDR) between dose fractions (Elkind repair, named after the discoverer of the phenomenon for single cells). The half time of repair T ½ is the time when half the repair has taken place and is usually about ½-1 hr for cells in culture but can be longer for tissues. Thus full repair may take 6-8 hours and can be longer in tissues (e.g. in CNS it may be 24+ hrs). The recovery ratio is a measure of SLDR, given by the survival of cells receiving a split dose divided by the survival of cells receiving the total dose as a single dose. Potentially lethal damage repair (PLDR) is another class of repair, assessed by delayed plating experiments. Contact inhibited (plateau phase) cells are irradiated, followed by incubation for various periods and subsequent reseeding, with analysis of cell survival by colony assay to obtain a measure of this type of repair.

The 'shoulder' or the curvature of a survival curve is usually considered to be a reflection of the repair capacity of a cell population. In terms of survival curve theory this can be thought of as arising from the concept that energy-deposition, sublesion-causing (DNA damaging) events must be accumulated to allow sublesion interactions for cell killing to occur. The possibility that lesion lesions can be repair between split doses then results in the shoulder in the low dose region of the curve. The increase in RBE with increasing LET is attributable to an increase in non-repairable lesions at high LET. Repair depends on dose and time, and the maximum repair velocity is observed when damage is saturating, analogous to enzyme kinetics. Repair during irradiation is negligible at the high dose rate of 1-5 Gy/min practiced in external beam therapy and high-dose-rate brachytherapy, but is very significant during the course of the 1.6 - 150 cGy/min practiced in lower-dose-rate brachytherapy. The successive increase of cell survival with declining dose rate is consistent with the role of time in repair. The dominance of repair at low dose rate eliminates the shoulder/curvature and results in a straight but shallower line on a semi-logarithmic plot, with good separation of survival

between cell lines with different repair capacity. This is a factor that is often the major cause of different radiosensitivities.

2.3.10. Cellular hyper-radiosensitivity (HRS) and induced repair (IRR)

Some but not all tumour cells cultivated in vitro show increased sensitivity per unit dose at doses up to 0.2-0.5 Gy compared to higher doses. This is known as hyper-radiosensitivity (HRS). The effect suggests that repair needs to be induced by a certain dose above about 0.5 Gy, so that smaller doses inflict greater damage and hence result in a steep decline of survival. The differential in low-dose/high-dose slopes is greater in radioresistant cell types, has been linked to G2 radiosensitivity and mutant p53. It is absent when using high LET irradiation. There have been attempts to exploit the HRS effect in clinical fractionation protocols but with little success to date.

2.3.11. Other molecular targets: bystander (epigenetic) effects

Recent studies have suggested that cells close to irradiated cells but not themselves exposed to radiation may exhibit damage similar to that caused by radiation, such as DNA damage and reduced survival (a bystander effect). Irradiation of Chinese hamster ovary cells (CHO cells) with α-particles below 5 cGy and analysis of hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutations indicated very low track traversals of 0.05-0.3/cell where most cells are not hit, but the number of cells showing mutations was greater than the hit cells by a factor of 5. Similarly, irradiation of 1 human fibroblast on a dish with He^{+} from a microbeam produced 80-100 damaged cells and irradiation of the cytoplasm only produced DNA dsb in non-irradiated cells. These findings have been variously interpreted as suggesting a role of gap junctions between cells to communicate damage response signals, or that damaging molecules can be released into the medium surrounding the cells and/or that energy deposition in DNA is not required to trigger a bystander response. Currently the literature on bystander effects remains controversial. For example, there have been reported difficulties in repeating the irradiated-medium transfer experiments, and this is a topic area that requires further research and clarification.

2.3.12. Radiation sensitisers

Oxygen is an effective positive modulator of radiosensitivity between pO_2 levels of 0 and about 20 mm Hg. At pO_2 levels decreasing below 10 mm Hg tissues are considered to be hypoxic and show increasing radioresistance. The oxygen enhancement ratio (OER) is given by the dose in hypoxia divided by the dose in air to achieve the same survival level. A survival fraction of 0.01 requiring 10 Gy in oxic and 28 Gy in hypoxic conditions would indicate an OER of 2.8. At a dose level of 2 Gy used in the clinic the OER is somewhat less and approximates to 2.0. Sensitization by oxygen has generally been explained by the oxygen fixation hypothesis, in which oxygen is argued to be capable of binding to radicals on the DNA and prevention their immediate restitution by interaction with reducing equivalents (H^{+}-donating molecules such as thiols). The absence of oxygen and the presence of reducing equivalents would hence lower radiation toxicity and there is evidence that higher levels of free sulphydryls in cells can increase the PO_2 level required for sensitization. Accumulated OER data show a wide variation between cell lines. Recent work demonstrating that hypoxia can modify gene expression including DNA repair genes suggests that other mechanism may also play a role in oxygen sensitization and consistently lower OERs have been reported for cells lacking homologous recombination (HR) repair and crosslink repair.

There are many other molecules that have been found to increase the radiosensitivity of cells using clonogenic assays, including molecules that enhance DNA damage, such as halogenated pyrimidines, inhibitors of DNA repair, modifiers of cell cycle checkpoints, such as caffeine and modifiers of mitogen-activated protein (MAP) kinase signalling pathways, such as inhibitors of RAS, epidermal growth factor receptor (EGFR), or protein kinase B (AKT). The study of such molecules can illuminate our understanding of cellular response to irradiation but their application in the clinic requires some expectation of specificity for tumour cells vs normal cells. Previous studies have focused on biological or pathophysiological differences between tumours and critical normal tissues such as hypoxia or proliferation. Recent studies have been focusing on molecular differences such as levels of gene expression or mutations in critical genes such as protein 53 (p53). Differential uptake of halogenated pyrimidines into DNA in place of thymidine in proliferating cells provides one rationale and both bromodeoxyuridine (BrdU) and iododeoxyuridine (IrdU) have been studied clinically. These molecules are slightly larger than thymidine and partially disrupt the structure of the DNA making it more susceptible to damage by X rays (or UV light), thereby radiosensitizing the cells when a significant fraction of the DNA has incorporated the molecule (usually requires several cell generations in the context of normal background thymidine levels). To date these molecules have not shown great gains in clinical application, because of the difficulty of obtaining sufficient differential uptake between tumour and exposed normal tissue. Inhibitors of EGFR have recently been tested in the clinic with some success. EGFR is highly expressed on some tumour types e.g. Head and Neck Squamous Cell Carcinoma (HNSCC) and Non Small Cell Lung Cancer (NSCLC), and inhibition of the signaling pathway is believed to reduce proliferation of tumour cells and block stimulation of this pathway by the radiation treatment but the exact mechanisms of the effect remain uncertain.

Other approaches being investigated experimentally include antisense oligonucleotides to inhibit the expression of anti-apoptotic factors such as Bcl-2; gene directed enzyme prodrug therapy (GDEPT) targeting DNA synthesis; radiation-activated molecular switches to drive specific promoters in tumours to increase expression of toxic molecules such as tumor necrosis factor alpha (TNF-α): inhibitors of checkpoint kinases (Chk1 and Chk2) required for expression of cell cycle blocks conceivably because blocking abrogation would inhibit repair.

2.3.13. Radiation protectors

In whole body irradiated mice, addition of cysteine or cysteamine is protective with a dose reduction factor (DRF) of 1.8. This means that the dose becomes less effective i.e. the LD 50/30 (the dose of radiation required to kill [LD=Lethal Dose] 50% of the test cohort within 30 days) increases by this factor. These factors can also be demonstrated in vitro. Other molecules giving similar levels of protection include: mercaptoethylamine, and Amifostine (or WR 2721), which is a phosphorothioate that can be activated in vivo by alkaline phosphatase to its thiol metabolite. This drug is currently used in the clinic as a normal tissue protector based on data which suggests that the drug permeates normal tissue but not much in the tumour because of hypoxia and chaotic vasculature. There is good evidence that it does provide some normal tissue protection in HNSCC and NSCLC patients receiving radiotherapy but there remains controversy about whether or not it it has been shown also to cause some tumour protection. Amifostine is also claimed to protect against mutation and carcinogenesis, as well as against nephrotoxicity from cisplatin. Sodium selenite, pentoxifylline, and vitamin E all show clinical benefits in reducing morbidity e.g. less xerostomia, mucositis, proctitis, enteritis, and fibrosis.

2.4. Tumour radiotherapy

2.4.1. Tumour growth

Tumour growth occurs because of the proliferation of the tumour cells and the development of supporting stroma and vasculature (by angiogenesis). Since cell division is a binary process it can be expected that tumour growth would be an exponential function with the volume increasing as a semilogarithmic function of time. This implies a constant time for the tumour to double in volume (*volume doubling time*). Small tumours often express this form of growth function but as they get larger the growth rate of a tumour usually declines (longer doubling time) due to nutrient deprivation and other conditions. Cell kinetic analysis of tumours has established that even in small tumours not every tumour cell is actively proliferating (i.e the *growth fraction* is less than unity) and that there is substantial cell loss from tumours. These factors do not in themselves influence the exponential nature of the growth curve, unless they change with time during growth, but they do influence the interpretation of the value of the volume doubling time calculated from such curves. If every tumour cell was in the division cycle and there was no cell loss the tumour doubling time would reflect the cell cycle time of the tumour cells (T_C). The reduced growth fraction means that the underlying *potential doubling time* (T_{pot}) of the tumour is longer than the cell cycle time and the cell loss means that the measured volume doubling time (T_D) is even longer. Thus human tumours have an average T_D that is in the range of 2-3 months (with wide variation for different tumour types) but the average T_C is 2-3 days and T_{pot} values are in the range of 4-20 days.

2.4.2. Tumour response to irradiation

The response of tumours to irradiation can be understood largely in terms of the response of the cells (both tumour and stromal) within the tumours. Widely used in situ techniques to assess tumour response to irradiation include determining growth delay, i.e. measuring the difference in time for treated and untreated tumours to grow to a defined size, and tumour control (Figure 2.4A and 2.4B**).** Both these parameters can be plotted as a function of dose to give a dose response curve. Tumour growth delay is the more commonly used endpoint because tumour cure experiments are much more time consuming and resource intensive. However, intrinsic to tumour growth is the concept that tumours contain a fraction of cells that have unlimited proliferative capacity (cancer stem cells). To achieve tumour control, all the cancer stem cells must be killed. Thus the tumour control endpoint directly assesses the sensitivity of the last surviving (most resistant) tumour clonogenic cells (or stem cells). Since treatments that only induce a growth delay use lower doses and do not kill all the tumour stem cells (by definition), it is necessary to assume that radiation modifiers that are tested by this approach would be able to affect the remaining surviving stem cells equally. This assumption has been questioned and it may not be correct if a proportion of the tumour stem cells are resistant to the tested treatment for unknown reasons.

The terms *radiosensitive* and *radioresistant* are often used to describe tumours that regress rapidly or slowly after radiation treatment. However, the rate of regression may not correlate with the ability to cure a tumour with tolerable doses of radiation so it is better to describe a tumour that regresses rapidly after treatment as *radioresponsive*. The response rate of a tumour depends on the proliferative rate of its cells because tumour cells often express their radiation damage (and die) by mitotic catastrophe. Thus, a tumour that contains a large proportion of proliferating cells will tend to express radiation damage in its cells early and will regress rapidly. Although radioresponsive, the tumour may contain surviving tumour stem cells that will be responsible for its recurrence.

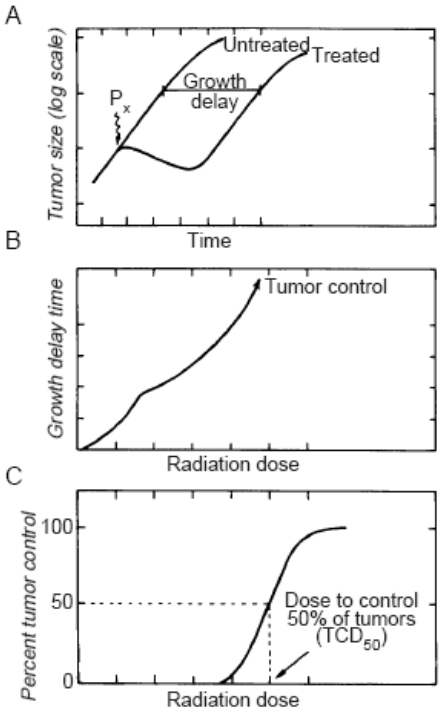

Fig. 2.4A, 2.4B and 2.4C Illustration of two assays for tumour response: In (A), growth curves for groups of treated and untreated tumours are shown and the measurement of growth delay indicated. Growth delay is plotted as a function of radiation dose in (B). After large doses some of the tumours may not regrow and the percentage of controlled tumours can be plotted as a function of dose as in (C) (Tannock et al, 2005).

As mentioned above the cancer stem cells within a tumour are unlikely to exhibit a uniform radiosensitivity. The microenvironment of the cells in the tumour can affect their sensitivity to radiation. This is well documented for hypoxia (see below) but there may also be interactions of the cells with the extracellular matrix (ECM). For example, interactions between the tumour cells and the ECM may influence cellular signalling such as the EGFR/MEK/ERK pathway that can affect cellular sensitivity to radiation. As discussed later, there is also increasing evidence that vascular damage and the induction of inflammatory cytokines play an important role in the responses of normal tissues to radiation treatment. The role of such factors in tumour response is largely unexplored. However, radiation-induced apoptosis of microvascular endothelial cells in a tumour has been suggested recently to play an important role in its response to radiation treatment.

2.4.3. Dependence of tumour control on dose and tumour size

Since tumour control depends on the killing of all the tumour stem cells, the proportion of such cells in a tumour (this may be as small as a few percent) and the tumour size can have a major influence on the dose required for tumour control. For a simple model, which assumes that the response of a tumour to radiation depends on the individual responses of the cells within it, the dose of radiation required to control a tumour only depends on: (1) the radiation sensitivity of the stem cells and (2) their number. The number of stem cells in a tumour can be estimated from its size and some assumption about the fraction of cancer stem cells that it contains. Equally from a knowledge of the radiation survival curve for the cells in a tumour, it is possible to calculate the expected level of survival following a given radiation dose. Because of the random nature of radiation damage there will be statistical fluctuation around this value (theoretically predicted by a Poisson distribution). From such calculations, it is

possible to construct a theoretical tumour control versus dose curve, which shows a sigmoid relationship with dose (Figure 2.4C). The position of the curve relative to dose will depend on the number of stem cells whereas the slope will depend on the radiosensitivity of the stem cells and as noted above on the extent of heterogeneity in these parameters. Thus in general it can be expected that larger tumours would need to be treated with larger doses for control to be achieved. This effect may be exacerbated by differential microenvironmental conditions, that can influence the radiosensitivity of the tumour stem cells, and may themselves vary with tumour size (e.g hypoxia – see below). A problem in the clinic is that normal tissue responses to irradiation also depend on the volume of tissue irradiated, such that it may be difficult to give larger doses to larger tumours.

2.4.4. Dose fractionation effects

The radiation tolerance of normal tissue is enhanced by fractionating the radiation dose over a number of days due to repair of radiation damage between the fractions and proliferation of surviving cells, thus higher doses can be given using this approach, which is the predominant mode of action of radiation therapy. However, the response of the tumour is also influenced by these factors and hence selecting the appropriate therapeutic approach depends on an appropriate balance between tumour response and normal tissue response (therapeutic ratio – see below). It is the response of late responding normal tissues that is usually the limiting factor in the dose that can be delivered to a tumour. Thus the finding that such tissues appear to have greater repair capacity than tumours is one factor favouring fractionated treatments. However, prolonging treatments over too long an interval may be counterproductive since proliferation and repopulation of the surviving tumour cells will occur during the treatment thus increasing the number of cells to be killed, whereas late responding normal tissue generally have low proliferation rates and extending the time will not greatly increase their radiation tolerance.

2.4.5. Predicting the radiation response of tumours

Multiple genetic and epigenetic changes occur in tumour cells during growth and it is well established that the microenvironment in tumours is very heterogeneous. Thus it is desirable to seek a way of assigning tumours to more homogeneous groups, so that patients with differences in prognosis can be identified. This is a major motivation for attempts to develop predictive assays. Studies of a wide range of cell lines derived from human tumours have shown intrinsic variations in radiation sensitivity. It is the size of the shoulder of the curves that varies most widely. Even small differences in the shoulder region can be important because they are magnified during the multiple fractionated daily doses of 1.8 to 2 Gy given in clinical radiotherapy. The cell survival following a dose of 2 Gy can vary widely in cells from different tumours from about 0.1 to 0.9 (Table 2.4). Consider a tumour for which the survival level following a dose of 2 Gy is 0.8. Assuming that each fraction of a multiple-dose treatment is equally effective, and that there is no cell repopulation between dose fractions (an assumption that ignores some of the issues to be discussed below), the survival following thirty fractions of 2 Gy would be $(0.8)^{30} = 10^{-3}$. In contrast, for a tumour in which the cell survival level following 2 Gy is 0.6, survival after 30 fractions would be $(0.6)^{30} = 2 \times 10^{-7}$. Thus, small differences in survival at low doses can translate into very large differences during a course of fractionated treatment. Estimates of the surviving fraction following a dose of 2 Gy for different histopathological types of human tumour show a trend toward higher levels of survival at 2 Gy for the cells from tumour groups expected to be less radiocurable.

The concept that tumour response for an individual patient can be predicted has been tested using the survival following 2 Gy of radiation (or another parameter that reflects radiosensitivity at clinically relevant low doses) to predict for the outcome of fractionated radiotherapy treatment. Using a clonogenic assay for cells from primary human cervix tumour biopsies grown in soft agar, West and co-workers in 1997 found that patients with tumours containing radioresistant cells (SF2>median) had significantly worse local control and survival than those with more tumours containing radiosensitive cells (SF2 <median; Figure 2.5**)** and similar results were later reported for head and neck cancers. However, other groups have not reported confirmatory results and the widespread application of clonogenic assays is limited by technical problems. Other proposed predictive assays evaluate radiation-induced apoptosis or senescence within solid tumours, or the expression of genes or proteins which relate to cell cycle control, cell death, and DNA repair. However, the predictive value of parameters such as the apoptotic index is uncertain given the limited correlation with cell death as assessed by a colony forming assay. Thus, although the evidence that tumour cells from individual tumours vary in radiosensitivity is strong and the concept of predicting the response of individual tumours remains appealing, a suitable, robust assay to detect such differences between individual patients has yet to be developed.

2.4.6. Tumour hypoxia

The cells in a tumour are influenced both by their interactions with the extracellular matrix (ECM) and by the pathological microenvironment of solid tumours, which is characterized by regions of nutrient deprivation, low extracellular pH, high interstitial fluid pressure (IFP), and hypoxia. These conditions in solid tumours are due primarily to the abnormal vasculature that develops during tumour angiogenesis. The blood vessels in solid tumours have highly irregular architecture, and may have an incomplete endothelial lining and basement membrane, which makes them more leaky than vessels in normal tissues. The oxygen concentration (pO_2) in most normal tissues ranges between 10 and 80 mm Hg, whereas tumours often contain regions where the pO_2 is less than 5 mm Hg. A proportion of tumour cells may lie in hypoxic regions beyond the diffusion distance of oxygen where they are exposed to chronically low oxygen tensions. Tumour cells may also be exposed to shorter (often fluctuating) periods of (acute) hypoxia due to intermittent flow in individual blood vessels. Tumour hypoxia is heterogeneous both within and amongst tumours and studies with both extrinsic and intrinsic markers of hypoxia have shown that hypoxic cells can occur close to blood vessels, presumably due to fluctuation in blood flow in individual vessels. Acute and chronic hypoxia can coexist in the same tumour and hypoxic regions in tumours are often diffusely distributed throughout the tumour and rarely concentrated only around a central core of necrosis. Hypoxia may play an important role in treatment outcome both because lack of oxygen results in cells being more resistant to irradiation and because hypoxia can affect the metastatic ability of some tumour cells.

TABLE 2.4 SURVIVING FRACTION AT 2 Gy FOR A VARIETY OF CELL TYPES

Tumour Cell Type[a]	Number of Lines	Mean Survival at 2 Gy (Range)
1. Lymphoma Neuroblastoma Myeloma Small Cell lung cancer Medulloblastoma	14	0.20 (0.08 – 0.37)
2. Breast Cancer Squamous cell cancer Pancreatic Cancer Colorectal cancer Non-small cell lung cancer	12	0.43 (0.14 – 0.75)
3. Melanoma Osteosarcoma Glioblastoma Hypernephroma	25	0.52 (0.20 – 0.86)

[a]Tumour types are grouped (*1-3) approximately in decreasing order of their likelihood of local control by radiation treatment. (Tannock *et al*., 2005)

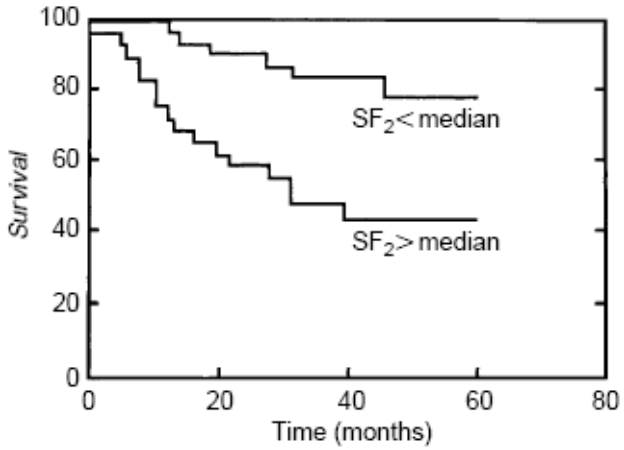

Fig. 2.5 Acturial survival in patients with cervical cancer treated by radical radiotherapy as a function of intrinsic radiosensitivity of tumours stratified as above or below the median survival following 2 Gy (SF2) of 0.41. Survival and local control (not shown) are significantly worse for patients with SF2>0.41. (Tannock et al., 2005).

The cells within hypoxic regions of tumours constitute an important target for cancer treatment since many such cells are viable and capable of regrowing the tumour if they survive treatment. Many tumours contain a proportion of hypoxic cells in the range 1 to 20%. These proportions represent the cells that are maximally resistant to radiation and there will also be a substantial proportion of cells in tumours that are at intermediate oxygen levels. Because of their resistance, the response of tumours to large single doses of radiation is dominated by the presence of the hypoxic cells within them, even if only a very small fraction of the tumour cells are hypoxic. Immediately after a dose of radiation, the proportion of the surviving cells that is hypoxic will be elevated. However, with time, some of the surviving

hypoxic cells may gain access to oxygen and hence become more sensitive to a subsequent radiation treatment. This process of reoxygenation can result in a substantial increase in the sensitivity of tumours during fractionated treatment. Nevertheless, many techniques have provided evidence that hypoxic cells in human tumours can affect the outcome of fractionated radiation therapy.

2.5. Normal tissue response to radiotherapy

2.5.1. Cellular and tissue response

Radiation treatment can cause loss of function in normal tissues. In renewal tissues, such as bone marrow or the gastrointestinal tract, loss of function may be correlated with loss of proliferative activity of stem cells. In other tissues, loss of function may occur through damage to more mature cells and/or through damage to supporting stroma and vasculature. Traditionally the effects of radiation treatment on normal tissues has been divided, based largely on functional and histopathological endpoints, into early (or acute) responses, which may manifest clinical symptoms within a few weeks of radiation treatment, and late responses where clinical symptoms may take many months or years to develop. Acute responses occur primarily in tissues with rapid cell renewal where cell division is required to maintain the function of the organ. Because many cells express radiation damage during mitosis, there is early death and loss of cells killed by the radiation treatment. Late responses tend to occur in organs whose parenchymal cells divide infrequently (e.g. liver or kidney) or rarely (e.g. central nervous system or muscle) under normal conditions. Depletion of the parenchymal cell population due to entry of cells into mitosis, with the resulting expression of radiation damage and cell death, will thus be slow. Damage to the connective tissue and vasculature of the organ may lead to progressive impairment of its circulation. If the damage to the circulation is severe enough, secondary parenchymal cell death may occur due to nutrient deprivation.

The radiosensitivity of the cells of a number of normal tissues can be determined directly using in situ assays. Survival curves obtained for the cells of different normal tissues in mice and rats are shown in Figure 2.6**.** Considerable variability in sensitivity is apparent and as with tumour cells, most of the difference appears to be in the shoulder region of the survival curve.

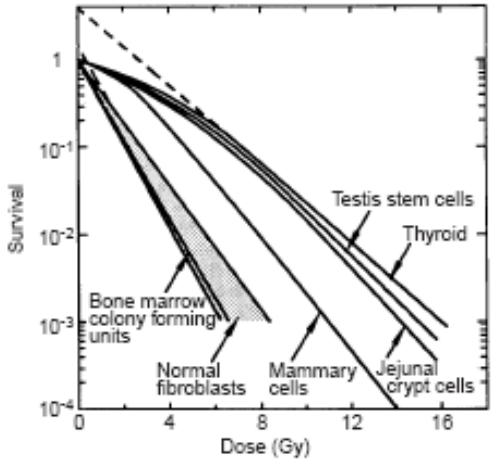

Fig. 2.6 Survival curves for cells from some normal tissues. Most of the curves are for cells from rodent tissues and the curves were produced using in vivo or in situ clonogenic assays. The range of survival curves for normal human fibroblasts are for cultured cell strains. (Tannock et al., 2005).

For study of the response of individual organs, one widely used approach is to define a level of functional deficit and to determine the percentage of irradiated animals that express at least this level of damage following different radiation doses. This approach results in sigmoidal dose response curves and dose-response relationships for normal tissues are generally quite steep and well defined.

Increased cytokine and chemokine expression has been observed within hours after irradiation in both early and late responding tissues, when there are no apparent functional or histopathological changes, and may recur and/or persist in cycles over many months, simulating a chronic inflammatory condition. These inflammatory factors may induce production of damaging radicals such as reactive oxygen species independently of those caused directly by the radiation treatment. The interplay between these various factors (cell killing, cytokine production, vascular damage) in producing the overall tissue damage remains poorly understood but is likely to vary from one organ to another.

2.5.2. Acute tissue responses

Acute radiation responses occur mainly in renewal tissues and have been related to death of critical cell populations such as the stem cells in the crypts of the small intestine, in the bone marrow, or in the basal layer of the skin. These responses occur within 3 months of the start of radiotherapy but are not usually limiting for fractionated radiotherapy because of the ability of the tissue to undergo rapid repopulation to regenerate the parenchymal cell population. Radiation-induced cell death in normal tissues generally occurs when the cells attempt mitosis, thus the tissue tends to respond on a time scale similar to the normal rate of loss of functional cells in that tissue and the demand for proliferation of the supporting stem cells. Radiation-induced apoptosis has also been detected in many cells and tissues, such as lymphoid, thymic, and hematopoietic cells, spermatogonia, and intestinal crypts. In lymphoid and myeloid tissue a substantial fraction of the functional cells can die by apoptosis and, thus, this mode of death plays an important role in the temporal response of these tissues to irradiation. In the crypts of the small bowel there is a fraction of stem cells that die by apoptosis, and the others die a mitosis-linked death. It has been proposed that radiation-induced endothelial cell apoptosis plays a role in early GI mucosal damage, but this remains a controversial issue and the significance of radiation-induced apoptosis in this tissue is unclear.

Following irradiation of skin, there is early erythema within a few days of irradiation and this is believed to be related to the release of 5-hydroxytryptamine by mast cells, increasing vascular permeability. Similar mechanisms may lead to the early nausea and vomiting observed following irradiation of the intestine. Expression of further acute skin reactions (moist desquamation and ulceration) depends on the relative rates of cell loss and cell proliferation of the basal cells, and they occur more rapidly in murine (7 to 10 days) than in human skin (2 to 3 weeks). The extent of these reactions and the length of time for recovery depend on the dose received and the volume (area) of skin irradiated, because early recovery depends on the number of surviving basal cells that are needed to repopulate the tissue. Erythema in human skin occurs at single doses greater than about 6 Gy, while moist desquamation and ulceration occur after single doses of 20 to 25 Gy. Increased cytokine levels have also been observed in skin and plasma following large doses of irradiation.

2.5.3. Late tissue responses

Late tissue responses occur in organs whose parenchymal cells normally divide infrequently and hence do not express mitosis-linked death until later times when called upon to divide.

They also occur in tissues that manifest early reactions, such as skin/subcutaneous tissue and intestine, but the nature of these reactions (subcutaneous fibrosis, intestinal stenosis) is quite different from the early reactions in these tissues. Late responses (usually regarded as those which occur more than 3 months after treatment) usually limit the dose of radiation that can be delivered to a patient during radiotherapy. The nature and timing of late reactions depends on the tissue involved and can be expressed as diminished organ function, for example, radiation-induced nephropathy (symptoms of hypertension, increased creatinine and blood urea nitrogen levels) or functional loss. However, one common late reaction is the slow development of tissue fibrosis that occurs in many tissues (e.g., subcutaneous tissue, muscle, lung, gastrointestinal tract), often a number of years after radiation treatment. Radiation-induced fibrosis appears to be associated with the aberrant and chronic expression of inflammatory cytokines, particularly TGF-β, following irradiation. This cytokine can stimulate proliferation of fibroblasts and their differentiation into fibrocytes that produce collagen. The volume of tissue or organ irradiated plays an important role in its response to irradiation but its roles may be different in different tissues, depending on the functional structure and functionality of the tissue. It is possible, for example, to given large doses to the whole of one kidney provide that the other kidney is functional and can take over the function of the damaged kidney. Similarly the dose required to cause functional impairment in lung depends on the volume of lung irradiated, with small volumes being able to tolerate quite large doses. The irradiated region will sustain severe damage and will develop fibrosis but the functional reserve of the lung will accommodate the loss of function of part of its volume. If this reserve is low, due to other damage, then lower doses can be tolerated. In contrast, in the spinal cord, giving a dose that severely damages the whole cross section of the cord to lengths of more than a centimetre is sufficient to disrupt the whole function of the cord and leads to myelitis.

Apoptosis has also been observed within hours after irradiation of a number of late responding normal tissues in rodents, such as the salivary glands, pulmonary and brain endothelial cells and spinal cord. For example, in rat spinal cord endothelial cell apoptosis following irradiation appears to initiate the disruption of the blood/spinal cord barrier, which may be an early lesion leading on to the development of white matter necrosis and myelitis. Apoptotic endpoints, however, have often not correlated with clonogenic survival or functional or histopathological endpoints, and the relevance of apoptosis in radiation-induced late normal tissue damage remains to be established.

2.5.4. Predicting normal tissue response

Patients receiving identical radiation treatments may experience differing levels of normal tissue injury; thus predictive assays might be useful in identifying those patients at greater risk of experiencing the side effects of radiotherapy. The enhanced radiosensitivity of patients with ataxia telangiectasia (AT) and Nijmegen breakage syndrome (NBS) supports a genetic contribution to individual variability in radiosensitivity. Studies of breast cancer patients have also shown individual correlation of acute and late skin reactions in one treatment field with those in a different treatment field. Several studies have quantitated the *in vitro* radiosensitivity of fibroblasts and peripheral lymphocytes as a potential predictive assay for normal tissue damage. These studies have shown variations in the radiosensitivity of fibroblasts from individual patients, but have been inconsistent in predicting late radiation fibrosis. While large differences in radiosensitivity, such as those observed in AT patients, are sufficient to cause discernable differences in late normal tissue effects, the differences in radiosensitivity of normal cells between most patients may not be sufficient to override the

effects of the other factors, such as cytokine induction, chronic inflammation and vascular damage that also influence the development of normal tissue damage.

2.5.5. Therapeutic ratio

All successful radiotherapeutic treatments depend on a favourable therapeutic ratio since the treatment involves exposure of normal tissues as well as the tumour. The concept is illustrated in Figure 2.7, which shows theoretical dose-response curves for tumour control and normal tissue complications. Tumour-control curves tend to be shallower than those for normal tissue response because of heterogeneity. In the clinic the therapeutic ratio is often defined as the percentage of tumour cures that are obtained at a given level of normal tissue complications (i.e., by taking a vertical cut through the two curves at a dose that is clinically acceptable, e.g., at 5% complications after 5 years, to give the *TD*5/5 value). In animal models it is more usual to define the therapeutic ratio in terms of the ratio of radiation doses *D*n/*D*t required to produce a given percentage of complications and tumour control (usually 50%). It is then a measure of the horizontal displacement on the dose axis between the two curves. It remains imprecise, however, because it depends on the shape of the dose-response curves for tumour control and normal tissue complications. The curves shown in Figure 2.7A depict a situation in which the therapeutic ratio is favourable because the tumour-control curve is displaced to the left of that for normal tissue damage. The greater this displacement, the more radiocurable is the tumour. Because the tumour control curve is shallower than that for normal tissue damage, the therapeutic ratio is more favourable for low and intermediate tumour-control levels. If the two curves are close together (Figure 2.7B) or the curve for tumour control is displaced to the right of that for complications, the therapeutic ratio is unfavourable because a high level of complications must be accepted to achieve even a minimal level of tumour control.

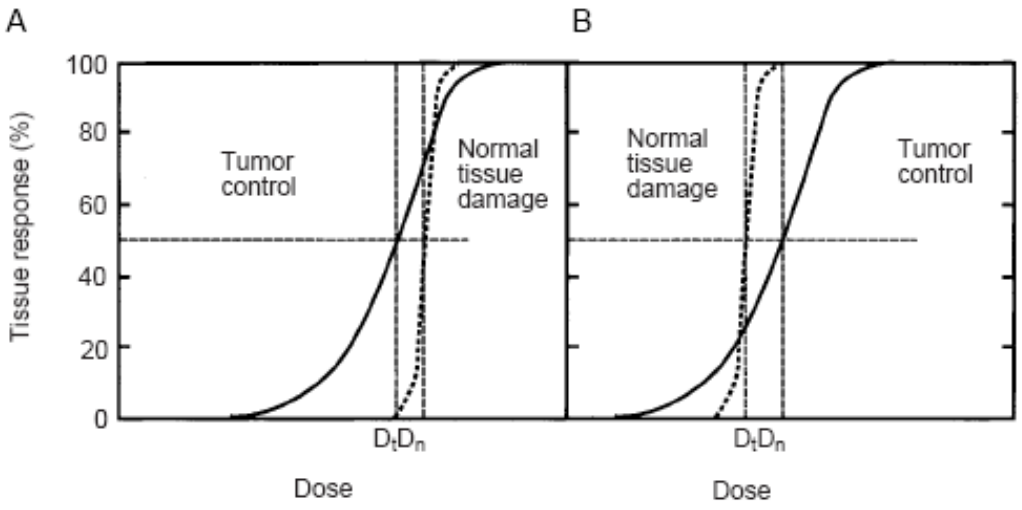

Fig. 2.7A and 2.7B Illustration of the concept of a therapeutic ratio in terms of dose-response relationships for tumour control and normal tissue damage (Tannock et al., 2005).

2.5.6. Whole body irradiation

The response of animals to single doses of whole body irradiation can be divided into four separate syndromes (prodromal, haematological, gastrointestinal, and neurovascular) that manifest following different doses and at different times after irradiation. Following doses greater than about 2 Gy, humans will develop early nausea and vomiting within hours of

irradiation (prodromal syndrome), which may be controlled with 5-hydroxytryptamine antagonists. The hematopoietic syndrome occurs at doses in the range of 2 to 8 Gy in humans (3 to 10 Gy in rodents) and is caused by severe depletion of blood elements due to killing of precursor cells in the bone marrow. This syndrome causes death in rodents (at the higher dose levels) between about 12 to 30 days after irradiation and somewhat later in larger animals, including humans. Death can sometimes be prevented by bone marrow transplantation (BMT) and cytokine therapy (e.g., GM-CSF, G-CSF, stem cell factor) provided that the radiation dose is not too high (<10 Gy) when damage to other organs may become lethal. There are substantial differences in the doses required to induce death from the hematopoietic syndrome (i.e., *LD*50 value) between different species of animals and even between different strains of the same species. The *LD*50 value for humans has been estimated at 4 to 7 Gy depending on the available level of supportive care (excluding BMT). The gastrointestinal syndrome occurs after doses greater than about 5 up to 15 Gy and in rodents doses at the upper end of this range usually result in death at about 1 week after irradiation due to severe damage to the mucosal lining of the gastrointestinal tract; this causes a loss of the protective barrier with consequent infection, loss of electrolytes and fluid imbalance. Intensive nursing with antibiotics, fluid, and electrolyte replacement can prevent early death from this syndrome in human victims of radiation accidents, but these patients may die later due to damage to other organs (e.g. kidney, lung). The neurovascular syndrome occurs following large doses of radiation (>20 Gy) and usually results in rapid death (hours to days) due to cardiovascular and neurological dysfunction.

2.6. Radiobiological basis of radiation protection

2.6.1. Health consequences after total body irradiation from radiation accidents

Radiation exposure of the total body with doses >2 Gy will cause clinical symptoms which, after higher doses may be so severe that they become life threatening. Such exposures are usually the consequence of accidents but such accidental exposures are rare. The most spectacular accidents were those in the nuclear industry which affected personnel such as the Tokaimura accident in 1999 in Japan when careless handling of sub-critical amounts of fissable material caused a chain reaction eventually killing 2 workers. The best known accident of the nuclear industry is the Chernobyl accident which lead to high total body doses in >200 rescue workers and firemen. Twenty eight of them died within 2 months from radiation sickness.

More frequent than accidents in the nuclear industry are accidental exposures of non-involved people from lost or discarded radioactive sources such as cesium from radiotherapy equipment (in Brazil (1987), iridium sources for testing the quality of welding in pipelines (in Algeria, 1978), or forgotten radioactive sources used for the training of military personnel (in Ukraine, 1973). In contrast to the described nuclear industry accidents, where radiation exposure of the body was acute and fairly homogeneous, radiation exposure from accidents with lost radioactive sources is usually very inhomogeneous and protracted over days and weeks.

The most dramatic “accidental” radiation exposures were caused by explosions of nuclear weapons. Two nuclear weapons exploded in Hiroshima and Nagasaki in August 1945, killing more than 100,000 people within a few weeks from mechanical injury and, above all, from thermal burns. These became even more lethal as a result severe radiation sickness. Other, unplanned accidental exposures to radiation from nuclear explosions occurred from weapons tests which caused high radiation doses in populations living at distances of >50 km from the

test site, such as near Semipalatinsk in Kazakhstan in 1948 and in the Marshall Islands in 1954.

The signs and symptoms of radiation sickness after an acute total body exposure are predominantly the consequences of radiation injury to the haemopoietic tissues in the bone marrow. Proliferating cells of the bone marrow decrease their proliferative activity after radiation exposure. Consequently, fewer cells are available for differentiation and maturation to white blood cells, red blood cells and platelets. Thus, the balance of cell production in the bone marrow and cell elimination from the peripheral blood is disturbed. Yet, mature cells of the myeloid line are not damaged by radiation exposures of a few Gy. Since mature granulocytes have a life span of only one day, the radiation-induced decrease of supply of granulocytes, called hypoplasia, will occur first, and followed by a decrease of the number of platelets. Since the time of granulocyte maturation between the last mitosis of myelocytes and the transit of early granulocytes into the blood is about four days – which is not disturbed in any way by radiation doses of a few Gy after total body irradiation - the decrease of granulocytes in the blood starts only after a delay of 4 days. Hypoplasia of the granulocytes (leukopenia or granulocytopenia) increases until day 12.

The further development of the haemopoietic radiation syndrome depends on the number of bone marrow stem cells which survived radiation exposure and are stimulated into very fast regeneration. If, after day 12, the concentration of granulocytes in the blood is maintained at a plateau of about 1,000/µl, this can be taken as a prognostically favourable sign, indicating a high probability that the number of surviving bone marrow stem cells was high enough to regenerate the bone marrow without any long term damage to haemopoiesis. The hypoplastic phase reaches a minimum of granulocytes 6 weeks after radiation exposure. If, however, the granulocyte count continues to decrease after day 12, there is a high risk that the number of surviving bone marrow stem cells is insufficient to lead to rapid regeneration of haemopoiesis before severe, potentially fatal consequences of leukopenia and of thrombopenia develop. In these cases, the only therapeutic option is allogeneic stem cell transfusion ("bone marrow transplantation"). In less severe cases when there is hope that sufficient bone marrow stem cells survived which can be stimulated, the therapeutic application of growth factors, in particular of G-CSF may be given to lead to maximal stimulation of proliferation and maturation of granulocyte progenitors.

The time course of decrease and recovery of platelets is similar to that of granulocytes but somewhat slower. Due to the long life span of erythrocytes, no significant anaemia is expected as a consequence of bone marrow hypoplasia, however, after the Chernobyl accident aneamia was common, caused by intravascular coagulation due to the widespread skin burns and the severe radiation injury of the skin from skin contamination with radioactive fission products.

In animal experiments, the severity of the haemopoietic radiation damage increased with dose between 2 and 10 Gy. In mice, some animals are likely to die from septicaemia after a dose of around 5 Gy, caused by severe agranulocytosis and diffuse interstitial haemorrhage. Deaths occur at the time of the nadir of granulocyte depletion in the blood, i.e. in the third week after acute radiation exposure. Half of the animals are likely to die after a total body dose of approximately 7 Gy (LD-50). After 9 Gy, the chances of survival are small unless a specific therapy is initiated. There are no data sufficiently reliable to define a LD-50 value for humans. Moreover, the probability of survival depends more on other concomitant risk factors such as chronic infections, and on the quality of medical interventions.

The medical treatment of acute radiation sickness after total body irradiation which may become clinically significant between 2 and 5 weeks after irradiation is purely symptomatic (with the exception of the rare cases where stem cell infusion is indicated). The pathogenesis of the haemopoietic radiation syndrome is very similar to the pathogenesis of bone marrow damage from cancer treatment with cytotoxic agents. This means that the treatment of radiation sickness should follow the principles and methods established by medical oncology for treating haematological toxicity in cancer patients. Since in most cases, spontaneous regeneration of the bone marrow from surviving stem cells is likely, the primary aim of medical intervention is to bridge the period of critical granulocytopenia and thrombopenia, i.e. to prevent septic infections and internal haemorrhage. Bacterial decontamination of the gut and the oropharynx, replacement of platelets, treatment of infections with antibiotics, and prophylactic treatment is to prevent fungal and herpes infections. Such conservative treatment following the principles established by medical oncology is very successful. This is proven by the fact that none of the victims of the Chernobyl accident succumbed primarily to the haemopoietic radiation syndrome. Those, who died fell victims to the extensive and severe thermal and radiation burns of large areas of the skin. In all radiation accidents since the Chernobyl accident, prophylactic treatment with haemopoietic growth factors, in particular with G-CSF, has been used. This led to rapid restoration of the granulocyte count in the blood. However, the overall impact on survival was less convincing.

Since the full signs and symptoms of the acute haemopoietic radiation syndrome after high radiation doses, which would require intensive treatment, does not occur until after a delay of 3 – 4 weeks, there is no time pressure for assessing the prognosis and for planning adequate treatment. Criteria for triage to assess the prognosis and the need for treatment are shown in Table 2.5**.** These criteria were used with great success after the Chernobyl accident. The clinical signs such as vomiting in the first few hours after exposure, as well as hair loss and lymphocyte counts in the first week after exposure, are more important for medical decision making than any results of physical or biological dosimetry.

TABLE 2.5 TRIAGE CRITERIA USED AFTER THE CHERNOBYL ACCIDENT

Severity	Vomiting time	Lymphocytes day 3	Hair loss within 2 weeks	Cytogenetic Radiation Dose	Lethality including skin burns
Mild	no	>600	no	< 2 Gy	0/105
Intermediate	after 1-2 h	300-600	no	2 – 4 Gy	0/53
Severe	after 30-60 min.	100-300	yes	4 – 6 Gy	6/23
Very severe	immediate	<100	yes	6 – 16 Gy	19/22

The determination of radiation dose from accidental exposure in the first few weeks after the accident is commonly done by a combination of physical reconstruction of exposure scenarios and calculation of organ doses and total body doses as well as by biological dosimetry. The preferred method of biological dosimetry which has proven its value in many minor and major accidents is the determination of the frequency of unstable chromosome aberrations in stimulated lymphocytes. The method has been well standardised: phytohaemagglutinin is added to 5 – 10 ml heparinised blood to stimulate resting lymphocytes into proliferation. After incubation for 48 hours at 37°C, cells entering mitosis are arrested in metaphase by

adding colchicine. It is important to arrest cells in their first mitosis since many of the severe chromosome aberrations which are used as "dosemeters" are eliminated in the first cell division. As a general rule, the number of dicentric chromosomes is counted in 500 arrested metaphases. If there are 25 dicentrics among 500 metaphases, a total body dose of 0.3 Gy can be assumed. After a dose of 3 Gy, there is, on average, one dicentric chromosome to be found in each metaphase. After homogeneous total body irradiation, the number of dicentric chromosomes per cell follows a Poisson distribution. Marked deviations from a Poisson distribution are an indicator of very inhomogeneous dose distribution which may have consequences for the prognosis.

The frequency of dicentric chromosomes decreases exponentially with time with a half time of approximately 3 years. Therefore, this method is less suited to assess radiation doses many years after exposure. Modern cytogenetic techniques, in particular the FISH technique (fluorescent in situ hybridisation, permits the evaluation of balanced translocations many years after radiation exposure. Since they are as characteristic of radiation exposure as dicentric chromosomes they have been used successfully in many situations where retrospective dosimetry was the aim.

2.6.2. Long term radiation risks from low radiation doses

The dramatic experience of the people of Hiroshima and Nagasaki in 1945 was the initiator for a proposal by the National Academy of Sciences of the USA to develop a programme for life-long follow-up of all A-bomb survivors. This programme, started in 1949 by the US Atomic Bomb Casualty Commission (ABCC) and continued by US-Japanese co-operation in the Radiation Effects Research Foundation (RERF) is arguably the largest, most comprehensive and most detailed epidemiological study ever performed – and it has been decided that even now, more than 60 years after exposure, follow-up will continue.

The results of this study are the most importance source of information on which rules and regulations of radiation protection are based. No other epidemiological study has comparable influence. Animal experiments and in vitro studies may provide mechanistic information but the RERF studies are the "gold standard" against which all other epidemiological and radiobiological studies on the long term effects of radiations on man have to be judged. The reason for this outstanding role is that in this study a large normal and healthy population of all ages and both sexes who have been exposed to a wide range of radiation doses to all organs of the body. Most important, however, is that through an incredibly massive effort, the radiation doses to all critical organs of each member of the cohort has been individually assessed by various methods of retrospective dosimetry. The various studies can be grouped into: (1) the Life-Span-Study (LSS). This prospective cohort study has been studying approximately 120,000 people. Radiation doses have been reconstructed in nearly 90,000. Approximately 5,000 had received a total body dose > 1Gy. The last comprehensive analysis of the fate of these A-bomb survivors was published by Preston et al (2003), and it encompasses all deaths from all diseases until December 1997 which could be attributed to radiation exposure. The extraordinary quality of the study is documented by the fact that only 0.2% of cohort members were lost from follow-up. 52% of the cohort population had died before 1.1.1998, and 9,917 of these deaths were from cancer or leukaemia. Cancer incidence has been studied in parallel based on the cancer registries of the provinces of Hiroshima and Nagasaki. Thus, both cancer incidence and cancer mortality can be studied in relation to radiation exposure. (2) The Adult Health Study comprises 20,000 people, a subgroup of the LSS, predominantly those exposed to higher doses. Each member of this cohort is invited to a free health check every two years in the outpatient clinics of RERF. This way, also non-fatal

health effects are detected and analysed in relation to radiation exposure, such as skin cancer, alterations of thyroid function etc. (3) The F-1 Study comprises approximately 70,000 children of parents who were exposed by the A-bomb explosions. This study is expected to give information on heritable radiation effects such as typical genetic diseases, but also about cancers in the offspring of irradiated people. (4) The In-Utero Study investigated the children born in Hiroshima and Nagasaki between September 1945 and May 1946 to detect potential radiation damage to the development of the embryo and fetus in utero.

2.6.3. Radiation-induced cancer in the A-bomb survivors

The most important and most significant long term health damage observed in the LSS of the A-bomb survivors is a dose dependent increased mortality from cancer. Among the 44,771 deceased members of the life span cohort with detailed dosimetric information available, there were 9,335 deaths from cancer and 582 deaths from leukaemia. By analysing the relationship with radiation exposure, it has been concluded that approximately 440 cancer deaths (i.e. approximately 4%) and nearly 100 leukaemia deaths (i.e. approximately 15%) can be attributed to the radiation exposure from the bomb in 1945. Significant relationships to radiation exposure were found for the following types of malignant disease (in decreasing probability of cancer mortality): stomach, colon, lung, leukaemia, breast, oesophagus, bladder, ovary, liver. It is remarkable that some of the most common types of cancer in the general population are not induced to any significant extent by radiation such as cancer of the prostate, of the cervix or of the rectum. Since, at the time of the last evaluation of data, nearly 50% of the cohort were still alive, it is not possible to make well-founded statements on the life-time risk of dying from radiation-induced cancer for people who were young at the time of exposure. However, cautious extrapolations have been made which are presented below.

It has been observed in the LSS that people who had been exposed to radiation as children or adolescents develop radiation-induced cancer after a longer latency than those who were exposed later in life, more specifically, radiation-induced cancers tend to occur at the time when the age-related increase of spontaneous cancer risk occurs. This observation was the basis for the commonly used risk projection model, the “relative risk model”. It states that irradiation causes a dose-dependent increase of the relative risk of developing certain types of cancer. The overall risk of a person is estimated by his or her spontaneous cancer risk at the age of estimation (taken from national cancer statistics) multiplied with the dose dependent risk factor. Since the spontaneous risk of dying from cancer steeply increases with age, most radiation-induced cancers, according to this model, even in those irradiated before adulthood, occur after very long latencies, at ages higher than 60 years. Based on these and some other assumptions it has been estimated (ICRP 60) that the life-time risk of dying from radiation-induced cancer after an acute exposure to 1 Gy (or 1 Sv) is 10%. If the dose is given over a period of weeks or months the risk factor is 5%, and if spread over a working life it is 4%.

From the information collected by the LSS, the dependence of the risk of dying from radiation-induced cancer on factors other than dose has been determined, such as age, sex and irradiated organ. The risk factors presented above are mean values for the general populations, a mix of old and young, of male and female. Therefore, they should not be used to estimate risk for an individual person.

In many situations of radiation exposure, such as in medicine or environmental radiation exposure, different organs receive very different radiation doses. Mainly based on the data of the LSS, but also some other epidemiological studies described in the next chapter, the relative contribution in the total radiation risk of radiation exposure of individual organs have been estimated. Three classes of organs have been identified and given organ weighting

factors (Table 2.6)**.** The mean radiation dose to each organ is multiplied with the respective organ weighting factor. All weighted organ doses are added up to arrive at the radiation dose which would result in the same radiation risk if given homogeneously to the total body. This sum of weighted organ doses is called the effective dose.

TABLE 2.6 ORGAN WEIGHTING FACTORS FROM ICRP IN 2007 (modified from ICRP ANNALS NUMBER 103)

	Organs	Organ weighting factor
High sensitivity	bone marrow, stomach, colon, lung breast	0.12
Intermediate sensitivity	Bladder, liver, oesophagus, thyroid	0.04
Low sensitivity	skin, bone surface remainder tissues (13)	0.01 0.12 (in total)
Genetic and Somatic effects from Gonadal exposure	gonads (ovary, testis)	0.08

It is expected that as new information arises from the continuing research on the LSS population, these factors may need further revision. Recent studies demonstrated a significant dose dependent increase in mortality from cardiovascular disease after latencies longer than 30 years. Also the re-evaluation of genetic risks may affect those numbers in future. These revisions are made after extensive consultations with radiation protection experts and radiation biologists by international committees, in particular by the International Commission on Radiological Protection (ICRP).

2.6.4. Epidemiological studies in other radiation-exposed populations

Other epidemiological studies which add important information for the assessment of the long term health consequences in radiation exposed people, mainly from radiation-induced cancer but also from other non-malignant diseases have been performed. Although, none individually has the same impact on radiation protection concepts as the Life Span Study, they help to specify the radiation risks from particular exposure scenarios (UNSCEAR 1994). The most important studies can be classified into:

- Radiation workers
- Studies in people who were exposed to radiation in the treatment of various diseases, such as
    1. Tuberculosis
    2. Ankylosing spondylitis (M. Bechtherew)
    3. Mastitis
    4. Cancer

- Studies in people who were exposed to high levels of naturally radioactive materials, in particular radon

    1. Miners

    2. People in homes

- Studies in people who were exposed to the fall-out of nuclear explosions and accidents in the nuclear industry

    1. Chernobyl

    2. Marshall Islands

    3. Techa River

2.6.4.1. Radiation workers

The first information on the risk of radiation-induced cancer was from studies comparing mortality of different medical professions. A significant increase in cancer mortality was found for radiologists who joined the profession before 1930, i.e. before strict rules and regulations were introduced to reduce occupational radiation exposure. Most occupationally exposed people today, also workers in the nuclear industry, receive very small radiation doses and no significant increase in cancer rates have been found with two exceptions which refer to the pioneering times of nuclear industry, i.e. workers in the reprocessing plant of Sellafield (UK) and workers in the plutonium factories of Mayak (former USSR). Particularly the latter is of great interest for radiation protection since it is the only major source of information on the radiation risks from plutonium.

2.6.4.2. Patients

Radiation played a bigger role in the treatment of various diseases up to the 1960s than today, with the exception of cancer for which the role of radiotherapy has been increasing steadily. Before the availability of powerful tuberculostatic drugs, pneumothorax with the aim of improving blood perfusion in the affected lung was a major treatment option. This required careful control of the collapsing lung which was done under permanent fluoroscopy. This way, the chest wall of patients accumulated very high radiation doses of up to >10 Gy. In females, a dose dependent increase in the risk of developing breast cancer was observed which depended strongly on age at exposure. Between the ages of 20 and 40 risk decreased dramatically, and there was little evidence that women after menopause were at any risk, if at all.

Mastitis, i.e. bacterial inflammation of the breast of women soon after giving birth has been one of the most successful indications for radiotherapy of non-malignant diseases. Total doses often were <2Gy. Compared to the unirradiated breast, radiation caused a significant, dose dependent increase of the risk of cancer in the irradiated breast later in life. The results of both studies are important to test the results of the LSS with regard to the risk of breast cancer. Japanese women have a very low base-line incidence rate of breast cancer which caused uncertainty of how to extrapolate those findings to European and American women. In general, the relative risk model (taking the country specific base-line cancer risks into account) and the age dependence of risk are similar in these studies.

The study on the risk of leukaemia and cancer among 14.000 British patients who suffered from ankylosing spondylitis (M. Bechtherew) treated with radiotherapy to the spine with total doses of 6 – 12 Gy was the first study which provided convincing evidence that low radiation doses which do not cause any acute or late normal tissue damage still may cause leukaemia and cancer.

It has long been discussed whether or not patients who have been successfully treated for cancer with radiotherapy have an increased risk of developing a second cancer. Second cancers, in general, are frequent after curative radiotherapy since the patient survives longer into an age when age-related cancer becomes increasingly frequent. From recent epidemiological studies it can be estimated that 80%-90% of second cancers after radiotherapy are due to the longer life span of a cured patient. The estimation of risk of radiation-induced cancer from radiotherapy of a first cancer can be derived from studies in patients with cancers which are frequent and have a similar chance of cure if treated with surgery or with radiotherapy, mainly cancer of the prostate, the breast and the cervix. From large prostate cancer study it can be concluded that if a patient is to be treated with radiotherapy, the risk of developing a radiation-induced second cancer is approximately 0.3%. (If this risk would be calculated using the organ weighting factors and the methodology described above for radiation protection purposes, the risk would be two orders of magnitude higher, because there is no allowance for cell kill after the high doses; therefore beware of doing this in people who are to receive radiotherapy for benign or for malignant diseases!). Half of the 0.3% risk of the radiation-induced cancers is in the low-dose regions such as lung and is probably induced by the same mechanism which is also responsible for the increased cancer risk of the A-bomb survivors. The other half is in the high-dose regions where radiotherapy frequently induced atrophy associated with chronic inflammation which is a well-known pre-cancerous lesion. The dose-incidence relationships of these two mechanisms are entirely different.

2.6.4.3. People exposed to high radon concentrations

Miners working in the hard rock mines in Saxony had long been known to die early from a wasting lung disease. More than 20 years before radioactivity was detected, in 1876 an epidemiological study in Schneeberg identified this lung disease as small cell lung cancer. This was related to the exposure of the miners working underground to an unknown carcinogenic agent in the air of the mines. It took 80 years until the cause of these lung cancers was identified as the decay products of the naturally radioactive noble gas radon. Numerous studies on miners, particularly working in uranium mines confirmed the early findings and defined a proportional relationship between the product of exposure time and radon decay product concentration in the air and the risk of lung cancer. As already proposed in the 19th century, forced ventilation of the mines reduced this risk to insignificant values.

Since high radon concentration may also occur indoors in some regions with special geological features, several large epidemiological studies have been performed to see whether the radon levels often measured in different rooms of normal houses may also be associated with an increased risk of lung cancer. The results of these studies are unequivocal in demonstrating that a mean radon concentration of >200 Bq/m³ is associated with an increased risk. This value is therefore chosen as an intervention level which, if exceeded should initiate measures to reduce radon levels in houses. Of particular importance is the finding, both in the miner studies and in the indoor radon studies that radiation risk and smoking risk are supra-additive, or even multiplicative.

2.6.4.4. Victims of nuclear accidents

The Chernobyl accident led to exposure of large populations, particularly in the most affected regions of Ukraine, Belarus and Russia with small to moderate radiation doses from radioactive fall-out. The determination of radiation doses to individuals was very complicated and still is associated with some uncertainty. Several hundred-thousand rescue workers (called liquidators) were exposed to estimated radiation doses between 0.05 and 0.25 Gy, mainly through external irradiation from radioactivity deposited to the ground. Several large epidemiological studies in the different Independent States are under way to determine the long term health effects, in particular leukaemia, cancer, and cardiovascular diseases. The identification of potential causes of disease among those often traumatised people is complicated by the social disruption the Chernobyl accident has caused to the affected populations and the rescue workers. So far, no undisputed results have been published, 20 years after the accident, although an increase in leukaemia rates look likely. On the other hand, there is no evidence of an increase in childhood leukaemia rates in any of the affected populations. The main health consequence of the Chernobyl accident is a massive increase in the rate of thyroid cancer among children. This was caused by uptake of large amounts of I-131 with milk in the weeks after the accident. Many cases have been documented, in those people who were small children at the time of the accident. Estimation of radiation doses to the thyroid has revealed a mean value of approximately 0.2 Gy in the thyroid cancer cases. There is a very pronounced age dependence of risk, the younger the child, the higher the risk. With international assistance, a very comprehensive state-of-the-art medical treatment programme, including treatment with I-131 became available to all affected children as a result of which, so far, the number of fatalities is "only" small but is bound to rise in years to come since there are still many surviving with metastatic disease. The dose dependence of risk of thyroid cancer among the Chernobyl children is similar to the dose dependence established before in various epidemiological studies on people who were irradiated with X rays for various diseases such as tinea capitis or thymic hyperplasia.

The United States of America tested their nuclear weapons between 1946 and 1956 on Bikini and Eniwetok in the Marshall Islands. This resulted in wide-spread contamination of distant atolls; however, radiation doses to the inhabitants were usually small, with the exception of one test in 1954 which went wrong, exposing the 82 inhabitants of Rongelap to external total body doses of approximately 2 Gy and 159 inhabitants of Utrik to 0.1 Gy by γ-rays from ground contamination. In addition, very high radiation doses to the thyroid of children resulted from incorporation of large amounts of I-131. Some Rongelapese children even developed clinical signs of myxoedema. Epidemiological studies described a high incidence of benign and malignant thyroid nodules in the most affected children. However, an epidemiological study on the entire population of the Marshall Islands did not provide undisputed evidence of an increased incidence rate of thyroid cancer as a result of radioactive fall-out from the bomb tests.

The plutonium factory of Mayak near Chelyabinsk in Siberia discharged, in the early years of its operation from 1946, large amounts of radioactive waste into the River Techa, this way exposing the populations downstream to high doses of γ-rays from sediments of the river banks. A large international effort has been initiated to study the health of these many thousands of people and relate this to estimated radiation doses in the various villages and fields. The importance of this study rests on the expectation that the population studied is similar in many respects to the LSS cohort in that it is a large normal, healthy population exposed to similar doses as in the LSS. However, the Techa river population accumulated the radiation dose over several years while the LSS population received the same doses within

seconds. The initial results of this study suggest that this expectation may be met, in order to answer one of the most import questions of radiation protection, namely whether radiation doses protracted over many months are equally, or more, or less effective than the same doses inflicted in a few seconds.

2.6.5. Mechanisms of radiation-induced cancer

The development of cancer occurs over a long period of time following a multi-step process. The extraordinary length of the silent or latent phase can be estimated from the results of the LSS of the A-bomb survivors. In the majority of cases diagnosed so far, there were more than 40 years between the radiation exposure which significantly contributed to the development of the cancers and their clinical manifestation.

The steps of carcinogenesis have been classified into initiation, promotion and progression. In some cancers, each step has been associated with specific mutational events, in particular the inactivation of tumour suppressor genes and the activation of oncogenes. In some radiation-induced leukaemias, the specific molecular changes have been identified. However, no typical "fingerprint-mutation" has been found so far for radiation-induced cancer, which would betray the causation by radiation exposure, The characteristic effects of radiation damage to the DNA permit the prediction that both the inactivation of tumour suppressor genes by deleting the whole gene or important parts of them, as well as activation of oncogenes by translocations of promotors to proto-oncogenes as a result of breakage/re-union mechanisms could occur after radiation exposure. So far, however, the results of basic radiobiological research do not permit the identification of the crucial molecular steps in radiation carcinogenesis which could also assist in defining the dependence of risk on low and very low radiation doses as they apply to modern medicine and industry and environmental radiation exposure.

There is strong evidence from animal studies and some human studies that the risk of radiation-induced cancer may be determined by various genes, such as mutations of the Rb gene. Other genes discussed in this context include BRCA 1 and 2. However, at the present state of knowledge, the role of genetic susceptibility on individual risks of radiation-induced cancer cannot be resolved definitively, although there is general agreement that it will be important. The implications of such findings on the selection of people for special occupations with a high risk of radiation exposure also need serious consideration.

2.6.6. Radiation effects in the developing embryo and fetus

In Hiroshima and Nagasaki, >1,500 children born between September 1945 and March 1946 were investigated at regular intervals between 1948 and 1964 to study the effects of radiation doses between 0.01 and >1 Gy on intra-uterine development at different stages of pregnancy. This study remains the only reliable source of information on the radiosensitivity of the unborn human.

Whereas experimental studies in mice demonstrated a wide range of characteristic malformations such as spina bifida, exencephaly or bone malformation of the extremities at doses well below 1 Gy, with the type of malformations showing very strict dependence on the stage of pregnancy, no such malformations were found to be increased in a dose dependent way in the children of Hiroshima and Nagasaki. However, there were 18 children who presented with microcephaly and severe mental retardation. The mothers of 15 children had been exposed to radiations from the bomb explosions at close distance from the hypocentre

when they were in week 8 to 15 of pregnancy, while 3 were exposed in later stages of pregnancy. Findings of dystopic grey matter by MRI investigations of some of those severely retarded people are in accordance with the results on experimental studies of the effects of radiation doses < 1 Gy given to pregnant mice in late pregnancy. The migration and maturation of immature neural cells during the development of the forebrain was severely disturbed. The result of this disturbance of migration is disorganisation of the formation of the structure of the synaptic network.

Damage to the intrauterine development of mice was found in none of the experimental studies after doses < 0.1 Gy. Also, in the studies of the Hiroshima children there is evidence for a threshold of 0.1 Gy. At higher doses, the risk of severe mental retardation increases rapidly to a value of 40% after 1 Gy. In later stages of pregnancy, the threshold dose may be higher. At the age of 10, all children who were exposed in utero had an IQ test. Also, the school performance at the same age was analysed. There was statistical evidence for a dose dependent decrease of the mean IQs as well as the mean school performance scores of those groups exposed in weeks 8 to 15 and 16 to 25 after doses >0.1 Gy. No decrease of intellectual development was recorded if irradiation had occurred before week 8 or after week 25, even if doses were >0.5 Gy.

Embryos in the pre-implantation stage are very radiosensitive. However, the radiation damage inevitably will lead to death of the conceptus and early abortion. Those embryos that survive develop normally. In human early fetus, also in the first few weeks after implantation during the period of major organogenesis, a comparable all-or-nothing effect is likely, i.e. either an early, spontaneous abortion or normal development. The results of these studies as well as of some follow-up studies and anecdotal reports after medical exposures demonstrate the high radiosensitivity of the developing embryo and fetus, in particular during the time of brain development. The findings of a probably threshold of 0.1 Gy will influence the advice to be given to pregnant women after a diagnostic radiology procedure. In particular after abdominal CT investigations, careful analysis of radiation doses in the uterus as well as medical anamnestic exploration has to be performed. A recommendation of termination of pregnancy because of possible radiation injury is very unlikely in most cases either because radiation did not occur in weeks 8 to 15 or because radiation doses to the uterus from most radiological procedures is well below 0.1 Gy.

2.6.7. Radiation-induced heritable diseases

Ever since the ground-breaking experiments of Muller in 1927 who was the first to describe that X rays would produce mutations and heritable disease in the fruit fly, the major concern of radiation protection was the possible deleterious effects of ionising radiation on the health of future generations. The dramatic experience of the A-bomb explosions in Japan initiated a very large research programme, to study and to assess the genetic risk to populations from increased radiation exposure of the general population. The large epidemiological programme among the children of the A-bomb survivors (the F-1 Study) was not informative nor was any of the other studies which investigated the children of radiation workers or of radiotherapy patients. The reason for these negative findings is that each person, with the exception of identical twins, is genetically unique and that any changes induced by mutagenic agents are diluted in the vast heterogeneity between individuals. For this reason, it requires genetically homogeneous mammals to investigate, quantitatively, the mutagenic effects of radiations. Those studies have been performed in few large institutions on millions of highly inbred mice. Most of the studies used the seven locus method, i.e. a breeding study in which irradiated wild-type animals (usually males) which were homozygous for seven un-mutated

genes which code for recessive traits were mated with unirradiated "tester" animals, usually females, which were homozygous for the same 7 genes in the mutated state. Since all seven genes are recessive, all offspring of this mating look normal since all will be heterozygous for all 7 genes, unless a mutation is induced in the irradiated animal. The typical features of homozygosity in those progeny are clearly visible signs such as fur colour or ear shape etc., permitting rapid scoring of large numbers of animals. Without irradiation, the spontaneous mutation rate is 1:100,000. With increasing radiation dose this mutation rate increased following a linear dose response curve if protracted radiation is given. The spontaneous rate is doubled by a dose of 1 Gy (doubling dose DD).

There is no good reason to assume that in humans, the doubling dose may differ significantly from that in mice. However, the mutation doubling dose does not give any useful information on the risk of heritable disease. Therefore, the mouse doubling dose is combined with information derived from human population genetics to estimate the risk of heritable disease in the progeny of irradiated people. Heritable diseases may occur as direct result of a mutation in a single gene (single gene disorders). Inheritance of these diseases follows the rules established by Mendel, and may be autosomal dominant, autosomal recessive or sex-linked recessive. For these "Mendelian" diseases, there is a straightforward relationship between mutation and disease and the pattern of transmission is simple and predictable. Data from human population genetics give an overall frequency of Mendelian diseases in the population of 2.4% (1.5% autosomal dominant, e.g. Huntington´s disease, 0.75% autosomal recessive e.g. Phenylketonuria, and 0.15% sex-linked recessive, e.g. Haemophilia). In addition, approximately 6% of live births are affected by a congenital abnormality with some genetic component and 65% of the population will develop, later in life, chronic disease with some genetic component as well, although environmental factors play a much bigger role. These are called multifactorial diseases and comprise common diseases such as diabetes, essential hypertension and coronary heart disease. This complexity of heritable diseases is incorporated in the present method of estimating the heritable risk among the progeny of irradiated people.

The equation to calculate genetic risk combines population genetic data in humans and radiation genetic data in mice as follows:

$$\text{Risk} = \text{Prevalence} \times 1/\text{Doubling Dose} \times \text{Mutation Component} \times \text{PRCF}$$

Risk is the probability that an offspring of the exposed person will develop heritable disease of one of the groups described above (Mendelian or multifactorial). The prevalence data are given above. For protracted irradiation, the accumulated dose in the gonads before conception is divided by 1. The mutation component is a factor which describes the relationship between the increase in the mutation rate and the rate of additional disease. Even for dominant diseases this is not 1, since the majority of existing mutations are inherited from parents and grandparents, often through many generations. A cautious estimate suggests that doubling of the rate of new dominant mutations will cause only a 30% increase of diseases with dominant inheritance in the first generation and 15% in the second generation. The same value of the Mutation Component is allocated to sex-linked recessive diseases. Since the development of single gene disease with recessive inheritance requires mutations in both alleles of the same gene, the relationship between a mutation and disease is very remote and the mutation component is therefore assumed to be close to zero. For multifactorial diseases, the relationship between mutation and disease is also not very close, and presently the mutation component is assumed to be 0.01. The potential recoverability correction factor (PRCF) has been introduced to account for the fact that the molecular structure of radiation-induced mutations differs markedly from the molecular structure of "spontaneous" mutations, in that

most spontaneous mutations are point mutations with a single base pair altered or a minute deletion whereas radiation-induced mutations are mostly large deletions, often affecting whole genes. These mutations are usually not compatible with inter-uterine development and most will lead to premature termination of pregnancy. The value of PRCF suggested today is 0.15 to 0.3.

Using this equation for estimating the risk of heritable diseases of a young man who had been exposed to a radiation dose of 1 Gy from radiotherapy, e.g. of pelvic lymph nodes of Hodgkin´s disease, the risk of radiation-induced dominant and sex-linked heritable disease would be:

$$\text{Risk} = 0.0165 \times 1 \times 0.3 \times 0.3 \times 0.5$$

The last factor of 0.5 has been introduced to account for the fact that only the father was irradiated. The result is a risk of less than 0.1%. The risk of multifactorial disease is similar at < 0.1% and the risk for radiation-induced recessive disease among the children is essentially zero.

Recent considerations of the potential molecular, genotypic manifestations of genetic diseases of genetic damage induced in the germ cells of irradiated individuals and transmitted to the progeny took account of the relationship between the observed molecular changes in the DNA of irradiated cells and gene function and gene position. It has been suggested that the major genetic effect of radiation exposure is related to microdeletions, i.e. deletions of multiple, functionally unrelated, yet physically contiguous genes that are compatible with survival of the individual receiving them. Such microdeletions are known to cause multisystem congenital abnormalities which share some common features: mental retardation, growth retardation, various malformations. Unlike the majority of congenital abnormalities which are typical multifactorial disorders, these abnormalities would show the same inheritance pattern as autosomal single gene diseases. These diseases are rare. It has been estimated that the risk of these multi-organ congenital disorders after exposure to 1 Gy is approximately 0.1%.

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3. EXTRA MODULE FOR RADIATION ONCOLOGISTS

3.1. Introduction

The text and figures in this section on the biological basis of radiotherapy are revised and condensed from various chapters in Tannock *et al* (2005).

Radiotherapy can involve either external beam treatment or brachytherapy with the choice depending on the type of tumour and location within the body. The dose of radiation delivered depends on whether the therapy is intended to be curative or palliative, on the volume of tissue to be irradiated and the expected toxicity to the surrounding normal tissues including factors such as the condition of the patient (age and other health problems that might increase the side effects of radiotherapy, e.g., connective tissue disorders, such as scleroderma). The relative radiosensitivity of the tumour cells is only rarely a factor in this decision. The side effects that may occur following local radiotherapy are directly linked to the normal structures and tissues within the irradiated volume and the effects increase with size of the dose fractions and the volume irradiated. Most curative radiotherapy regimens consist of daily fractions in the range of 1.8 to 3 Gy per day over a period of 5 to 8 weeks. Using modern planning techniques, doses up to about 75 Gy to the tumour can usually be achieved without causing severe side effects. The dose to normal tissues is usually limited to reduce the level of severe complications to no more than about 5 percent of the population after a period of 5 years (known as the *TD*5/5 value. Increased radiotherapy dose is associated with increased local control so this dose limit may be increased if radiotherapy is the only curative treatment option for the patient particularly for small fields. Palliative radiotherapy is given when the disease is incurable in order to achieve better pain control, to control bleeding, or to prevent tissue destruction or ulceration. These radiotherapy treatments are usually of short duration and consist of 1 to 3 fractions of 5 to 8 Gy or 5 to 10 fractions of 3 to 4 Gy.

3.2. Physics

External beam conformal radiotherapy employs three-dimensional planning using radiation beams given from different angles to maximize tumour dose while minimizing normal tissue irradiation. Imaging is used to localize the tumour and critical normal tissues in order to define the gross tumour volume (GTV). The final plan will deliver the maximum radiation dose to a slightly larger volume (the planning target volume, PTV) to account for microscopic disease beyond the detectable edge of the tumour, for body or organ movement, and for issues pertaining to the physics of the radiation beam. The plan is developed by computer simulation based on the energy and number of radiation beams and their orientation. The dose delivered to the different regions of the field by each of the beams is calculated and summed to create an isodose contour map. Verification images can be used to track successful delivery of the treatment and special techniques and markers are sometimes used to track organ movement within the body (e.g., movement of a lung tumour during normal breathing). Recent improvements in radiotherapy planning involve the use of intensity modulated radiation therapy (IMRT) in which the radiation beams are differentially regulated within the area of irradiation so that there are relatively low- and high-dose volumes of irradiation. The combination of multiple beams allows for better dose distributions resulting in a decreased volume of normal tissue in the high dose region (PTV) without compromising dose to the tumour. A recent application of this approach is stereotactic radiosurgery, which uses highly focused irradiation beams of charged particles (e.g., proton beams), γ-rays, or high energy X rays precisely collimated to target the tumour site.

These techniques often produce non-uniform dose distribution within the normal organs which makes it difficult to determination a precise relationship between normal tissue response and dose. Currently this is address by generating a dose-volume histogram (DVH) for each exposed organ in a patient as part of a modern radiotherapy plan. These DVHs can then be reduced to create one of more parameters (e.g. either an effective volume [Veff] irradiated to a reference dose or an effective dose [equivalent uniform dose; EUD] uniformly applied to the whole volume), which are used in a model for predicting normal tissue complication probability (NTCP). Several models have been proposed; however, currently the quality of clinical data available for such predictions is rarely sufficient to alter radiotherapy practice. One important complexity with IMRT plans is that increased volumes of normal tissue are exposed to lower doses within the entire body and this raises concerns as to the possibility of increased radiation-induced second malignancies.

3.2.1. Brachytherapy, radionuclides, and radioimmunotherapy

Low-dose rate radiation sources implanted into or beside the tumour (known as brachytherapy) can be used either alone or in combination with external beam radiotherapy for accessible tumours such as those of the cervix, prostate, head and neck, breast, bladder, lung, esophagus, and some sarcomas. Tissues close to the implanted source will receive a high dose and tumour cell killing will be high. Further from the source, normal cell killing will be less due to lower dose rates and a decreased total dose over the duration of treatment. Recently computer controlled brachytherapy systems have been designed to deliver short pulses of radiation (pulsed-dose brachytherapy) using a high-dose source traveling along a catheter track within the tumour. Radiobiological modelling suggests that the acute and late reactions are similar to traditional (continuous) brachytherapy as long as the gaps between pulses are less than 1 hour. Injected radionuclides can also be used if there is selective uptake by the tumour so that local irradiation may lead to death of the tumour cells. Iodine-131 (^{131}I) is used to treat well-differentiated thyroid cancer, radiolabeled (e.g., indium-111) somatostatin analogues for the treatment of neuroendocrine tumours, and strontium-89 (^{89}Sr) to treat bone metastases mainly in prostate cancer. The conjugation of radionuclides to specific antibodies allows targeted radiotherapy to tumours containing cells expressing the relevant antigens or receptors and is termed radioimmunotherapy. Radionuclides emitting α-particles or short-range beta particles or electrons (e.g. Auger electrons) that can kill cells within a radius of 1 to 3 cell diameters of the bound isotope are optimal for localized treatment. Currently, in patients, this approach is limited by the lack of specific uptake in tumour cells and the difficulties of accurate dosimetry and treatment planning.

3.2.2. Charged particles and high LET radiotherapy

Charged particles (protons, heavy ions) have a physical advantage because they can give improved depth-dose distributions for deep-seated tumours. Much of their energy is deposited in tissue at the end of particle tracks (i.e., in the region of the Bragg peak). Uncharged neutron beams do not demonstrate a Bragg peak and their depth-dose distributions are similar to those for low-LET radiation. There is also a potential biological advantage in that the oxygen enhancement ratio is reduced with high LET ions, so hypoxic cells are protected to a lesser degree. There is also reduced capacity for repair following high-LET radiation relative to that following low-LET radiation, a property partially responsible for the increased relative biological effectiveness (RBE) for high-LET radiations. The expected gains with protons are largely confined to improved dose distribution, while for neutrons any gains are likely to be related to the biological factors. One potential difficulty in using high-LET radiation is that because late-responding tissues demonstrate greater repair capacity than early-responding

tissues, the reduction in repair capacity following high-LET irradiation may result in relatively higher RBE values for late-responding tissues. Clinical studies with fast neutrons have been associated with an increase in complications, particularly subcutaneous fibrosis, and randomized trials have not demonstrated therapeutic gain. In contrast, protons have demonstrated an advantage for treatment of tumours, such as choroidal melanomas and skull-base tumours, which require precise treatment of a highly localized lesion and other tumour sites might also benefit. Proton therapy planning can also be combined with IMRT planning techniques to give finely contoured dose distributions, but cost limitations (i.e., the requirement of a cyclotron or synchrotron) currently preclude proton therapy as a common approach to radiotherapy. Similar problems limit the use of ions although there is currently increasing interest in the use of carbon ion beams.

3.2.3. Boron neutron capture therapy (BNCT)

Compounds enriched with boron-10 are administered prior to irradiation with a thermal neutron beam. Thermal neutrons interact preferentially with the ^{10}B atoms in the tumours, and, a fission reaction produces high-energy charged particles (^{7}Li and ^{4}He) resulting in tumour cell killing. This technique has been investigated particularly for treatment of brain tumours. For an improved therapeutic ratio with BNCT, relatively high concentrations of ^{10}B must be achieved in the tumour, with low concentrations in normal tissues. However, several studies show high concentrations of ^{10}Bo in the vascular cells as well. New boronated compounds and new strategies for delivering the compounds have improved the differential concentrations achievable in tumours and surrounding normal tissues with encouraging results. However, the depth-dose distribution for the thermal neutron beam is relatively poor and this remains a limitation in the clinical use of this treatment approach.

3.3. Molecular and cellular biology

3.3.1. Techniques

The cloning of the human genome and subsequent technical improvements have made it possible to isolate any specific gene. It is currently easier to isolate a gene than its protein product but the nucleotide sequence of an isolated gene can be used to deduce the amino acid sequence of its product. Small peptides corresponding to the proposed amino acid sequence of the product can then be synthesized and antibodies made against these peptides. Often the antibodies will react with the complete protein, allowing the subsequent isolation and purification of the gene product. Techniques commonly used for the genetic analysis of tumours are described below.

3.3.1.1. Blotting techniques

Southern blotting is a widely used method for analyzing the structure of DNA that involves the blotting of DNA on to a supporting matrix. The DNA to be analyzed is cut into defined lengths using restriction enzymes, denatured and the DNA fragments are separated using electrophoresis of an agarose gel; the smallest fragments migrate farthest and the largest remain near the origin. Pieces of DNA of known size may be electrophoresed at the same time to act as a molecular weight scale. A nylon membrane is placed on top of the gel and fluid is drawn through the gel (by vacuum suction) causing the DNA to migrate onto the nylon membrane, where it is immobilized. To determine the size of the fragment of DNA that carries a particular gene, a piece of the gene is separately cloned and made radioactive to create a probe. The nylon membrane containing all the fragments of DNA is incubated in a

solution containing the radioactively labeled (single-stranded) DNA probe. Under these conditions, the probe will anneal with homologous DNA sequences present on the membrane. Gentle washing will remove the single-stranded, unbound probe; hence the only radioactive DNA fragments remaining on the membrane will be those (homologous sequences) that hybridized with the labeled probe. To detect the region of the membrane containing the radioactive material, the nylon sheet can be placed on top of a piece of X ray film, enclosed in a dark container and placed at -70°C for several hours to expose the film. The film is then developed and the places where the radioactive material is located show up as dark bands. An almost identical procedure can be used to characterize messenger RNA. The mRNA is separated by electrophoresis, transferred to nylon membranes, and probed with a labeled, cloned fragment of DNA. The technique is called ***Northern blotting*** and is used to evaluate the expression patterns of genes. An analogous procedure, called ***Western blotting***, has also been devised to characterize proteins. Following separation by denaturing gel electrophoresis, the proteins are immobilized by transfer to a charged synthetic membrane. To identify specific proteins, the membrane is incubated in a solution containing a specific primary antibody that will bind to the protein of interest, then incubated with a secondary antibody that is conjugated to horseradish peroxidase (HRP), biotin or a fluorochrome. The protein antibody conjugate can be detected by exposure to chemoluminescence detection reagents (or directly) as the emitted fluorescent light can be identified by short exposure to X ray film, allowing the bands of interest to be identified. This last step may now be done with high resolution imagers designed to detect radioactivity or light.

3.3.1.2. Assays for DNA breaks

The nuclei of cells can be digested and the DNA in a gel subjected to a constant or pulsed electric field to separate the fragments into bands in the gel. This provides a sensitive assay for radiation-induced double-strand breaks. In the Comet assay, nuclei can be partially digested and subjected to an electric field which stretches the radiation-damaged DNA into a comet-like tail. Digestion under alkali conditions reveals damage characteristic mainly of single-strand breaks (SSBs), and digestion under neutral pH reveals damage characteristic mainly of double-strand breaks (DSBs).

3.3.1.3. The polymerase chain reaction

Blotting techniques required many cells to produce enough DNA or RNA for hybridization analysis. The polymerase chain reaction (PCR) addresses this problem. A unique DNA polymerase enzyme called *Taq* polymerase (which is resistant to denaturation at high temperatures) and specific oligonucleotide primers are used to amplify the amount of cell DNA for further analysis. Usually DNA of about 200 to 1000 base pairs is amplified. Analysis by PCR requires precise knowledge of the sequences flanking the region of the gene of interest. Two short oligonucleotides complementary to the flanking regions can then be synthesized, and these are used as primers for *Taq* polymerase. All components of the reaction (target DNA, primers, deoxynucleotides, and *Taq* polymerase) are placed in a small tube and initially heated (~95°C) to denature (separate) the DNA duplex. Then incubation at ~50°C allows hybridization of the primers to the single-stranded DNA followed by incubation at ~70°C to allow *Taq* polymerase to synthesize new DNA from the primers. This cycle is repeated every few minutes to create multiple rounds of amplification. The precise time of each cycle depends on the nature of the primers and the length of DNA to be amplified. Twenty cycles can theoretically produce a million-fold amplification. PCR can also be used to study gene expression or screen for mutations in RNA. It is first necessary to use reverse transcriptase to make a complementary single-strand DNA copy (cDNA) of an mRNA prior

to performing the PCR. The cDNA is then used as a template for a PCR reaction as described above. Reverse transcriptase PCR (RT-PCR), allows amplification of cDNA corresponding to both abundant and rare RNA transcripts, thereby providing a convenient source of DNA that can be screened for mutations.

3.3.1.4. Single nucleotide polymorphisms (SNPs)

DNA sequences can differ at single nucleotide positions within the genome as frequently as 1 out of every 1000 base pairs and if SNPs are present in exons they may affect protein structure and function. For example, SNPs may be involved in altered drug metabolism due to their modifying effect on the Cytochrome P450 metabolizing enzymes. Most methods to characterize SNPs require PCR amplification of the sample prior to analysis, thus influencing the number of fragments that can be analyzed simultaneously. The sample is denatured and mixed with a known DNA sample and then partially renatured causing the formation of homoduplexes and heteroduplexes. Denaturing high-performance liquid chromatography (DHPLC) allows the automated detection of these different duplexes caused by pair mismatches due to single base substitutions, insertions, or deletions. Suspected polymorphic/mutated sites are then sequenced to verify the presence of such genetic variation.

3.3.1.5. DNA sequencing

The most frequently used method to sequence DNA is dideoxy-chain termination. This method is analogous to DNA replication in vitro, but it uses dideoxynucleotide triphosphates (ddNTPs) in the reaction. DNA sequencing is carried out in four separate reactions each containing one of the four ddNTPs (i.e., ddATP, ddCTP, ddGTP, or ddTTP) together with the other normal nucleotides. In each reaction the sequencing primers bind and start the extension of the chain at the same place. The extended chains, however, terminate at different sites when dideoxynucleotides are incorporated. This produces fragments of different size terminated at every nucleotide. Separation of the newly synthesized radioactive DNA on polyacrylamide gels allows visualization of each fragment produced in the sequencing reaction. Usually a sequence of 200 to 500 bases can be read from a single gel. In automated fluorescent sequencing, fluorescent dye labels are incorporated into DNA extension products and automated DNA sequencers can detect fluorescence from four different dyes that are used to identify A, C, G, or T extension reactions. The sequence of a strand of DNA can be compared to data available on public databases (such as www.ncbi.nlm.nih.gov) to check for regions of sequence similarity.

3.3.1.6. Microarray analysis

This technique involves the production of DNA arrays or chips on solid supports for large-scale hybridization experiments. There are two basic types of chip: in one, DNA probe targets are immobilized to a solid inert surface such as glass and exposed to a set of fluorescently labeled sample DNAs; in the second, an array of different oligonucleotide probes is synthesized in situ on the chip. The array, which may contain tens of thousands of probe targets or oligonucleotide probes, is exposed to fluorescently-labeled DNA samples, or cDNAs made from mRNA isolated from cells. The complementary sequences which hybridize to the chip are determined by digital imaging. DNA microarray analysis allows large-scale gene discovery, gene expression, gene mapping, and gene sequencing studies as well as detection of mutations or polymorphisms. Microarrays are very useful but they are also very misleading; better data analysis tools need to be developed to improve the accuracy of the microarrays.

3.3.1.7. Modifying gene expression

Gene function can often be studied by transferring the gene into a cell different from the one from which it was isolated. A mutated oncogene, isolated from a tumour cell, may be transfected into a normal cell to determine whether it causes malignant transformation. A number of transfection protocols have been developed for efficient introduction of foreign DNA into mammalian cells, including calcium phosphate or Diethylaminoethyl-Dextran (DEAE-dextran) precipitation, spheroplast fusion, lipofection, electroporation, and transfer using viral vectors. For all methods, the efficiency of transfer must be high enough for easy detection, and it must be possible to recognize and select for cells containing the newly introduced gene. It is usually necessary to select for retention of the transferred genes before assaying for expression. For this reason, a selectable gene, such as the gene encoding resistance to the antibiotic neomycin, can be introduced simultaneously by taking advantage of the fact that frequently cells that can take up one gene will also take up another. For *lipofection*, plasmid DNA is complexed with a liposome suspension in serum-free medium. This DNA/liposome complex is added directly to cells grown in tissue culture, and after a three- to five-hour incubation period, fresh medium containing serum is added. The cells are incubated to allow expression of the transfected gene. Electroporation entails administration of an electrical current to a cellular-DNA mixture. Viral vectors are useful because they can be targeted to a variety of cell types. Retroviruses are very stable because their complementary DNA integrates into the host mammalian DNA, but only relatively small pieces of DNA (up to 10 kilobases) can be transferred. Adenovirus vectors take larger inserts and have a very high efficiency of transfer. Nonviral vectors, such as liposomes, can be used for transient expression of introduced DNA.

An alternative approach to studying gene function is by its inactivation by introducing a DNA or RNA sequence that will specifically inactivate the expression of the gene of interest. This can be achieved by introducing DNA or RNA molecules with a base sequence where the order of the bases is opposite to that of the usual complementary strand (i.e. 3'→ 5') instead of 5'→ 3') within the target gene. So-called antisense RNA or DNA molecules can combine in vitro specifically with their homologous sequences in mRNA and interfere with the expression of that gene. Small complementary RNA molecules that can directly interfere with gene expression (RNA interference: RNAi), leading to the specific disappearance of the selected gene products, can also be used. RNAi interferes with the stability of the complementary mRNA transcript by initiating a degradation process. Specific gene inactivation in this way has the potential for therapy of tumours; for example, by inhibiting the expression of an oncogene. A limitation of the above technologies is that a high concentration of molecules must be efficiently delivered to all the tumour cells and must persist inside the cells for a prolonged period of time. Once the nucleic acids enter a cell, they are vulnerable to a variety of cellular nucleases.

3.3.1.8. Proteomics

Proteomics is the large-scale study of proteins, particularly their structure and function. Studying proteins requires two stages of sample preparation. Proteins are separated using 2-dimensional electrophoresis, followed by identification using mass spectrometry (MS).

***Protein separation*:** 2-dimensional electrophoresis separates proteins based on size, as in regular electrophoresis, but also based on charge, or isoelectric point (pI). The first step is isoelectric focusing (IEF). The mixed protein sample is run on an immobilized pH gradient; the range of the gradient used depends on the expected proteins in the sample. The sample is added to the gradient and an electric current is applied. Proteins will be positively charged at

pH's below their pI and negatively charged at pH's above their pI. When the protein is at the point in the gradient where the surrounding pH is equal to its pI, there will be no charge on the protein and it will stop moving. Once enough time has passed for the proteins to settle in the gradient, the current is removed and the gradient is laid horizontally along an SDS-PAGE gel. An electric current is then applied and the proteins move horizontally out of the IEF gradient and into the polyacrylamide gel where they are separated based on molecular weight. This method can reproducibly separate mixtures of proteins. Once the proteins have been separated, they can be analyzed quantitatively as long as there is a reference sample. The amount of protein in cells under two conditions (e.g. aerobic and anaerobic) can be measured by staining with a fluorescent dye. The brighter the fluorescence, the more protein is present. Proteins that are expressed at different levels are then taken for further analysis and identification.

***Protein identification by mass spectrometry*:** The spots are cut out of the gel and digested into smaller polypeptide fragments (5 to 10 amino acids) by enzymes. The polypeptide fragments are analyzed by mass spectrometry, which will give the molecular weight of each fragment. Once the masses of the fragments have been determined, they are run through a sequence database and compared to find the actual amino acid sequence, and thus identity, of the protein.This method of protein analysis also gives information on any post-translational modifications that have occurred, such as alternate splicing, glycosylation or phosphorylation. Monitoring expression by this method is useful for measuring levels of active proteins in a cell and may give clues to the metabolism, signaling or other activities of a cell under varying conditions.

3.3.2. Cell signaling

Changes in the physical or chemical environment of the cell results in responses are brought about by elaborate networks of intracellular signals, caused by changes in protein phosphorylation and enzymatic activity, localization, and the formation of protein-protein complexes. Cellular responses are triggered by the recognition of extracellular signals at the cell surface that result in the activation of linked cytoplasmic and nuclear biochemical pathways. These signal transduction pathways control cellular processes from cell proliferation and survival to specialized functions such as the immune response and angiogenesis. When they are dysregulated, normal signaling pathways contribute to malignant transformation in human cells.

3.3.2.1. Extracellular growth factors

Cellular regulation can occur through direct cell-to-cell contact or cell contact with its surrounding extracellular matrix, but much of our knowledge of signal transduction pathways comes from studying the interaction of soluble growth factors with complementary growth factor receptors expressed on responsive cells. Growth factors influence cellular processes such as growth, proliferation, differentiation, survival, and metabolism via their interaction with specific transmembrane receptor protein tyrosine kinases (RPTKs). Most growth factors are small monomeric (i.e., single chain) polypeptides, such as epidermal growth factor (EGF). There are also dimeric polypeptide growth factors (i.e., those containing two chains of amino acids), such as platelet derived growth factor (PDGF). Growth factors may be freely diffusible or can reside in spacially restricted domains either through binding to components in the extracellular matrix or because they are membrane-anchored molecules that reside on the surface of the producing cells. Receptors for growth factors are membrane-spanning cell surface molecules that share the ability to phosphorylate themselves and other cytoplasmic

proteins on tyrosine residues, thereby activating a signaling cascade. Binding of the growth factor or ligand induces conformational changes in the extracellular domain of the receptor that facilitates dimerization (i.e., joining together) or clustering of receptor tyrosine kinases. The consequent conformational changes in the growth factor receptor bring together two intracellular catalytic domains, resulting in intermolecular autophosphorylation (transphosphorylation) of tyrosine residues. Phosphorylation of key residues within the kinase activation loop induces the opening of the catalytic site and allows access to ATP and protein substrates, while phosphorylated residues in noncatalytic regions create docking sites for downstream signaling molecules that are essential for signal propagation. Abnormal RPTKs involved in cancer are deregulated making their catalytic activity independent of ligand binding. For example, the HER2/neu (human epidermal growth factor gene 2) proto-oncogene encodes an RPTK (receptor protein tyrosine kinase) that is frequently amplified in human breast and other tumours. Increased expression is thought to increase the concentration of active dimers generating continuous and inappropriate cellular signaling.

3.3.2.2. Cytoplasmic signaling molecules

Signaling pathways downstream of activated RPTKs are constructed through interactions of specific proteins that create networks of signaling molecules. A unifying feature of cytoplasmic signaling proteins is the presence of one or more conserved noncatalytic domains that mediate sequence specific protein-protein interactions. Many of these domains bind specifically to short (typically less than 10 amino acids) contiguous regions of their target protein. Activation of growth factor receptors results in the autophosphorylation of the receptor at multiple tyrosine residues, and results in the creation of a number of docking sites for cytoplasmic proteins. The activity of both receptor and cytoplasmic tyrosine kinases is tightly regulated. The opposing action of protein tyrosine phosphatases can eliminate docking sites for proteins or inhibit tyrosine kinase activity by dephosphorylation of regulatory phosphorylation sites in the kinase activation loop. Regulation of receptor levels at the cell membrane is another mechanism used to regulate activity. The rapid removal of receptors from the cell surface by endocytosis allows a cell to return to an unstimulated, basal state after receiving and responding to a specific signal.

3.3.2.3. RAS proteins

Three distinct mammalian RAS protein isoforms—H-RAS, K-RAS, and N-ras—are part of a large family of small (low molecular weight) guanine nucleotide triphosphate (GTP) binding proteins. The three RAS proteins have a molecular weight of 21 kilodaltons (hence the designation p21RAS) and share 85 percent sequence homology. The RAS proteins are GTPases that cycle between an active GTP-bound ‘on’ and an inactive GDP-bound ‘off’ configuration in response to extracellular signals, essentially functioning as a molecular binary switch (Figure 3.1). In the active GTP-bound form, RAS proteins bind to a number of distinct effector proteins that in turn, activate downstream signaling cascades. One of the best characterized is its interaction with the protein kinase Raf-1 activates its kinase activity, and consequently the downstream cascade of protein kinases, including MEK (mitogen activated kinase kinase) and ERK (extracellular signal-regulated kinases) and the p110 catalytic subunit of PI-3K (phosphatidylinositol 3-kinase; see below). For normal function RAS proteins are post-translationally modified by a protein farnesyl transferase that adds an isoprenoid chain group to a cysteine residue in the carboxy terminus of RAS proteins. This covalently linked farnesyl group is required for RAS association with intracellular membranes. Both H-ras and N-ras are also subsequently modified by the addition of two palmitoyl long chain fatty acids that are important for the correct localization of these proteins to specific membrane locations.

RAS proteins regulate cell cycle progression, cell survival, and cytoskeletal organization (Figure 3.1). Oncogenic mutations of *RAS* have been identified that inhibit its intrinsic GTPase activity, trapping it in the activated GTP-bound state and leading to cell transformation. Mutated K-RAS has been identified in a number of different cancers including those of the lung, colon and pancreas.

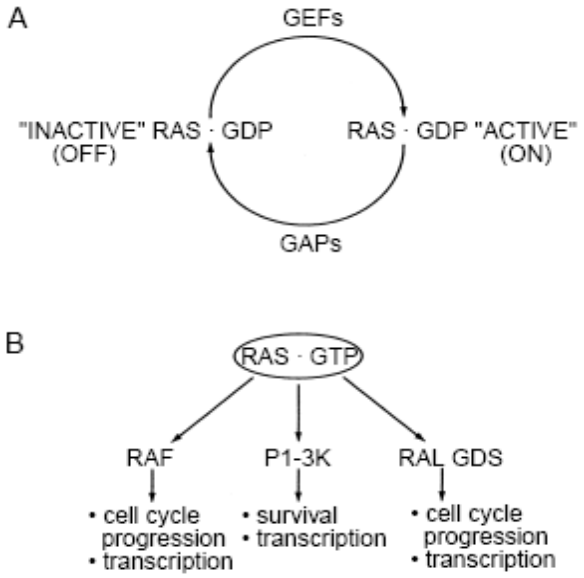

Fig. 3.1 Ras protein activation and downstream signaling: (A) Ras cycles between an inactive GDP bound state and the active GTP bound state. Ras activation is regulated by guanine nucleotide exchange factors (GEFs) that promote exchange of GDP for GTP. GTP hydrolysis requires GTPase activating proteins (GAPs) which enhance the weak intrinsic GTPase activity of RAS proteins, (B) Once in its active GTP bound form, RAS interacts with different families of effector proteins including RAF protein kinases, phosphoinositide 3–kinases (PI-3K), and RALGDS, a GEF for the RAS related protein RAL. Activation of these downstream pathways leads to cellular responses including gene transcription, cell cycle progression, and survival (Tannock et al. 2005).

3.3.2.4. Mitogen activated protein kinase (MAPK) signaling pathways

These are highly conserved signaling pathways in all eukaryotic cells that respond to divergent signals, including growth factors and environmental stresses such as osmotic stress and ionizing radiation. All MAPK pathways include a core three-tiered signaling unit, in which MAPKs are activated by the sequential activation of linked serine/threonine kinases. The MAPK is activated by phosphorylation of threonine (Thr) and tyrosine (Tyr) residues by a family of dual specificity kinases, referred to as MEKs or MKKs (MAPK-kinase). MEK activity is regulated by serine and threonine phosphorylation catalyzed by kinases called MAP3Ks (MAPK-kinase-kinase). Three distinct MAPK pathways in mammalian cells are the extracellular signal regulated kinase 1 and 2 (ERK1/2), the c-Jun N-terminal kinase or stress activated protein kinase (JNK/SAPK), and p38 (Figure 3.2). Activation of Ras proteins causes the activation of Raf-1, a MAP3K upstream of ERK1/2. ERK kinase activation is part of a final common pathway used by growth factor receptors such as those for EGF and by more diverse stimuli from cytokine receptors and antigen receptors. Raf-1 directly activates MEK-1/2 by phosphorylating it on serine residues, which enhances the availability of the catalytic site to potential substrates. Activated MEK-1/2 is a dual specificity kinase that phosphorylates the ERK kinases, which induces both catalytic activation of ERK and its translocation to the

nucleus. Nuclear ERK interacts with specific transcription factors leading to their phosphorylation and activation of specific transcriptional targets. The SAPK (stress activated protein kinase) and p38 pathways mediate responses to cellular stresses such as extremes of heat, exposure to ultraviolet and ionizing radiation, anticancer drugs, and exposure to potentially damaging biologic agents such as the cytokines IL-1 and tumour necrosis factor (TNF). The core components of the SAPK and p38 parallel those of the ERK pathway, although the upstream activation steps are less well defined. Deregulation of MAPK signaling has been implicated in malignant transformation. Increased levels of activated ERKs are found frequently in human tumours, and often are attributable to the presence of mutations in Ras or other upstream components in the growth factor signaling cascades. Activating mutations in BRAF, a Raf-1 related kinase, are found in greater than 60 percent of human melanomas and at lower frequency in a wide range of other human tumours.

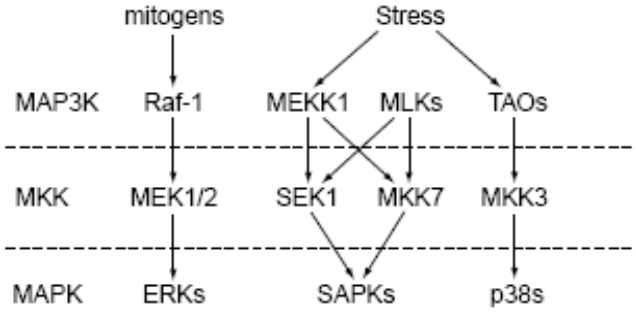

Fig. 3.2 MAPK signaling pathways. Parallel signaling pathways include MAP3Ks that respond to distinct stimuli, and activate the dual specificity MKKs, which in turn activate the MAPKs. The activated ERKs, SAPKs, and p38 family members induce distinct cellular responses as described in the text. (Tannock et al., 2005)

3.3.2.5. Phosphoinositide signaling pathways

Phosphoinositides are phospholipids of cell membranes that are dynamically regulated in response to growth factor signalling. They contribute to signal propagation by serving as precursors of the second messengers IP3 (inositol triphosphate) and DAG (diacylglycerol), or by binding to signaling proteins that contain specific phosphoinsitide binding modules. In response to growth factor signaling phosphoinositides can be phosphorylated or dephosphorylated by lipid kinases and phosphatases at distinct positions on the inositol ring. Activation of phosphoinositide 3-kinase (PI-3K), which specifically phosphorylates the 3-position, leads to the rapid production of phosphatidylinositol-3, 4, 5-trisphosphate (PtdIns (3, 4, 5) P3). The protein serine/threonine kinases PDK1 (pyruvate dehydrogenase kinase, isozyme 1), and Akt/PKB are recruited in the vicinity of activated receptors where PKB (protein kinase B) is activated by conformational changes evoked by phospholipid binding and its subsequent phosphorylation by the constitutively active PDK1. Substrates for activated PKB include regulators of apoptosis, or regulators of cell growth. PDK1 also phosphorylates other protein targets including ribosomal p70 S6-kinase, part of the mTOR pathway and a key regulator of cell growth through control of the protein translation machinery. The importance of phosphoinositides in human cancers was clearly revealed by the discovery that numerous human malignancies are associated with inactivating mutations in the PTEN gene (a human gene that acts as a tumor suppressor). PTEN is a 3-phosphoinositide phosphatase, that functions as a major negative regulator of PI-3K (phosphoinositide-3 kinase) signaling.

3.3.2.6. Transcriptional response to signaling

Activation of signaling pathways leads to transcription of new genes that coordinate cell growth, cellular differentiation, cell death, and other biological effects. Transcription of genes is catalyzed by the enzyme RNA polymerase II and regulated by transcription factors that can activate or repress gene expression by binding to specific DNA recognition sequences, typically six to eight base pairs in length, found in the promoter regions at the start of genes. The activity of transcription factors can be modified, most often by phosphorylation, through the activity of many of the signaling pathways described above, but most notably by the MAPKs. Transcription factor activity may also be enhanced through interaction with small molecules (e.g., steroid hormones) or by signal-induced release from inhibitory interactions such, as for NF*k*B (normally bound by the inhibitor I*k*B, which is released upon phosphorylation). Both transcription activators and repressors exert their effects by binding to multisubunit co-activators or corepressors that act to modify chromatin structure and assembly of DNA polmerase complexes. Enzymes that regulate histone acetylation and phosphorylation are key components of transcriptional activator and repressor complexes.

3.3.2.7. DNA/Chromatin structure and function

The structure and activity of chromatin can be altered by posttranslational modifications (e.g., acetylation, phosphorylation, methylation, and ubiquitylation). The acetylation status of histones, the core proteins of nucleosomes, can regulate gene expression by altering chromatin coiling. Histone acetylation near the promoter regions of genes facilitates the interaction of the DNA with transcription factors whilst deacetylation results in more condensed chromatin structures that inhibit assembly of the transcription machinery at the promoter. Histone deacetylase (HDAC) inhibitors promote acetylation, leading to the uncoiling of chromatin and depending on the cell type, can lead to transcriptional activation of about 2 percent of human genes, including tumour suppressor genes. Treating cells with HDAC inhibitors can increase cell cycle arrest, induction of apoptosis, and differentiation in cancer cells. Methylation of cytosine bases in DNA can also play an important role in inactivating genes and has been implicated in the inactivation of many of the tumour suppressor genes that cause familial cancers. Usually, there is a gain of methylation in normally unmethylated CpG islands within the DNA (genomic regions that contain a high frequency of CG dinucleotides). Methylation-induced transcriptional silencing begins early during the process of genetic instability and can affect many genes that are important in tumour progression. Given that methylation is a potentially reversible state, this creates a target for novel cancer therapeutic strategies involving gene reactivation. Both retinoic acid and 5-aza-deoxycytidine can reverse DNA methylation and re-activate expression of normal regulatory genes, thereby leading to the regression of some human leukaemias. Several HDAC inhibitors have shown impressive antitumour activity *in vivo* at nanomolar concentrations and are currently in phase I and phase II clinical trials alone, or in combination with demethylating agents.

3.3.3. Oncogenes and tumour suppressor genes

There is substantial evidence that multiple genes must be mutated or deregulated in a single cell to cause malignant transformation and cancer growth. Thankfully this is a rare event. Most cells that harbour even a single mutation are either targeted for repair or are cleared from the organism by protective mechanisms including immune surveillance or activation of cellular suicide programs (apoptosis). Not all mutations contribute to tumour development: the genetic material in each of our cells is estimated to encode approximately 30,000 genes,

and mutations in less than 10 percent of these genes contribute to the carcinogenic process. Genes that may contribute to tumourigenesis play key roles in regulating critical cellular processes such as cell division, lifespan, differentiation, angiogenesis, invasion, and death. They can be divided into two categories: oncogenes and tumour suppressor genes.

3.3.3.1. Oncogenes

Two copies or alleles of each gene exist in every cell. Specific mutation of one allele converts the normal "protooncogene" to the activated, transforming oncogene that can contribute to the carcinogenic process. The oncogene is dominant over the protooncogene and generally results in a protein product that is deregulated and/or constitutively active. Oncogenic conversion is a gain to loss or inactivation of both alleles is required for transformation. Tumour suppressors are also known as recessive oncogenes or anti-oncogenes and their inactivation represents a loss-of-function mutation. Both oncogene activation and tumour suppressor inactivation collaborate in the stepwise progression to tumourigenesis. Here we will focus on these cancer genes and outline how they have been identified, the genetic abnormalities associated with deregulation, the nature of the specific gene products, their mechanisms of action, and recent advances to counteract these tumour-promoting lesions with novel anticancer therapeutics.

3.3.3.2. Tumour suppressor genes

Approximately 1 percent of all human cancers arise in individuals with a hereditary cancer syndrome. Even though such conditions are relatively rare, investigations of the affected individuals and of mutations of genes associated with their disease have proven invaluable in understanding the genetics and etiology of cancer. Most inherited cancer syndromes are a consequence of germline transmission of inactivating, loss-of-function mutations in tumour suppressor genes. Unlike oncogenes, whose mutations are associated with sporadic tumours and act in a dominant manner, mutations of tumour suppressor genes are recessive at the somatic level, and the remaining wild-type allele is inactivated during cancer development. Phenotypic and clinical manifestations of numerous inherited cancer predisposition syndromes, together with the known genetic events associated with them, are catalogued in an expanding Online Mendelian Inheritance in Man (OMIM) database (www.ncbi.nlm.nih.gov/omim). Studies by Knudson investigating the epidemiology of familial retinoblastoma, an autosomal dominant hereditary form of retinal cancer led to the two hit hypothesis (Knudson, 2001), highlighting the importance of recessive mutations in tumourigenesis. In contrast to sporadic cases of retinoblastoma (Rb), patients with familial disease were likely to develop a more severe, bilateral or multifocal disease at an earlier age of onset. Based on these observations, Knudson proposed that two mutations, or two hits, were required for retinoblastoma to appear in both sporadic and familial cases. In familial retinoblastoma, the first mutation (in one of the alleles of the Rb gene) is transmitted through the germline and is present in all cells, whereas the second mutation needs to occur somatically. Thus a second hit (in the other allele of the Rb gene) in only one retinal cell is sufficient for the tumour to arise, in agreement with the dominant inheritance of familial retinoblastoma. In sporadic (noninherited) retinoblastoma, both mutations and hits have to occur within the same somatic cell, statistically a far less likely event. Genetic defects of tumour suppressor genes also occur frequently in sporadic cancer, both during tumour initiation and progression.

Cytogenetic studies in lymphocytes of patients with familial cancers provided important information about the chromosomal location of tumour suppressor genes. For instance, 5 percent of retinoblastoma patients had interstitial deletions on chromosome 13q14, whereas Wilms tumour patients frequently had deletions on chromosome 11p13, pointing to the

chromosomal position of tumour suppressor genes associated with these diseases. Recent studies have indicated that small deletions and point mutations are more commonly found than large deletions in tumours. Information relating to genetic alterations in cancer can be accessed through an interactive, database (http://cgap.nci.nih.gov/Chromosomes/Mitelman). Genetic material in all somatic cells is comprised of equal contributions of maternal and paternal chromosomal material. Small DNA sequence differences (polymorphisms) between parental chromosomes, termed *heterozygosity*, are present at most genetic loci. In tumours, the wildtype allele of a tumour suppressor gene is commonly replaced by a mutated one through the processes of mitotic recombination, chromosomal nondysjunction, or gene conversion, resulting in the absence of DNA polymorphism, termed loss of heterozygosity (LOH). Loss of heterozygosity can also arise from a complete absence of the wild-type allele, as in the case of large deletions or partial or complete chromosomal loss.

3.3.3.3. The p53 gene

The p53 tumour suppressor gene is mutated in more than 50 percent of all human cancers and provides a selective growth advantage for cells harbouring its mutations. Genetic studies in mice have demonstrated that p53 is not essential for normal growth and development but mice carrying p53 mutations are highly susceptible to tumour development, particularly of lymphoid origin, confirming the role of p53 as a tumour suppressor. Human carriers of a heterozygous p53 mutation suffer from Li-Fraumeni syndrome (OMIM #151623), a rare autosomal dominant disorder characterized by early onset mesenchymal and epithelial malignancies at multiple sites. Tumours in Li-Fraumeni patients display LOH and absence of the wild-type p53 allele in tumour cells. p53 structural features suggest a function in regulation of gene expression. Numerous genes that contain DNA elements specifically targeted by p53 within their promoters have been discovered, indicating that p53 might have multiple functions in tumour suppression. In response to cellular stresses induced by DNA damage, hypoxia, or oncogene activation, intracellular levels of p53 increase and activation of p53 induces an arrest of cells in G1 phase of the cell cycle. It is believed that a transient p53-induced G1 arrest allows DNA repair to occur before replication, thus preventing cells with damaged DNA from entering S phase. One of the most prominent transcriptional targets of p53 is p21 (Cip1/Waf1), a 21 kDa inhibitor of cyclin-dependent kinases important for proper G1-S cell cycle transition. Cells lacking p53 have an increased incidence of genetic instability and gene amplification. p53 also has a function in programmed cell death or apoptosis and in response to agents that induce double strand DNA breaks, p53 initiates an apoptotic program in a number of cell types. p53 protein is also a common target of DNA tumour virus proteins, including SV40 large tumour antigen, the adenoviral E1B protein, and the papillomavirus E6 protein, as well as certain cellular oncogenes. The transforming potential of these molecules is often directly dependent on their ability to interact with p53 and impair its function. Thus, molecules involved in the regulation of p53 form an elaborate oncogene-tumour suppressor gene network. The existence of such networks has been demonstrated for the majority of known tumour suppressor genes.

3.4. The cell cycle

Cells can be recognized by morphological criteria in mitosis (M). DNA synthesis takes place only in a specific period in the cell cycle termed the synthesis or S phase. The gaps (G)

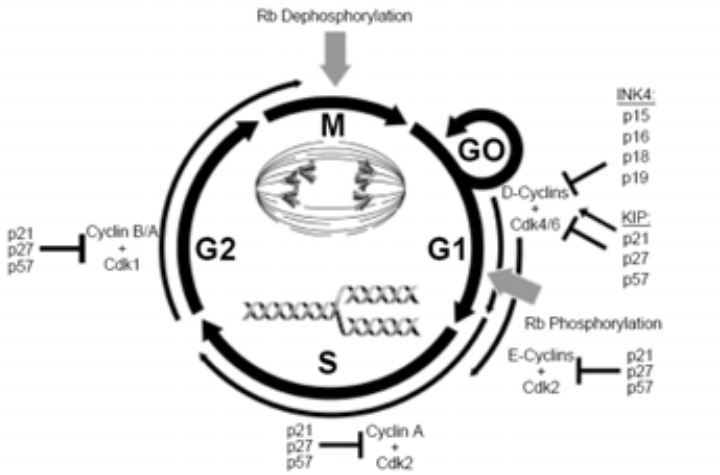

Fig. 3.3 Overview of the cell cycle. The cell cycle is classically divided into the G_1 (G, gap), S (DNA synthesis), G_2 and M (mitotic) phases. The majority of cells in living organisms are in a quiescent G_0 phase. Transition between these phases is governed by positive effectors (cyclins and cyclin-dependent kinases) and negative (INK4 and KIP family) regulators (Cdk inhibitors). Phosphorylation of the retinoblastoma protein (Rb) removes its restraining effect on the G_1-to-S phase transition. p21 and p27 play both activating and inhibitory roles for Dtype cyclin-Cdk (Tannock et al., 2005)

between M and S phase and between S phase and M are called, respectively, the G_1 and G_2 phases (Figure 3.3). Following M, cells may also enter a quiescent G_0 phase in the absence of stimuli triggering further cell division cycles. Many cells in normal adult tissues are in a quiescent G_0 state. In the presence of a sustained mitogenic stimulus, cells in G_0 or G_1 progress to a *restriction point* (R), beyond which a cell is committed to enter S phase. After the R point, growth factors present in the environment are no longer required for progression into S phase and completion of G_2 and M phases. In cancer cells, deregulation of multiple control mechanisms results in cells with different degrees of autonomy from extracellular growth-stimulatory or growth-inhibitory signals, making them more likely to meet the requirements for transition through the R point. Progression through the cell cycle is coordinated by a tightly regulated series of events involving the synthesis, assembly, and activation of key cell cycle regulatory complexes comprised of cyclins and cyclin-dependent kinases (Cdks), followed by their subsequent disassociation and degradation. Multiple mechanisms regulate the timing of these processes, including transcriptional and translational controls, posttranslational control via ubiquitin-mediated proteolysis, as well as regulation of the subcellular localization of proteins.

3.4.1. Cycle-dependent kinases and cyclins

Mammalian Cdks comprise a family of ten serine-threonine protein kinases (Cdk 1–10) that catalyze different cell cycle transitions. A Cdk molecule binds to an activator molecule known as a cyclin, which is an absolute requirement for Cdk activation. In addition, Cdks are regulated by site-specific phosphorylation and by the binding of Cdk inhibitors. Activation of Cdks requires the phosphorylation of a conserved threonine residue that is catalyzed by the Cdk-activating kinase (CAK). This phosphorylation elicits a conformational change that, together with cyclin binding, is required for kinase activity. Cdk-activating kinase is active throughout all phases of the cell cycle, but its access to the Cdk substrate is cell cycle regulated. Inhibition of CAK action on, or access to the Cdks, prevents phosphorylation and activation of Cdks and leads to cell cycle arrest. These processes are controlled through the

cell cycle by the interplay between the Wee-1 and Myt-1 kinases and a group of cell division cycle (Cdc) phosphatases Cdc25A, B, and C.

3.4.2. Activation of Cdks by binding to cyclins

The family of mammalian cyclins (A to H) all shares a conserved sequence of about 100 amino acids, referred to as the cyclin box. Different cyclins bind and activate different Cdks, and activated cyclin-Cdk complexes, in turn, phosphorylate various target proteins to ultimately mediate progression through the different cell cycle phases. Cyclin levels change significantly during cell cycle progression, allowing for precise timing of Cdk activation and ensuring that these kinases are catalytically active only at specific times during the cell cycle (Figure 3). This is regulated by both the specific subcellular localization and the timed expression and degradation of various cyclins and Cdk inhibitors throughout the cell cycle. In general, the peak nuclear expression of a specific cyclin occurs at or just prior to the peak activity of the partner kinase, and following activation, the respective cyclins are degraded rapidly by the ubiquitin-mediated proteosomal pathway. In most cells, cyclin D-Cdk complexes are activated by mitogenic stimuli early in G_1 followed by activation of cyclin E-Cdk2 in mid G1 phase. The D- and E-type cyclins promote movement through the G_1/S transition. Cyclin A-Cdk2 activation in late G1 phase follows cyclin E-Cdk activation and is essential for initiation of and progression though S phase and for the onset of mitosis. In mammalian cells, two B-type cyclins (cyclin B1 and cyclin B2) associate with Cdk1 to regulate entry into and exit from mitosis. One of the important substrates of the cyclin-D–, cyclin-E–, and cyclin-A–associated kinases is the retinoblastoma protein, pRb. In early G_1 phase, pRb is hypophosphorylated and bound to a member of the E2F family of transcription factors. The pRb/E2F complexes recruit additional molecules such as histone deacetylases (HDACs) to repress the transcription of genes whose products are required for DNA synthesis such as dihydrofolate reductase. However, activation of cyclin D and later cyclin-E–dependent kinases during progression from G_1 to S phase leads to the accumulation of pRb in a hyperphosphorylated state. This relieves the pRb-HDAC–mediated transcriptional repression and allows E2F family members to dissociate from the hyperphosphorylated pRb, and activate transcription of genes required for S phase entrance, such as cyclin E and cyclin A, as well as enzymes needed for DNA synthesis. The phosphorylation of the retinoblastoma protein is one indicator of cell cycle progression through the restriction point. The retinoblastoma protein is dephosphorylated in mitosis, prior to G_1 phase of the next cell cycle.

3.4.3. Inhibitors of cyclin-dependent kinases

In addition to phosphorylation and binding to cyclins, Cdks are subject to regulation by the binding of Cdk inhibitory (CKI) proteins. There are two families of CKIs: the *K*inase *I*nhibitory *P*rotein (KIP) family and the *In*hibitor of Cd*k4* (INK4) family. Overexpression of either KIP or INK proteins leads to G_1 arrest. The KIP family members, which include p21 (Cip1/Waf1), p27, and p57 share homology at their N-terminal Cdk inhibitory domain, and in vitro they can inhibit all cyclin-Cdk complexes. The KIP proteins have binding sites for both the cyclin and Cdk, and only a single KIP molecule is required for cyclin-Cdk inhibition. In vivo, KIP expression and activity is tightly regulated. As noted earlier p21 is a protein whose gene expression is upregulated by p53 following cellular stress or DNA damage. Members of the INK4 family act in G_1 phase to inhibit primarily Cdk4 and Cdk6. The four members, p15, p16, p18, and p19 are structurally related and act to destabilize the association of the D-type cyclins with Cdk4 or Cdk6. The p16 protein plays an important role in the proliferative arrest of cells at senescence and the p16 gene is frequently deleted in human cancers. Studies in

mice have suggested that this protein plays a tumour suppressor role since cell lines derived from p16-null mice undergo spontaneous immortalization with high frequency.

3.5. DNA damage and repair

***DNA repair disorders*:** A number of human disease syndromes are associated with pronounced cellular sensitivity to DNA-damaging agents due to hereditary deficiencies in DNA repair or to deficiencies in signaling pathways that are activated by DNA damage. Patients with several of these syndromes show marked chromosomal instability and predisposition to malignancy. For example, the disorders, xeroderma pigmentosum (XP), ataxia telangiectasia (AT), Bloom's syndrome (BS), and Fanconi's anemia (FA) are all autosomal recessive, cancer-prone diseases that are associated with defective DNA repair. Patients suffering from XP are sun-sensitive and have an extreme predisposition to skin cancer—an increase in incidence of perhaps 1000-fold. Patients with AT have a very high incidence of lymphomas often, before the age of 20. The incidence of lymphomas is also increased markedly in FA and BS patients. Human hereditary non-polyposis colon cancer (HNPCC) is caused by a deficiency in DNA mismatch repair.

***Fidelity of repair*:** An important property in DNA repair is the fidelity of the repair pathway leading to the concepts of error-prone, and error-free, DNA repair. Many DNA lesions can block transcription of RNA, thereby inactivating the gene which contains damaged DNA. Persistent blockage of RNA synthesis can lead to cell death but these lesions are often repaired through the transcription-coupled repair pathway (TCR). For lesions that block DNA replication, several error-prone DNA polymerases have been described which have low fidelity to allow for replicative bypass (i.e., translesion DNA synthesis) of the damage contained within DNA. These polymerases can temporarily be used by the cell following DNA damage and can then be substituted by more accurate DNA polymerases. Spontaneous oxidative damage is known to occur in cells, producing 10^4 to 10^5 oxidative residues, (e.g., 8-oxo-guanine) per cell per day among the 3×10^9 bases in the genome. If a cell cannot repair this continual onslaught of base damage, malignant transformation may occur. DNA base damage, occurring as a result of endogenous oxidative processes or exogenous DNA damage (e.g., ionizing radiation) is repaired by the base excision repair pathway.

***Base excision repair*:** Base excision repair (BER) involves the enzymatic removal of the damaged DNA base by DNA glycosylases leaving an apurinic or apyrimidinic (AP) site which allows for resynthesis and insertion of new bases complementary to the opposite strand. The major BER pathway is short-patch BER and involves the replacement of a single nucleotide following DNA backbone cleavage at the AP site. A minor BER pathway is the long-patch BER pathway, which exists for the repair of two to thirteen damaged nucleotides.

***Nucleotide excision repair*:** Nucleotide excision repair (NER) is a complex DNA repair pathway designed to repair ultraviolet-induced photoproducts or cyclobutane pyridimine dimers (CPD). The process of NER is highly conserved in eukaryotic cells and consists of four steps: (1) recognition of the damaged DNA; (2) excision of an oligonucleotide of twenty-four to thirty-two residues containing the damaged DNA by dual incision of the damaged strand on each side of the lesion; (3) filling in of the resulting gap by DNA polymerase; and (4) ligation of the nick. Nucleotide excision repair has two subpathways termed global genome repair (GG-NER) that is transcription-independent and removes lesions from the entire genome, and transcription-coupled repair (TCR-NER). The GG-NER pathway surveys the entire genome for lesions which distort the DNA. These lesions are removed rapidly. In contrast, CPDs are removed more efficiently from the transcribed strand of expressed genes by TCR-NER. TCR-NER focuses on DNA lesions that block the activity of RNA

polymerases and overall transcriptional activity. The elongating transcriptional machinery is thought to facilitate the recognition of DNA lesions on the transcribed strand in TCR-NER.

Double strand break repair*:** In human cells, repair of DNA double strand breaks (dsb) occurs either by ***homologous recombination (HR) or ***nonhomologous end joining (NHEJ).*** In homologous recombination (Figure 3.4), extensive homology is required between the region of the DNA dsb and the sister chromatid or homologous chromosome from which repair is directed (Figure 3.4). This pathway is thought to predominate in repair of DNA dsbs in germline tissues and during S and G_2 phases of the cell cycle of somatic cells. The HR pathway results in error-free repair of DNA dsbs because the intact undamaged template is used to pair new DNA bases between the damaged and undamaged strands during DNA synthesis. Nonhomologous end joining (Figure 3.5) does not require homology and the NHEJ proteins simply link the ends of DNA breaks together; this usually results in the loss or gain of a few nucleotides. Nonhomologous end joining is error-prone and operates predominantly to repair damage in somatic cells during the G_1 phase of the cell cycle. There is a separate, but related, DNA dsb repair pathway that ligates DNA dsbs using small pieces of microhomologous DNA and involves the MRE11 and BRCA2 proteins. The BRCA2 breast cancer susceptibility protein may also play a role in the homologous repair of DNA dsbs. Both BRCA1 and BRCA2 proteins form discrete nuclear foci during S-phase at the sites of DNA damage following exposure to DNA damaging agents. Although RAD51 colocalizes at subnuclear sites with BRCA1, only 1 to 5 percent of BRCA1 in somatic cells associates with RAD51. In contrast, a significant fraction of the total intracellular pool of BRCA2 binds to RAD51 and BRCA2-deficient cells have ten-fold lower levels of homologous recombination when compared to BRCA2-proficient cells. One model suggests that a BRCA2-RAD51 complex promotes the accurate assembly of DNA repair proteins required to offset DNA breaks that accumulate during DNA replication; these could otherwise lead to gross chromosomal rearrangements, loss of heterozygosity at tumour suppressor gene loci, and carcinogenesis.

Major protein complexes implicated in the NHEJ pathway are the DNA-PK protein kinase and XRCC4/Ligase IV complexes. Human DNA-PK consists of a ~460 kilo Daltons DNA-PK catalytic subunit (DNA-PK_{CS}), and a DNA end-binding KU heterodimer (consisting of 70 kD and 80 kD protein subunits). The catalytic subunit shows homology to the PI-3K kinase superfamily at its C-terminus, which contains the protein kinase domain required for phosphorylating DNA-PK associated proteins during repair. Mutations in either *DNA-PK_{CS}* or in one of the *KU* genes results in sensitivity to ionizing radiation and reduced ability to repair radiation-induced DNA dsbs. XRCC4 forms a stable complex with DNA ligase IV, and probably links the initial lesion detection by KU 70/80 and DNA-PK_{CS}, scaffolding to the actual ligation reaction carried out by ligase IV. The NBS1/MRE11/RAD50 protein complex acts in both HR and NHEJ pathways and also in maintenance of telomeres. Mutations in the NBS1 gene result in Nijmegen Breakage Syndrome *(*NBS), a recessive disorder with some phenotypic similarities to ataxia telangiectasia (AT) including chromosomal instability, radiosensitivity, and an increased incidence of lymphoid tumours. Mutations in human MRE11 have been linked to the ataxia telangiectasia-like disorder (ATLD). Cells from NBS, AT, and ATLD patients are hypersensitive to DNA dsb-inducing agents and show radioresistant DNA synthesis after exposure to ionizing radiation. The relative levels of DNA-PK_{CS} and KU80 protein expression vary widely among different tissue types. However, there is no evidence that tumour cell radiosensitivity is simply correlated to the relative expression level of DNA-PK_{CS} protein expression.

Ionizing radiation leads to rapid phosphorylation of a nucleosomal histone protein, H2AX (γ-H2AX is the phosphorylated form) that can be measured as an intracellular marker of DNA double-strand breaks. This early event precedes the actions of repair enzymes involved in homologous recombination and nonhomologous end-joining of these breaks. Nuclear foci, each containing thousands of γ-H2AX molecules covering about 2 megabases of DNA surrounding the break, can be detected using antibody staining and fluorescence microscopy. Foci of γ-H2AX are believed to recruit repair enzymes to sites of DNA damage and the number of γ-H2AX foci has been directly correlated to the number of DNA double strand breaks. It is probable that residual nuclear foci at late times following irradiation (>12 hrs) represent nonrepaired DNA double-strand breaks that lead to subsequent cell lethality.

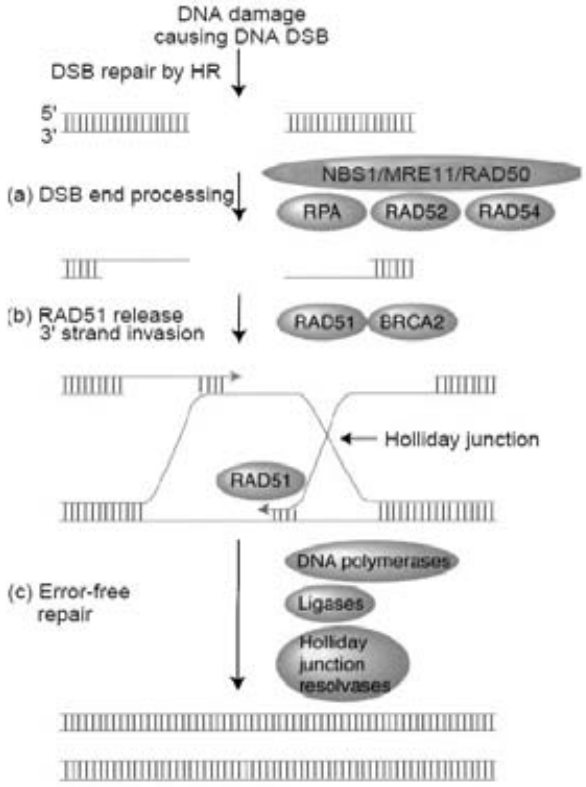

Fig. 3.4 Homologous recombination (HR) repair of DNA double strand breaks (DSB). The initiating step for homologous recombination is thought to be the processing of the 3` end of the DNA break by the NBS1/MRE11/RAD50 protein complex. Following binding of the RAD52 and RAD54 proteins, the Replication Protein A (RPA) facilitates the assembly of the Rad51-BRCA2 complex on the single-strand 3` DNA overhang to form a RAD51-nucleoprotein filament. The RAD51 nucleofilament DNA is then able to pair with a homologous region in duplex DNA forming a Holliday junction after alignment of sister chromatids. Complex chromatin alterations and configurations are required to unwind the DNA and allow for DNA strand exchange. After identification of the identical sister chromatid sequences, the intact double-stranded copy is then used as a template to repair the DNA break by subsequent DNA synthesis using DNA polymerases, ligases and Holliday junction resolvases. Homologus recombination results in error-free (i.e. high fidelity) repair of DNA double-strand breaks and predominates in the S and G2 phases of the cell cycle (modified from van Gent et al., 2001)(Tannoch et al, 2005).

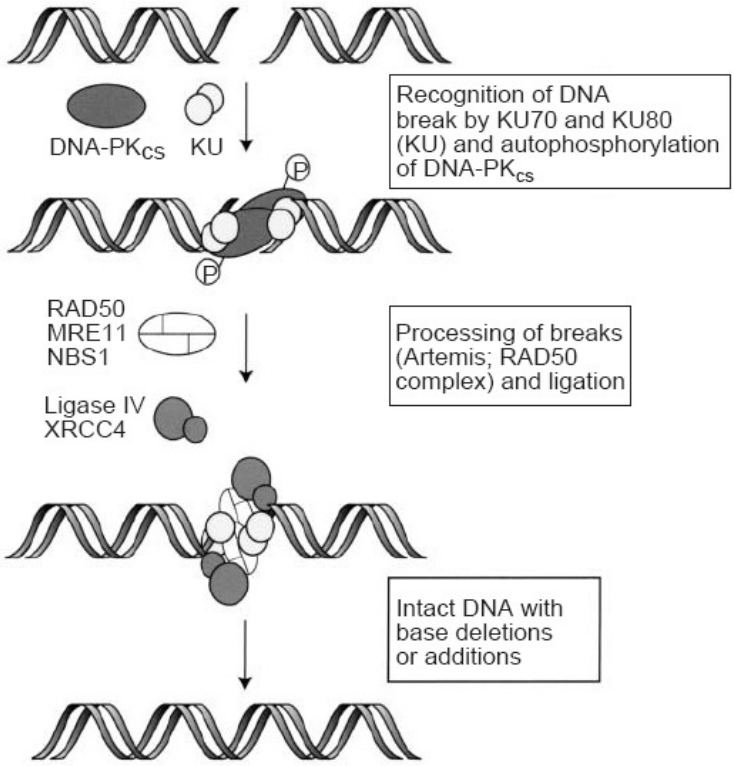

Fig. 3.5 Non-homologous end-joining (NHEJ) repair of DNA double-strand breaks. DNA-PKcs, KU70, KU80, DNAligase IV, and XRCC4 are all critical components of the NHEJ repair pathway. DNA-PK is composed of a heterodimeric DNA-binding component named KU70/KU80 which binds to either blunt or staggered DNA ends at the double-strand break and recruits the large catalytic subunit kinase, DNA-PKcs to the break. DNA-PKcs undergoes autophosphorylation after binding to the DNA break and may recruit additional proteins to the damaged site as potential phosphorylation substrates. The NBS1-MRE11-RAD50 protein complex and the Artemis protein may be involved in processing of DNA ends during the initial binding and activation of DNA-PK kinase activity. The XRCC4-ligase IV heterodimer finally ligates the breaks to create intact DNA strands. Non-homologous recombination can result in error-prone (i.e., low-fidelity) repair of DNA double-strand breaks and predominates in the G1 phase of the cell cycle (modified from van Gent et al., 2001) (Tannoch et al., 2005).

3.6. Tumour growth and cell kinetics

3.6.1. Tumour growth

In normal tissues that undergo cell renewal, there is a balance between cell proliferation, growth arrest and differentiation, and loss of mature cells by programmed cell death or apoptosis. Tumours grow because the homeostatic mechanisms that maintain the appropriate number of cells in normal tissues are defective, leading to imbalance between cell proliferation and cell death, so that there is expansion of the cell population. However, the proliferative rate of tumour cells varies widely between tumours, nonproliferating cells are common, and there is often a high rate of cell death.

***Tumour growth*:** Tumour growth can be determined by estimating tumour volume as a function of time. Exponential growth will occur if the rates of cell production and of cell loss

or death are proportional to the number of cells present in the population. Exponential growth implies that the time taken for a tumour to double its volume is constant and may leads to the false impression that the rate of tumour growth is accelerating with time. Increase in the diameter of a human tumour from 0.5 to 1.0 cm may escape detection, whereas increase in the diameter of a tumour from 5 to 10 cm is more dramatic and is likely to cause new clinical symptoms. Both require three volume doublings and during exponential growth they will occur over the same period of time. Estimates of the growth rates of untreated human tumours are limited but there are published estimates of the growth rate of many human tumours. In general these estimates indicate that there is a wide variation in growth rate, even among tumours of the same histologic type and site of origin. There is a tendency for childhood tumours and adult tumours that are known to be responsive to chemotherapy (e.g., lymphoma, cancer of the testis) to grow more rapidly than less responsive tumours (e.g., cancer of the colon) and metastases tend to grow more rapidly than the primary tumour in the same patient. Representative mean doubling times for lung metastases of common tumours in humans are in the range of 2 to 3 months.

Tumours are unlikely to be detected until they grow to about 1 gram, and tumours of this size will contain about one billion (10^9) cells. There is indirect evidence that many tumours arise from a single cell, and a tumour containing about 10^9 cells will have undergone approximately thirty doublings in volume prior to clinical detection (because of cell loss, this will involve more than thirty consecutive divisions of the initial cell). After ten further doublings in volume, the tumour would weigh about 1 kilogram (10^{12} cells), a size that may be lethal to the host. Thus, the range of size over which the growth of a tumour may be studied represents a rather short and late part of its total growth history. There is evidence (e.g., for breast cancer) that the probability of metastatic spread increases with the size of the primary tumour, but the long preclinical history of the tumour may allow cells to metastasize prior to detection. Thus, early clinical detection may be expected to reduce but not to prevent the subsequent appearance of metastases.

3.6.2. Cell kinetics

Early studies of cell population kinetics were based on autoradiography to detect the selective uptake of radioactive thymidine into cellular DNA, although these methods have been supplanted by automated techniques based on flow cytometry. The proportion of thymidine-labeled cells at a short interval after administration of tritiated thymidine (the labeling index) is a measure of the proportion of cells in S phase. Typical values for the proportion of cells in S phase are in the range of 3 to 15 percent for many types of human solid tumours. Higher rates of cell proliferation are evident in faster-growing malignancies, including acute leukemia and some lymphomas. However, the rate of cell proliferation is usually less than that of some cells in normal renewing tissues, such as the intestine or bone marrow. Thus, accumulation of cells in tumours is not due simply to an increased rate of cell proliferation as compared to the normal tissue of origin. Rather, there is defective maturation and the population of malignant cells increases because the rate of cell production exceeds the rate of cell death or removal from the population. In the percent-labeled-mitoses (PLM) method, serial biopsies (or serial specimens from identical animals) are taken at intervals after a single injection of 3H-thymidine, and the proportion of mitotic cells that are labeled is estimated from autoradiographs. Most tumours contain nonproliferating cells, and the term growth fraction describes the proportion of cells in the tumour population that is proliferating. The occurrence of extensive necrosis in solid tumours and of apoptotic cells and the ability of tumour cells to metastasize from a primary tumour indicate that there is considerable cell death or loss from many tumours. The rate of cell loss from tumours can be estimated by

comparing the rate of cell production (from assessment of the labeling index or fraction of S phase cells by flow cytometry) with the rate of tumour growth. The overall rate of cell production may be characterized by the potential doubling time of the tumour (Tpot), which is the expected doubling time of the tumour in the absence of cell loss.

Flow cytometry: Flow cytometry is a method that allows the separation and sorting of cells based on cellular fluorescence. Cells can be stained with a fluorescent dye whose binding (to DNA) is proportional to DNA content, and flow cytometry then allows enumeration of cells containing different amounts of DNA. Several fluorescent dyes are available which stain DNA, including ethidium bromide, propidium iodide, acridine orange, and Hoechst 33342. Most dyes require fixation of the cells to allow access of dye to the DNA, although selected DNA specific dyes (e.g., Hoechst 33342) can enter viable cells; the Hoechst dye allows isolation of viable cells according to DNA content. Often, fluorescent reagents are applied concurrently or sequentially to allow for analysis or separation of cells on the basis of two or more criteria (such as the expression of a specific protein and DNA content). Computer analysis of a fluorescent DNA distribution provides estimates of the proportion of cells with 2N DNA content (i.e., G_1 and most nonproliferating cells), with 4N DNA content (G_2 and mitotic cells), and with intermediate DNA content (S phase cells). In tumours, the presence of aneuploidy (i.e. a G_1-phase DNA content different from that of normal cells) and of variable DNA content among G_1 cells complicates analysis of DNA distributions and the estimation of cell cycle parameters. The proportion of S phase cells obtained from a DNA distribution is analogous to the thymidine labeling index and gives a broad indication of the proliferative rate. Flow cytometry can be used to estimate cell cycle phase distribution, growth fraction, and kinetic properties of cell populations. Precursors such as 5-bromodeoxyuridine (BrdUrd), can be incorporated into newly synthesized DNA (like tritiated thymidine) and can be recognized by flow cytometry using commercially-available fluorescently-tagged monoclonal antibodies. Analysis of BrdUrd staining and DNA content at different times later allows analysis of the tagged cells as they move through the cell cycle. Several methods allow proliferating and nonproliferating cells to be distinguished by flow cytometry. A variety of cellular antigens [e.g., that are recognized by the monoclonal antibody, Ki-67 and proliferating cell nuclear antigen (PCNA)] appear to be expressed uniquely in cycling cells and can be recognized by fluorescently-labelled antibodies. The Ki-67 antigen has been used most often as a marker for proliferating cells although its function remains poorly understood.

A number of estimates of the duration of S phase (T_s) and of potential doubling time (T_{pot}) in human tumours have been derived from PLM studies or BrdUrd (or IUrd) labeling and flow cytometry. Mean values for T_s tend to be in the range of 12 to 24 hours and values of the mean cell cycle time are in the range of 2 to 3 days, but the distribution of cell cycle times is broad, and both the PLM and BrdUrd techniques tend to give information about the faster proliferating cells in the population. T_{pot} values (range of 4.5 to 20 days) are much longer than estimates of mean cycle time T_c, implying that many human tumours have a low growth fraction (usually in the range 5-30% for solid adult tumours but may be much higher for childhood tumours and adult tumours such a lymphoma or cancer of the testis). The mean values of T_{pot} are also much lower than estimates of volume doubling time for common human tumours (typically 2 to 3 months) because the rate of cell loss in many human tumours is in the range of 75 to 90 percent of the rate of cell production. Not surprisingly, well-nourished cells close to blood vessels have a more rapid rate of cell proliferation than poorly nourished cells close to a region of necrosis. Slowly proliferating cells at a distance from functional blood vessels may be resistant to radiation because of hypoxia and to cytotoxic chemotherapeutic drugs because of their low proliferative rate and limited drug access.

***Stem cells in tumours*:** Evidence for the monoclonal origin of human tumours is provided by the observation that a unique identifying feature (a clonal marker) may be found in all of the constituent cells. Initial evidence accrued from analysis of X-linked genes or gene products in cells from tumours in women who are heterozygous at these genetic loci. One of the X chromosomes becomes inactivated at random in all cells of females during early life. The normal tissues of heterozygous females are therefore mosaics that contain approximately equal number of cells in which one or the other (but not both) of the two alleles of a gene on the X chromosomes are expressed. However, cells in tumours arising in such individuals usually express only one allele of such genes—for example they express only one form (isoenzyme) of the X-linked glucose-6-phosphate dehydrogenase. Other clonal markers include chromosomal rearrangements such as the Philadelphia chromosome in chronic myelogenous leukemia; uniquely rearranged immunoglobulins or T-cell receptors expressed by B-cell lymphomas or multiple myelomas and T-cell lymphomas; and molecular markers whose detection has been facilitated by the availability of gene sequencing. The above techniques have demonstrated clonality in at least 95 percent of the wide range of tumours that have been examined.

Renewal tissues such as bone marrow and intestinal mucosa represent a hierarchy of cells produced by cell division and differentiation from a small number of stem or early precursor cells. Most tumours arise in renewal tissues, and there is substantial evidence that many tumours contain a limited population of stem cells with the capacity to regenerate the tumour after treatment. Other cells in the tumour population may have lost the capacity for cell proliferation (e.g., through differentiation) or have only limited potential for cell proliferation (analogous to morphologically recognizable late precursor cells in bone marrow, such as myelocytes). Recent experiments have suggested that there is a population of cells in some human tumours that express distinct markers on their cell surface and have the properties of stem cells, including self-renewal. A variety of cell surface markers have been reported to be associated with stem-like cells in tumours including CD44 (breast, colon, pancreas, prostate), CD133 (brain, colon, prostate, kidney), CD166 (colon), however, the specificity of these markers remains in doubt since in most cases they have only been shown to be capable of enriching the tumour cell population for stem-like cells. The stem cell model has major implications for the treatment of human tumours since cure or long term control requires the eradication of the stem cells. If stem cells represent a small subpopulation within some tumours then short term changes in tumour volume may not reflect the effects of treatment on stem cells.

3.6.3. Cell proliferation in normal tissues

Cell proliferation in a variety of normal tissues has also been studied using thymidine labeling and flow cytometry. Acute effects of radiation injury are observed in rapidly proliferating tissues, because radiation-damaged cells often die when they attempt mitosis. Chemotherapy toxicities that are common to many drugs (e.g., myelosuppression, mucositis, hair loss, and sterility) are also observed in these tissues, reflecting the greater activity of most anticancer drugs against proliferating cells. The cell kinetics of hemopoietic cells in the bone marrow and epithelial cells in the intestine are examples of renewal tissues where cell proliferation is an important determinant of anticancer therapy.

Bone marrow: Cells in bone marrow and blood have an orderly progression of differentiation from myeloblasts to polymorphonuclear granulocytes, from pronormoblasts to red blood cells, and from megakaryocytes to platelets. The earlier bone-marrow precursor cells cannot be recognized morphologically, but can be enriched by flow cytometry using fluorescent markers

to antigens that are expressed selectively on their surface. For example, stem cells may be recognized by the expression of the CD34 antigen and the tyrosine kinase receptors known as c-kit and Flk-2/Flk-3. The stem cells may undergo self-renewal or may produce progeny that are early precursor cells for lymphocytes or for cells which under appropriate conditions in culture will form colonies containing cells of the granulocyte, erythroid, megakaryocyte, and monocyte series. The growth factors that stimulate hemopoietic precursor cells to proliferate and differentiate into lineage specific cells include stem-cell factor (Kit-ligand), Interleukin-1 (IL-1), IL-3, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and erythropoietin. Granulocyte-macrophage colony-stimulating factor and G-CSF decrease the duration and extent of myelosuppression after chemotherapy and erythropoietin is used to treat anemia and accompanying fatigue.

Thymidine-labeling studies demonstrated that recognizable precursors of granulocytes and red cells are among the most rapidly proliferating cells in the human body, with a mean duration of S phase (*T*s) and mean cell cycle time (*T*c) of about 12 and 24 hours. Stem cells and other early precursor cells proliferate slowly under resting conditions, and their more rapidly proliferating progeny provide replacement for the normal loss of mature cells. However, stem cells may proliferate rapidly following depletion of more mature functional cells (e.g., by cancer chemotherapy) or after bone-marrow ablation and transplantation. The pattern of proliferation and differentiation in the bone marrow provides an explanation for the decrease in mature granulocytes at 10 to 14 days after cycle-active chemotherapy and their recovery by 21 to 28 days. The rapidly proliferating intermediate precursor cells are most likely to be killed by chemotherapy but changes in the numbers of cells in the peripheral blood are not seen immediately because the later maturing cells are nonproliferating and tend to be spared by chemotherapy. The bone marrow is generally regarded as the most critical tissue for radiation sensitivity following whole body irradiation due to the sensitivity of the stem cells and doses greater than 6-8 Gy are usually lethal without a bone marrow transplant.

Intestine: The villi of the small intestine are lined by a single layer of differentiated epithelial cells that do not proliferate, and cell death and shedding of cells into the lumen occurs at the top of the villi. These cells are replaced by upward migration of cells lining the crypts, which lie between and at the base of the villi. Cell proliferation in the upper two thirds of the crypts is high but occurs more slowly at their bases. Slowly proliferating cells in this region act as precursors for the entire crypt and surrounding villi. Control of cell proliferation in the intestine is complex and proliferation of stem cells in the crypts is influenced by a number of growth factors including EGF, keratinocyte growth factor (FGF-7), and IL-11. Some cycle dependent drugs (e.g., cytosine arabinoside, CPT-11) and radiation may cause severe mucosal damage and diarrhea. Following whole body exposure to radiation, damage to the intestine can be lethal within a few days if the dose is sufficiently high (>10 Gy for humans)

3.7. Cell death mechanisms

Many types of cells do not show morphological evidence of radiation damage until they attempt to divide. Following doses of less than about 10 Gy, lethally-damaged cells may undergo permanent growth arrest (senescence), interphase death or lysis during radiation-induced apoptosis, or cell lysis as a result of mitotic catastrophe (often after a number of abortive mitotic cycles). A radiation survival curve based on colony-forming ability represents the total cell death within an irradiated cell population as a result of all types of cell death. For the majority of normal and tumour cells, death secondary to mitotic catastrophe accounts for most of the cell kill following irradiation. However, in some radiosensitive cells and the cancers that arise from them — notably lymphocytes, spermatocytes, thymocytes, and

salivary gland epithelium—irradiation causes the cells to undergo an early (within a few hours) interphase death. This death is associated with the biochemical and morphologic characteristics of apoptosis (i.e., cell membrane blebbing, the formation of nuclear apoptotic bodies, and specific DNA fragmentation patterns). Depending on the type of cell, the intracellular target(s) for the induction of the apoptotic response may be either the cell membrane or the DNA or both.

Why some cells undergo extensive radiation-induced apoptosis within a few hours after irradiation, while others do not, is unclear, but may relate to radiation-induced expression of proteins which trigger an apoptotic response. For example, in hematopoietic cells, radiation can lead to upregulation of pro-apoptotic genes (such as *fas, bax,* and *caspase-3*) and/or downregulation of anti-apoptotic genes, (such as *bcl-2*). In endothelial cells ionizing radiation can initiate a sphingomyelin-dependent signaling pathway within the cell membrane which can induce apoptosis in the absence of DNA damage. Ceramide is generated from sphingomyelin (SM) by the action of acid sphingomyelinase (ASM), or by de novo synthesis coordinated through the enzyme ceramide synthase. In the radiation response, ceramide serves as a second messenger in initiating apoptosis, while some of its metabolites block apoptosis. In certain cells, such as endothelial, lymphoid and hematopoietic cells, ceramide mediates apoptosis, while in others ceramide may serve only as a co-signal or play no role in the death response. The ceramide-mediated apoptotic response to radiation can be inhibited by basic fibroblast growth factor.

Altering the apoptotic response of tumour cells may be one strategy to sensitize tumours to radiotherapy. Some tumours may evade radiation therapy-induced apoptosis by carrying *p53* gene mutations or by lacking *p53* expression or function; restoration of wild-type *p53* function using gene therapy may potentiate radiation cell kill. However, the induction of apoptosis following irradiation does not account for the therapeutic effect of radiation in solid epithelial tumours, as it does not correlate with eventual clonogenic cell survival as measured by colony-forming assays. The other modes of cell death (i.e. mitotic catastrophe and/or terminal growth arrest) account for this difference. Most tumour cell lines have retained the capacity of normal cells to undergo accelerated senescence after irradiation, and although the cell-cycle-related *p53* and *p21Waf1* genes can act as positive regulators of treatment-induced senescence, they are not required for this response in tumour cells. Senescent or terminal-arrested cells are metabolically active but do not proliferate and do not form colonies following irradiation. They eventually die, days to weeks following irradiation, by necrosis. This may explain the relatively slow resolution, yet ultimate cure, of some tumours following radiotherapy.

The random nature of the energy deposition events means that damage can occur in any molecule in a cell. Biochemical processes, such as DNA, RNA, or protein synthesis, respiration, or other metabolism can be inhibited by irradiation but this usually requires doses in the order of 10 to 100 Gy. DNA is a major target of ionizing radiation because of its biological importance to the cell and even relatively small amounts of DNA damage can lead to cell lethality. Focal areas of DNA damage can arise because of the clustering of ionizations within a few nanometers of the DNA. These “local multiply-damaged sites” (LMDS) include combinations of single- or double-strand-breaks in the sugar-phosphate backbone of the molecule, alteration or loss of DNA bases, and formation of crosslinks (between the DNA strands or between DNA and chromosomal proteins). It has been estimated that approximately 10^5 ionization events can occur within the cell per Gy of absorbed radiation dose, leading to approximately 1000 to 3000 DNA-DNA or DNA-protein crosslinks, 1000 damaged DNA bases, 500 to 1000 single-strand DNA breaks and 25 to 50 double-strand DNA breaks. The

vast majority of the ionization events do not cause DNA damage and most of the DNA lesions caused can be repaired by a variety of DNA repair pathways, probably acting together to repair clustered LMDS-associated lesions. Nevertheless, a small number of DNA strand breaks may remain unrepaired (residual breaks). High-LET irradiation causes an increase in both the number and complexity of DNA-clustered lesions and is more difficult to repair. The results from assays of DNA double-strand breaks suggest that clonogenic cell survival following radiation is correlated with the residual level of such breaks.

If a cell does survive and goes on to proliferate after irradiation, delayed chromosomal instability may sometimes be observed in its descendants. One factor that seems to perpetuate the unstable phenotype in irradiated cells is the continued production of reactive oxygen species. Such species or other factors may be released from an irradiated cell and cause damage to neighboring nonirradiated cells (e.g. ***bystande**r* cells). For example, transfer of media from irradiated unstable cell clones to a nonirradiated cell population has been reported to lead to cell death in some of the nonirradiated cells within 24 hours. Similarly, targeting of 10 to 30 percent of a cellular population with high-LET irradiation using a microbeam can lead to cell death in the nontargeted surrounding cells within the culture dish. These data are consistent with clinical studies that have shown chromosomal changes in circulating peripheral lymphocytes in patients who received only localized radiotherapy. Similarly the serum from people who have been exposed to whole body irradiation has been reported to be clastogenic for lymphocytes in culture. This ***bystander effect*** of radiation has implications for assessment of radiation risk and for health risks associated with radiation exposure as the total cell kill within an irradiated cell population may be greater than that calculated simply on the basis of the number of cells that were directly irradiated.

3.8. *In vitro* and i*n vivo* assays for cell survival

Inhibition of the continued reproductive ability of cells is an important consequence of the molecular and cellular responses to radiation, as it occurs at relatively low doses (a few grays) and it is the major aim of clinical radiotherapy. A tumour is controlled if its stem cells (i.e., clonogenic cells) are prevented from continued proliferation. A cell that retains unlimited proliferative capacity after radiation treatment is regarded as having survived the treatment, while one that has lost the ability to generate a clone or colony is regarded as having been killed, even though it may undergo a few divisions or remain intact in the cell population for a substantial period. Colony formation following irradiation is an important endpoint for radiobiologists and radiation oncologists, as it relates to a cell's ability to repopulate normal or tumour tissues following exposure to ionizing radiation. In the assay that is used most often to assess colony formation, cells grown in culture are irradiated either before or after preparation of a suspension of single cells and plated at low density in tissue-culture dishes. Following irradiation, the cells are incubated for a number of days, and those that retain proliferative capacity divide and grow to form discrete colonies of cells. After incubation, the colonies are fixed and stained so that they can be counted easily. Cells that do not retain proliferative capacity following irradiation (i.e., are killed) may divide a few times but form only very small abortive colonies. If a colony contains more than 50 cells (i.e., derived from a single cell by at least six division cycles), it is usually capable of continued growth and can be regarded as having arisen from a surviving cell. The plating efficiency (PE) of the cell population is calculated by dividing the number of colonies formed by the number of cells plated. The ratio of the PE for the irradiated cells to the PE for control cells is calculated to give the fraction of cells surviving the treatment (cell survival). If a range of radiation doses is used, then these cell-survival values can be plotted to give a survival curve.

Cells taken directly from animal or human tumours can also be grown in culture, allowing the in vitro assay method to be extended to the study of the radiation sensitivity of tumour cells treated in vivo. Untreated cells rarely have a PE of 1 (more usually it is 0.5 to 0.8 for cells passed for many generations and much lower for cells derived from spontaneous tumours). The techniques described above have been used to obtain survival curves for a wide range of malignant and normal cell populations. In general, for low-LET radiation (e.g., x- or γ-rays), these curves are plotted as cell survival on a $\log_{10}$ scale (y-axis) with dose on a linear scale (x-axis). Such semilogarithmic curves usually have a shoulder region at low doses but at higher doses, the curve either becomes steeper and straight so that survival decreases exponentially with dose or appears to be continually bending downward. The accuracy of the experimental data obtained is usually such that either shape could fit the data adequately over the first few decades of survival. The difference in survival curves for x- or γ-rays (low-LET) and for fast-neutron (high-LET) irradiation is that, in general, both the slope and the shoulder of the survival curve are reduced for higher LET radiation. The biological effectiveness of different types of radiation can be characterized by a parameter known as the relative biological effectiveness (RBE). The RBE is defined as the ratio of the dose of a standard type of radiation to that of the test radiation that gives the same biological effect. The standard type of radiation was usually taken as 200- or 250-kilovolt (peak) X rays, but now Cobalt 60 γ-ray energies are used mainly as the standard for comparison. Their RBE relative to 250-kilovolt (peak) X rays is about 0.9. Because the shoulder of the survival curve is reduced for high-LET radiation, the RBE varies with the dose or the survival level at which it is determined.

Many different mathematical models have been used to produce equations that can fit survival-curve data within the limits of experimental error. Two of the more commonly used models are the old target-theory model and the newer linear-quadratic models of cell survival. The target-theory model of cell survival (SF) was based on the hypothesis that a number of critical targets had to be inactivated for cells to be killed. Cell killing by radiation is now recognized to be more complex, but the equation and parameters derived from the model are still used to describe the shape of cell survival curves (Figure 3.6).

$$SF = N/N_0 = 1 - (1-e^{-D/D_0})^n$$

Because survival curves often have an initial slope this equation is often modified to add a single-hit component (Figure 6).

$$SF = e^{-D/D_s}(1 - (1-e^{-D/D_0})^n)$$

The linear-quadratic model of cell kill is based on the idea that multiple lesions, induced by radiation, interact in the cell to cause cell killing. The lesions that interact could be caused by a single ionizing track, giving a direct dependence of cell killing on dose, or by two or more separate tracks, giving a dependence of lethality on higher powers of dose. The assumption that two lesions must interact to cause cell killing gives an equation that can fit most experimental survival curves quite adequately, at least over the first few decades of survival.

$$SF = N/N_0 = \exp -(\alpha D+\beta D^2)$$

N and N_0 represent the number of surviving cells and the number of starting cells respectively, D represents the radiation dose; while the parameters (D_0, n) represent the inverse slope and extrapolation number of the target theory equation (Figure 3.7), and the parameters (α and β) represent the probabilities that the lesions that interact to cause cell killing are produced by a single track or by two interacting tracks in the linear-quadratic

equation (Figure 3.7). Such mathematical models are useful when comparing cellular radiosensitivity among a variety of cell types or when the shape of the survival curve is altered following treatment with drugs or changes in the environment (e.g., hypoxia). From these models, it has been observed that there is greater variation in the low-dose or shoulder region of the radiation survival curves obtained for mammalian cells as compared to the variation in the slopes of the high-dose region of the curves.

Non-clonogenic assays have also been used to estimate the relative radiosensitivity of cells, although assays that measure short term growth or programmed cell death/apoptosis often do not correlate with the longer-term clonogenic assay. Assays for apoptosis may predict clonogenic survival within some cancer cell lines e.g., neuroblastoma, lymphoma, and testicular, as these cell types tend to die uniformly by apoptosis following irradiation. Assays that evaluate cellular growth for a short period (e.g., 24 to 48 h) following radiation include the MTT assay that determines cellular viability by colorimetric assessment of the reduction of a tetrazolium compound. They are of limited value for radiosensitivity studies because it is rarely possible to assess more than one decade of cell kill and they usually do not correlate with the clonogenic assay. At present, clonogenic survival remains the 'gold standard' for determining the radiosensitivity of cells in vitro. Methods have also been developed for assessing the ability of cells to form colonies in vivo. One of these is the spleen-colony method, which has been used to assess both the radiation and drug sensitivity of bone marrow stem cells. In this assay bone marrow from treated animals is injected into irradiated hosts and colonies from surviving bone marrow stem cells can be then counted in the spleen. Other colony-forming assays have been developed to study the radiation response of stem cells in situ in certain proliferative tissues, including skin, gastrointestinal tract, testis, cartilage, kidney, and certain tumours.

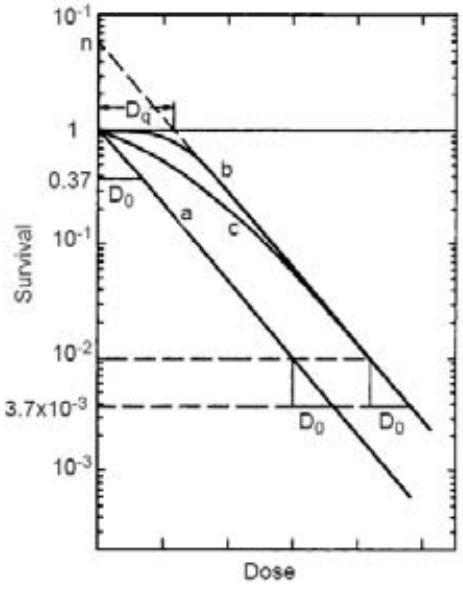

Fig. 3.6 Survival curves defined by the singlehit and multitarget models of cell killing. Curve a: *Single-hit (single-target) survival curve* Curve b: *Multitarget survival curve.* Curve c: *Composite (two-component) survival curve resulting from both multitarget and single-hit components. Also shown is how the parameters* D*0,* n*, and* Dq *can be derived from the survival curves. (Tannock et al., 2005)*

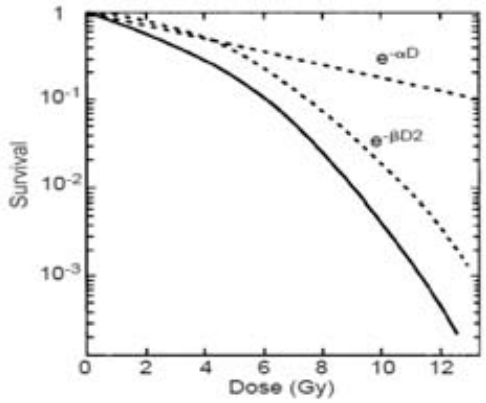

*Fig. 3.7 Survival curve (*solid line*) as defined by the linear-quadratic model of cell killing. The curves defined by the two components of the equation are shown separately as the dashed lines. (Tannock et al., 2005)*

3.9. Repair of radiation damage

The repair of cellular damage between radiation doses is the major mechanism underlying the clinical observation that a larger total dose can be tolerated when the radiation dose is fractionated. The shoulder of the survival curve reflects the accumulation of sublethal damage that can be repaired. When the interval between two fixed doses of radiation is varied, there is a rapid rise in survival as the interval is increased from zero (single dose) to 2-6 hours due to repair of ***sublethal damage*** (SLDR). Because cells that survive radiation tend to be synchronized in the more resistant phases of the cell cycle, their subsequent progression (inevitably into more sensitive phases) may lead to a small reduction in survival at 4-8 hrs before continued repair and repopulation increase survival at later times(12-24 hrs). This pattern of SLDR has been demonstrated for a wide range of cell lines and the timing of the secondary fall and subsequent rise is somewhat variable depending on the cell kinetics. The repair capacity of the cells of many tissues in vivo has been demonstrated using cell-survival and functional assays in vivo. An increase in total dose is required to give the same level of biological damage when a single dose (D_1) is split into two doses (total dose D_2) with a time interval between them. The difference in dose (D_2-D_1) is a measure of the repair by the cells in the tissue. The capacity of different cell populations to undergo SLDR is reflected by the width of the shoulder on their survival curve (D_q) or the (D_2-D_1) value. Survival curves for bone marrow cells or cells derived from the radiosensitive disorders AT (ataxia telangiectasia) and NBS (Nijmegen Breakage Syndrome) (or cells which lack DNA-repair enzymes) have no shoulder and demonstrate little or no evidence of cellular repair. Recent data suggest that AT and NBS cells may also have increased residual DNA double-strand breaks following irradiation, suggesting a defect in DNA DSB-repair. Other cells (e.g., jejunal crypt cells) can demonstrate a large repair capacity (D_2-D_1 value of 4-6 Gy).

To maximize SLDR capacity tissue or cells can be irradiated under low-dose rate conditions as in brachytherapy. The effect of radiation on tissues and cells for the same dose differs widely for exposure over a short time (acute irradiation) and for continuous irradiation over an extended period of time (irradiation given at a low-dose rate). Dose rates above about 1 Gy per minute can be regarded as acute (single-dose) treatment. As the total dose of x- or γ-rays is delivered at decreasing dose rates, the DNA damage in the cell (i.e., yield of chromosome aberrations and DNA-double strand breaks) progressively diminishes due to repair of the damage during the treatment. As a result, the shape of the radiation survival curve changes from one exhibiting pronounced curvature at high dose rates to one approaching linearity at

low-dose rates. Cell lines with a greater capacity to repair sublethal damage will demonstrate a large dose-sparing effect relative to those cells that have limited capacity to repair the damage. Most of the effect of cellular repair occurs in the range of dose rates of 1.0 to 0.01 Gy per minute. Below about 0.1 Gy per minute, the effects of cell cycle progression (redistribution and the G_2 block) become apparent; below about 0.01 Gy per minute, the effects of cell repopulation will start to become evident as the radiation damage is not severe enough to trigger cell cycle arrest in other phases of the cell cycle. At lower dose rates, the processes of repair and cellular repopulation within the cell culture predominate.

Cell survival can also be increased by holding cells after irradiation under conditions of suboptimal growth such as low temperature, nutrient deprivation, or high cell density. The latter conditions may reflect those experienced by G_0/G_1 populations of cells in growth deprived regions of tumours. The increased survival is due to the repair of ***potentially lethal damage*** (PLDR), which usually results in a change in the slope of the cell-survival curve. Such repair may contribute to increased radiation survival observed in vivo for some transplantable cell lines when compared to the radiosensitivity of the same cells growing in vitro.

Adaptive radiation responses and low-dose hyper-radiosensitivity*:** Following very low doses of radiation, mammalian cells may have an inducible radioprotective response that acts both in vitro and in vivo. This so-called adaptive response appears to be triggered by a threshold level of radiation damage. For example, some mammalian cells appear to be hypersensitive to doses of ionizing radiation in the range 0.01-0.3 Gy as compared with higher radiation doses. Following doses of radiation above about 1 Gy, this ***hyper-radiosensitivity (HRS) is not observed. The exact mechanism responsible for this effect is unclear and it does not seem to occur in all cells. An adaptive radiation response is observed in rodents for irradiated normal skin and kidney cells. For example, the use of multiple radiation fractions of less than 1 Gy can decrease the total dose required for the same biological effect in vivo by a factor of 2 to 4 (relative to that required with fractions of 2 Gy). It has been argued that differences in the radiosensitivity of human tumour cells might be explained in part by the variation in the adaptive response observed for different human tumour cell lines. However, a recent in vivo experimental study using ultrafractionated treatment of tumours, failed to demonstrate any evidence that HRS influenced sensitivity in vivo although effects on tumour cell proliferation during treatment may have influenced the result.

***Genomic and proteomic studies of irradiated cells*:** Cellular damage following ionizing radiation can affect the expression of a number of genes involved in the response of cells to stress. Some early-response genes, such as the early growth response factor (*EGR*-1) and *p21Waf1* cdk-inhibitor protein, contain radiation responsive regulatory domains in their promoter regions that can facilitate their rapid induction by ionizing radiation. These sequences have been proposed for use in radiation-induced gene-therapy vectors to drive expression of suicide genes within an irradiated field for tumour therapy. Synthetic enhancers of gene expression designed for use with radiation utilize short motifs of sequence CC(A/T)6GG (i.e., radiation-responsive CArG elements) derived from the EGR1 gene. Such constructs can be responsive to radiation at doses of 1 to 5 Gy. These tumour-targeting vectors might be used in clinical situations where the irradiation volume can be tightly controlled to spare normal tissues using conformal radiotherapy planning and have shown early promise in animal models.

Irradiation can also modify intracellular signaling through modification of the activity of tyrosine kinases, MAP-kinases, SAP-kinases, and ras-associated proteins. An example is the

activation of the c-abl pathway which phosphorylates Rad51, a DNA repair protein, at sites of DNA damage. Other genes induced by radiation include those encoding cell cycle related proteins [e.g., growth arrest after DNA damage *(GADD)* genes, *p34cdc2, cyclin B, p53*], growth factors, and cytokines [e.g., platelet-derived growth factor *(PDGF),* transforming growth factor beta *(TGF-β),* basic fibroblast growth factor *(bFGF)*], and enzymes (e.g., plasminogen activator]. Liberation of inflammatory cytokines such as *TGF-β,* tumour necrosis factor *(TNF-α)* and interleukin-1 (IL-1) by cells following radiation damage may lead to a continuing cascade of cytokine production, and may be responsible for the acute inflammation and late onset fibrosis observed in some irradiated tissues. Studies using cDNA microarrays have found that radiation-induced gene expression can be cell-type specific.

Cell cycle sensitivity and DNA damage checkpoints: Mammalian cells have evolved complex interrelated responses to DNA damage including cell cycle checkpoints, DNA repair, and apoptosis. Cell cycle checkpoints are sites of cell cycle arrest in the G_1, S, and G_2 phases that ensure successful and accurate DNA replication and repair prior to mitosis. Two general types of cell cycle checkpoints exist. The mitotic spindle assembly checkpoint is responsible for ensuring that the mitotic spindle is correctly formed prior to division. Additionally, there are DNA integrity checkpoints that delay the cell cycle in response to DNA damage or to defects in DNA replication (i.e., G_1 to S, intra-S, and G_2 to M checkpoints). Delaying cell cycle progression could allow for the repair of DNA damage in cells prior to undergoing DNA replication or mitosis and is thought to prevent genetic instability. Early kinetic studies reported a rapid decrease in the mitotic index in an irradiated cell population, as both lethally damaged and surviving cells ceased to enter mitosis, while cells already in mitosis continued their cell cycle progression. After a period of time, which depends on both the cell type and the radiation dose, surviving cells re-enter mitosis; this time is known as the mitotic delay. Mitotic delay appears to be due largely to a block of cell cycle progression in G_2 phase, although cells in G_1 and S phases are also delayed in their progression, albeit to a lesser extent. There is approximately 3–4 hours of G2 delay per 1 Gy in a diploid cell. Cells may continue to experience delays in their progression through the next and subsequent cell cycles.

As a result of radiation induced delays in the cell cycle and the fact that cells in different phases of the cell cycle have different radiosensitivities, cell populations can be partially synchronized by irradiation. If a single radiation dose is given to cells in different phases, then a pattern of cell survival as a function of cell cycle position is obtained. Many cell lines appear to have a resistant period in S phase and a sensitive period in G_2 phase following irradiation *in vitro*, however, some cell lines have different patterns of sensitivity throughout the cell cycle. Some oncogene-transfected cells (e.g., overexpressing the *ras* oncogene) show increased resistance in the G_2 phase, whereas other cells, including DNA repair-deficient cells, show similar sensitivity throughout all phases of the cell cycle. The pattern of radiosensitivity throughout the cell cycle can be different for the same tumour cells growing in vivo or in vitro, indicating the influence of cell-cell interactions on cell survival.

The ATM (ataxia telangiectasia mutated) protein plays a role in initiating checkpoint pathways in all three cell cycle phases. G_1 cell cycle arrest following irradiation depends on an intact ATM-p53/Cdc25A-Rb pathway and decreased activity of cyclin D and E complexes. This leads to continued hypophosphorylation of the Rb protein at the G_1/S interface and blocking of the initiation of DNA replication. Radiation-induced G_1 arrest is abrogated in cells that lack functional p53, ATM, or Rb proteins. Most data suggest that cells having altered p53 protein function (and an abrogated G_1 checkpoint) acquire relative radioresistance in comparison with those cells having normal p53 protein function. The radioresistant phenotype has been correlated with the level of expression of mutant p53 protein in

transformed cells. Acquired radioresistance may also result from the inactivation of normal p53 function by viral proteins such as the HPV-E6 protein, which can bind to and degrade the normal p53 protein. Cells lacking ATM function exhibit a defective G_2 checkpoint after irradiation. DNA repair activity has been detected during the radiation-induced G_2 delay and this checkpoint probably allows damaged chromosomes to be repaired prior to mitosis. Tumour cells often exhibit an aberrant G_1 cell cycle checkpoint while the G_2 cell cycle checkpoint remains intact. Drugs that abrogate the G_2 checkpoint (i.e., caffeine, methylxanthines, UCN-01) lead to the induction of premature mitosis and mitotic catastrophe in the treated cells. UCN-01 preferentially sensitizes p53-mutated, radioresistant tumour cells to ionizing radiation.

***Molecular repair of DNA damage*:** Data from a number of studies indicate that the base excision repair (BER) and DNA-dsb repair pathways are involved in repairing the majority of ionizing radiation-induced DNA damage. For DNA-dsb repair, the main pathways of repair include homologous recombination (HR), which is operational during S and G_2, and nonhomologous and joining (NHEJ), which is operational during G_1. There is no simple relationship between expression of DNA repair genes or proteins and the relative radiosensitivity among unselected normal or tumour cells. However, in defined cell models, DNA repair capacity can influence cellular radiosensitivity as indicated by the extreme radiosensitivity of cells from some patients with DNA repair deficiency syndromes such as Ataxia Telangiectasia and the Nijmegen breakage syndrome. Similarly, isogenic cells defective in the expression of the BRCA1 and BRCA2 proteins can have decreased HR-related repair of DNA-dsbs and decreased radiation cell survival. A reduced capacity for repair of DNA double-strand breaks is also observed among X ray–sensitive mutant Chinese hamster ovary (CHO) cells and among radiosensitive fibroblasts derived from severe combined immunodeficient mice (SCID) in which deficient NHEJ was correlated to a lack of DNA-PK_{CS} kinase expression. Indeed, mouse cells made deficient for NHEJ (i.e., mouse knockouts for DNA-PK_{CS} or Ku70 genes) have exquisite radiosensitivity and defective rejoining of DNA-DSBs. The understanding of the relationship between deficient DNA repair and radiosensitivity has led to strategies designed to radiosensitize tumour cells. In human fibroblasts, small silencing RNAs (siRNA) have been used to decrease endogenous DNA-PKcs or ATM expression and result in a reduced capacity for repair of radiation-induced chromosome breaks and an increased yield of acentric chromosome fragments. These chromosomal rearrangements are associated with increased radiation cell killing. Similarly, antisense RNA or specific pharmacological approaches have been used to ablate DNA repair protein expression with resulting radiosensitization. Inhibitors of DNA repair appear to be a promising area of development and may have clinical value if the repair of DNA-dsbs in tumour tissues is reduced preferentially to that in normal tissues following irradiation (i.e., improve the therapeutic ratio).

***Effects of oncogenes and tumour suppressor genes on radiation response*:** Aberrant expression of oncogenes or tumour suppressor genes may increase the intrinsic cellular radioresistance of human and rodent cells. For example, increased radiation survival has been observed in selected cell lines following the transfection of a single oncogene, such as activated *RAS, SRC*, or *RAF*. This has led to studies designed to radiosensitize tumour cells by the inhibition of oncogene function using inhibitors of intracellular signaling pathways or antisense techniques (or siRNA) to decrease oncogene overexpression. When the *ras* oncogene undergoes mutation, it is permanently activated in the GTP-bound signaling state, providing proliferative signals in the absence of growth factor ligands, leading to altered cell growth, transformation, and radioresistance. However, increased radioresistance is more commonly observed in cells transfected with an activated *ras* gene in combination with a

nuclear cooperating oncogene, such as *c-myc* or mutant *p53*. Inhibitors of *ras* protein prenylation or function (farnesyl transferase inhibitors) have been reported to enhance radiation-induced cytotoxicity among preclinical models of human breast, lung, colon, and bladder cancer cells expressing mutated *H* or *K-ras* genes. Improvements in *ras*-pathway specificity are required for future development of farnesyl transferase inhibitors, but early clinical studies have shown success with minimal toxicity using these drugs in combination with radiotherapy in the treatment of advanced lung, and head and neck cancers. Downstream to *ras*, the *raf-MEK-ERK* and phosphatidylinositol-3 kinase *(PI-3K)-Akt/PKB* pathways are two separate signaling pathways that have also been linked to tumour radioresistance. Using antisense oligonucleotides against human *RAF* increased radiosensitivity in a human squamous cancer cell line and inhibitors of *PI-3K* signaling, such as LY294002 and wortmannin, enhanced the response to radiation in lung, bladder, colon, breast, HNSCC, and cervical cancer cells. The target specificity of these agents remains a concern as they can also inhibit other important PI-3K related proteins (ATM, ATR, and DNA-PKcs) in normal tissues.

The tyrosine kinase activity of the epidermal growth factor receptor (EGFR) is increased following cellular exposure to ionizing radiation and addition of exogenous EGF to cells in culture renders them relatively radioresistant. Both EGFR and the related HER-2/neu receptor are overexpressed in a wide variety of epithelial tumours (head and neck squamous cell cancers (HNSCC), gliomas, breast, lung, colorectal, and prostate cancers) and this overexpression has been associated with poor clinical outcome following radiotherapy. Targeting EGF and HER-2/neu receptor signaling using monoclonal antibodies or specific inhibitors of EGFR or HER-2 leads to radiosensitization *in vitro* and *in vivo* and initial clinical studies are positive for inhibitors of EGFR in HNSCC.

3.10. Tumour biology and host/tumour interactions

***Dose response and tumour control relationships*:** The emphasis on the molecular and cellular effects of radiation treatment reflects the belief that the response of tumours can be understood largely in terms of the response of the cells within those tumours. Tumour response to radiation treatment can be assessed by techniques that do not measure tumour cell survival directly. One such endpoint is growth delay that is determined by measuring the size of untreated and irradiated tumours as a function of time to generate growth curves. The delay in growth is the difference in time for treated and untreated tumours to grow to a defined size. The time difference is a measure of tumour response and can be plotted as a function of radiation dose. The shape and position of this curve will be different for different treatments. If groups of animals receive different radiation doses to their tumours, the percentage of controlled tumours can be plotted as a function of dose to give a dose control curve (Figure 3.8).

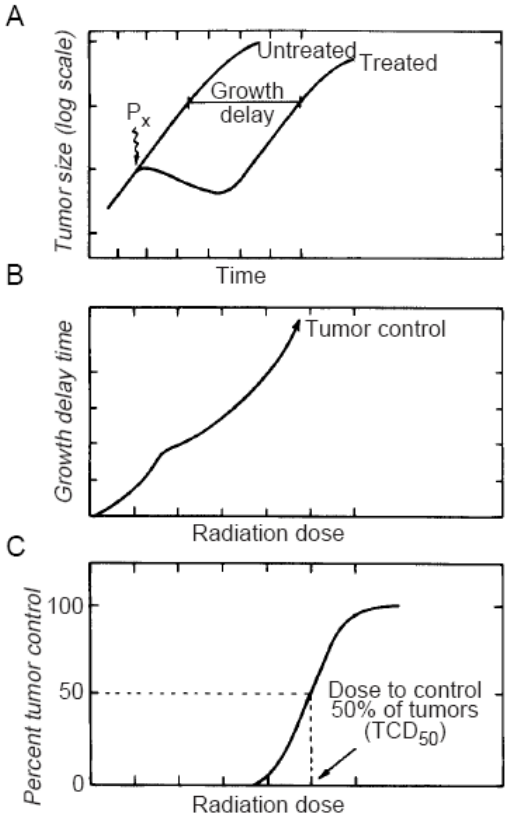

*Fig. 3.8 Illustration of two assays for tumor response: In (*A*), growth curves for groups of treated and untreated tumors are shown and the measurement of growth delay indicated. Growth delay is plotted as a function of radiation dose in (*B*). At large doses some of the tumors may not regrow and the percentage of controlled tumors can be plotted as a function of dose as in (*C*) (Tannock et al., 2005).*

Intrinsic to tumour growth is the concept that tumours contain a fraction of cells that have unlimited proliferative capacity (i.e., tumour stem cells). To achieve tumour control, all the tumour stem cells must be killed. For a simple model, which assumes that the response of a tumour to radiation depends on the individual responses of the cells within it, the dose of radiation required to control a tumour only depends on: (1) the radiation sensitivity of the stem cells and (2) their number. From a knowledge of the survival curve for the cells in a tumour, it is possible to predict the expected level of survival following a given single radiation dose. A simple calculation, using typical survival curve parameters for well-oxygenated cells (D_0 = 1.3 Gy, Dq = 2.1 Gy), indicates that a single radiation dose of 26 Gy might be expected to reduce the probability of survival of an individual cell to about 10^{-8}. For a tumour containing 10^8 stem cells, this dose would thus leave, on average, one surviving cell. (Note that a tumour containing $5x10^8$-10^9 total cells would be expected to have a volume of about $1cm^3$). Because of the random nature of radiation damage there will be statistical fluctuation around this value. The statistical fluctuation expected from random cell killing by radiation follows a Poisson distribution; the probability (P_n,) of a tumour having n surviving cells when the average number of cells surviving is *a* is given by:

$$P_n = (a^n e^{-a})/n!$$

For tumour control, the important parameter is P_0, which is the probability that a tumour will contain no surviving stem cells (i.e., $n = 0$). From the above equation $P_0 = e^{-a}$ so for $a = 1$, as

in the example above, the probability of control would be $e^{-1} = 0.37$. Different radiation doses will, of course, result in different values of *a*. For example, for identical tumours each containing 10^8 cells, a dose that reduces the survival level to 10^{-9} will give $a = 0.1$ (i.e., 10 cells surviving in 100 tumours) with an expected probability of control of $e^{-0.1} = 0.90$. From such calculations, it is possible to construct a theoretical tumour control versus dose curve, which shows a sigmoid relationship (Figure 3.9).

The above discussion assumes that the tumour stem cells exhibit uniform radiosensitivity within a tumour that reflects their radiosensitivity *in vitro*. However, the microenvironment of the cells in the tumour can significantly affect their sensitivity to radiation. This is well documented for hypoxia but there may also be interactions of the cells with the extracellular matrix (ECM) and/or interactions with growth factors. Interactions between the tumour cells and the ECM might also influence cellular signaling such as the EGFR/MEK/ERK pathway that can affect cellular sensitivity to radiation. There is increasing evidence that vascular damage and the induction of inflammatory cytokines play an important role in the responses of normal tissues to radiation treatment. The role that such factors may play in tumour response is largely unexplored. However, radiation-induced apoptosis of microvascular endothelial cells in the tumour might play a role in its response to radiation treatment.

The terms radiosensitive and radioresistant have often been used to describe, respectively, tumours that regress rapidly or slowly after radiation treatment. This can be misleading because the rate of regression may not correlate with the ability to cure a tumour with tolerable doses of radiation. A better term to describe a tumour that regresses rapidly after treatment is radio-responsive. The rate of response of a tumour depends on the proliferative rate of its cells because most tumour cells express their radiation damage when they attempt mitosis. Thus, a tumour that contains a large proportion of proliferating cells will tend to express radiation damage to its cells early and will regress rapidly. Although radioresponsive, the tumour may contain surviving stem cells that will be responsible for its recurrence.

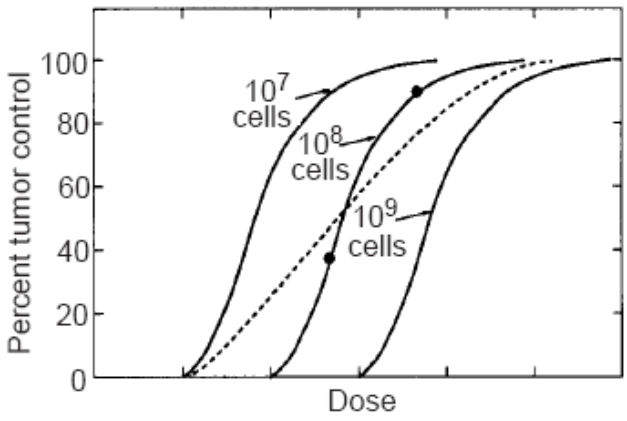

Fig. 3.9 Percentage tumor control plotted as a function of dose for single radiation treatments. Theoretical curves for groups of tumors containing different numbers of tumor stem cells are shown. The points on the curve labeled "10^8 cells" are derived as discussed in the text. The composite curve (dashed) was obtained for a group containing equal proportions from the three individual groups (Tannock et al., 2005).

***The oxygen effect and radiosensitivity*:** The biological effects of radiation on cells are enhanced by oxygen. There is some uncertainty about exact mechanisms but O_2 can interact with radicals formed by radiation, resulting in products which cause damage to DNA that is more difficult for the cell to repair. For this effect oxygen must be present in the cells at the

time of or within a few milliseconds of the radiation exposure. Cells irradiated in the presence of air are about three times more sensitive than cells irradiated under conditions of severe hypoxia. At very low levels of oxygen the cells are resistant but, as the level of oxygen increases, their sensitivity rises rapidly to almost maximal levels at oxygen concentrations above about 35 micromoles per liter (equivalent oxygen partial pressure 25 mmHg). The oxygen concentration at which the sensitizing effect is one half of maximum (the *K*m value) varies among cell lines but is usually in the region 5 to 17 micromoles per liter (3 to 10 mmHg equivalent partial pressure). The degree of sensitization afforded by oxygen is characterized by the oxygen enhancement ratio (OER), which is defined as the ratio of doses required to give the same biological effect in the absence or the presence of oxygen. For doses of *X*- or γ-radiation greater than about 3 Gy, the OER for a wide range of cell lines in vitro and for most tissues in vivo is in the range 2.5 to 3.3. For *X*- or γ -ray doses less than 3 Gy (i.e., in the shoulder region of the survival curve), the OER is reduced in a dose-dependent manner. A reduction of the OER at low doses is clinically important because the individual treatments of a fractionated course of radiation are usually 2 Gy or less. The OER is also dependent on the type of radiation, declining to a value of 1 for radiation with high LET values greater than about 200 keV/μm.

***Tumour Hypoxia*:** The cells in a tumour are influenced both by the microenvironment of solid tumours, which is characterized by regions of nutrient deprivation, low extracellular pH, high interstitial fluid pressure (IFP), and hypoxia. The oxygen concentration (pO_2) in most normal tissues ranges between 10 and 80 millimeters of mercury, depending on the tissue type, whereas tumours often contain regions where the pO_2 is less than 5 millimeters of mercury. These conditions in solid tumours are due primarily to the abnormal vasculature that develops during tumour angiogenesis. The blood vessels in solid tumours have highly irregular architecture, and may have an incomplete endothelial lining and basement membrane, which makes them more leaky than vessels in normal tissues. The leakiness of tumour blood vessels and a lack of functional lymphatic vessels is believed to be responsible for the increased IFP in tumours. A proportion of tumour cells may lie in hypoxic regions beyond the diffusion distance of oxygen where they are exposed to chronically low oxygen tensions. Tumour cells may also be exposed to shorter (often fluctuating) periods of (acute) hypoxia due to intermittent flow in individual blood vessels. Tumour hypoxia has been found to be heterogeneous both within and amongst tumours, even those of identical histopathological type, and it does not correlate simply with standard prognostic factors such as tumour size, stage, and grade. Studies with both extrinsic and intrinsic markers of hypoxia have shown that hypoxic cells can occur close to blood vessels, presumably due to fluctuation in blood flow in individual vessels resulting in regions of hypoxia for short periods of time (minutes to hours). Acute and chronic hypoxia can coexist in the same tumour and hypoxic regions in tumours are often diffusely distributed throughout the tumour and rarely concentrated only around a central core of necrosis.

Hypoxia may play an important role in treatment outcome for many tumour types due to radiation resistance but hypoxia can also affect the metastatic ability of some tumour cells. This latter effect is probably due to altered gene expression associated with exposure to hypoxia. The expression of as much as 1.5 percent of the genome may be modified by exposure to hypoxia. Many of these genes are involved in cellular functions such as anaerobic respiration and include glycolytic enzymes and cell membrane proteins such as glucose transporters (e.g., GLUT-1) and enzymes that control carbonate levels (e.g., carbonic anhydrase IX, CA-IX). Genes that modify the oxygen carrying capacity of blood (e.g., erythropoietin) or increase vascularity, such as the angiogenic growth factors like vascular endothelial growth factor (VEGF) are also upregulated, as are survival factors and invasive

factors. Many of the genes upregulated by hypoxia contain a hypoxia response element (HRE) in their promoter region that is responsive to the transcription factor, hypoxia-inducible factor 1 (HIF-1), a dimeric protein containing α and β subunits. HIF-1α is unstable under oxic conditions but is stabilized and expressed at increased levels in cells exposed to hypoxia. It is often overexpressed in tumours. Hypoxia-inducible factor 1 may also act in concert with other transcription factors to modify the expression of genes. Cells expressing activated oncogenes (such as *Ras* or *src*) demonstrate increased expression of angiogenic factors such as VEGF under hypoxic conditions and there is also evidence that signaling pathways such as the PI-3K/AkT or MEK-ERK pathways) can increase the expression of HIF-1 responsive genes by enhancing the transcriptional activity of HIF-1. While increases in HIF-1α during hypoxic exposure appear to occur in all cell types, these secondary effects are cell type specific.

Prolonged exposure to hypoxia can lead to cell death by apoptosis. Cells that have a mutated p53 gene have been found to acquire genetic resistance to hypoxia-mediated apoptosis. This suggests that hypoxia may promote tumour progression by selecting for cells with p53 mutations. Other studies suggest that the cells which are exposed to an hypoxic tumour environment are more likely to develop genomic instability and acquire mutant genotypes. There is also evidence that exposure to hypoxia may reduce the functionality of DNA repair proteins, such as MSH-2, that are involved in mismatch repair. These observations suggest that cells growing within hypoxic regions of tumours constitute an important target for cancer treatment. Evidence that cells in the hypoxic regions of tumours are viable and capable of regrowing the tumour is provided by analysis of cell survival curves. For most tumours the terminal slope of such curves is characteristic of that for hypoxia cells (Figure 3.10). The proportion of viable hypoxic cells in tumours can be estimated from the ratio (*S*air/*S*anox) of the cell survival obtained for tumours in air-breathing animals irradiated with a large dose to the cell survival obtained for tumours irradiated with the same dose under anoxic conditions (e.g., tumour blood supply clamped or animal killed prior to the irradiation). It is assumed that the tumours made deliberately anoxic contain 100 percent hypoxic cells and that the radiation survival curve for the naturally occurring hypoxic cells is the same as that for the cells made deliberately anoxic. Most tumours treated in air-breathing animals contain a proportion of hypoxic cells, in the range 10 to 20%. These proportions represent the cells that are maximally resistant to irradiation (radiobiologically hypoxic). There will also be a substantial proportion of cells in tumours which are at intermediate oxygen levels.

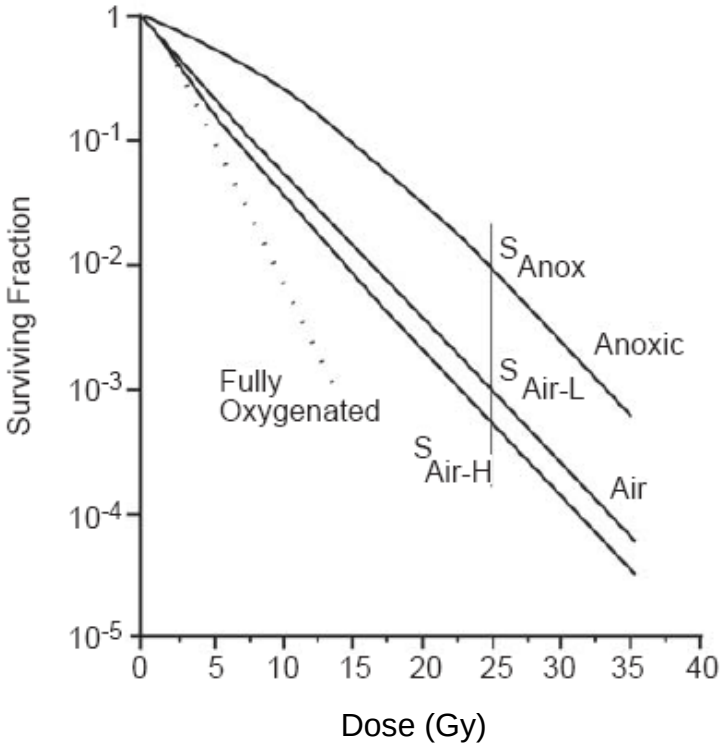

*Fig. 3.10 Effect of a subpopulation of hypoxic cells on the survival curve obtained for an irradiated tumor. The four curves shown are for a well-oxygenated population of cells (dotted line), two curves derived from tumors irradiated under air-breathing conditions (H and L) and a curve for tumors irradiated under anoxic conditions. The hypoxic fraction can be estimated by taking the ratio of the survival obtained under air-breathing conditions (**S**air) to that obtained under anoxic conditions (**S**anox) at a dose level where the survival curves are parallel, as illustrated. For the H curve this value is about 0.06 (6%) and for the L curve it is about 0.12 (12%) (Hill et al, 1971)*

The most commonly used method to measure pO_2 in human tumours has been polarographic oxygen electrodes (usually the Eppendorf oxygen electrode). These electrodes can measure microregional pO_2 (in a volume estimated to be equivalent to about 500 cells) in multiple locations giving a distribution of values. Measurements of tumour pO2 using this technology have revealed wide pO2 variations both within and between tumours. The oxygen electrode has the disadvantage that it is invasive and it is difficult to distinguish between measurements made in viable versus nonviable tissue regions. Studies with intrinsic markers of hypoxia (such as HIF-1α, GLUT-1, and CA-IX) have the advantage that they can be applied to existing tissue blocks for retrospective analysis of previous clinical studies. Increased levels of these markers have been associated with poorer treatment outcome in different tumour types. The most commonly used extrinsic markers are pimonidazole and EF-5, which have provided further evidence for substantial heterogeneity in hypoxia both within and between tumours. Further studies are required to establish whether these extrinsic markers will be reliable predictors of tumour hypoxia and treament outcome. The correlations between measured pO2 values, and/or between extrinsic and intrinsic markers have not been very consistent possible due to the large degree of heterogeneity in hypoxia observed in tumours.

***Increasing oxygen delivery to tumours*:** Because hypoxic cells represent a radiation-resistant subpopulation in tumours that is not present in most normal tissues, the therapeutic ratio might be improved by techniques to reduce the influence of hypoxic cells on tumour response. Clinical studies have demonstrated the negative effect of anemia on prognosis, and in many centres, blood transfusions are used to maintain patients at normal hemoglobin levels during treatment. A small randomized study in patients with carcinoma of the cervix showed improvement of local control with blood transfusions. However, there is little evidence that erythropoietin can improve local control or disease-free survival following radiotherapy. Experimental studies have suggested that low arterial oxygen tensions may also influence tumour response by affecting the level of hypoxia. Carbon monoxide in cigarette smoke reduces the oxygen carrying and unloading capacity of the blood and may result in reduced tumour oxygenation. Patients with head and neck cancer who continue to smoke during radiotherapy have been found to have decreased local control and survival after radiation treatment.

In earlier studies oxygen delivery to tumour cells was increased by giving patients oxygen under hyperbaric conditions (200 to 300 kPa) during radiation treatment. An increase in the dissolved oxygen concentration in blood plasma should result in greater diffusion of oxygen into the hypoxic regions. Clinical studies with HPO as an adjuvant to radiation therapy have demonstrated significant improvement in local tumour control and survival for patients with cancers of the head and neck and cervix but this has not been observed in the limited studies of tumours at other sites. Other recent strategies for improving tumour oxygenation include the use of a combination of nicotinamide, which has been shown to increase tumour perfusion, and carbogen (95% O2 and 5% CO2) breathing. This combination (called ARCON therapy) has been reported to improve outcome in head and neck cancers treated with radiation therapy. Paradoxically, there is evidence in animal tumour models that treatment with anti-angiogenesis agents can improve oxygenation in some tumours, possibly due to regularization of the vasculature. Studies combining such agents with radiation treatment of experimental tumours have indicated improved treatment response but it remains uncertain whether these improved responses are due to improved oxygenation or to factors such as direct tumour cell kill induced by the anti-angiogenesis treatment.

3.11. Radiobiology of normal tissue damage

Radiation treatment can cause loss of function in normal tissues. In renewal tissues, such as bone marrow or the gastrointestinal tract, loss of function may be correlated with loss of proliferative activity of stem cells. In other tissues, loss of function may occur through damage to more mature cells and/or through damage to supporting stroma and vasculature. For example, head and neck irradiation can lead to altered swallowing or a dry mouth (xerostomia), while irradiation of pelvic structures may lead to nausea or a change in bladder and bowel function. Whole body radiotherapy, which is sometimes given in addition to chemotherapy during bone marrow transplantation, can lead to nausea and vomiting, decreased blood counts, and altered humoral and cell-mediated immune responses.

Traditionally the effects of radiation treatment on normal tissues has been divided, based largely on functional and histopathological endpoints, into early (or acute) responses, which occur within a few weeks of radiation treatment, and late responses that may take many months or years to develop. Acute responses occur primarily in tissues with rapid cell renewal where cell division is required to maintain the function of the organ. These tissues are examples of what is known as the hierarchical model as they consist of a hierarchy of stem cells, proliferating, maturing cells and functional differentiated cells that are usually incapable

of further division. Because many cells express radiation damage during mitosis, there is early death and loss of proliferating, maturing cells killed by the radiation treatment. The lack of cells to feed into the functional compartment leads to reduced tissue function. Late responses tend to occur in organs whose parenchymal cells divide infrequently (e.g., liver or kidney) or rarely (e.g., central nervous system or muscle) under normal conditions. Depletion of the parenchymal cell population due to entry of cells into mitosis, with the resulting expression of radiation damage and cell death, will thus be slow. In tissues such as liver or thyroid the cells may have no strict compartments and in such 'flexible' tissues all cells including those in the functional compartment may divide to repair damage. Damage to the connective tissue and vasculature of the organ may lead to progressive impairment of its circulation. If the damage to the circulation is severe enough, secondary parenchymal cell death may occur due to nutrient deprivation. The loss of functional cells may induce other parenchymal cells to divide, causing further cell death as they express their radiation damage. In flexible tissue this may result in sudden onset of organ failure due to rapid loss of functional cells. Consequential late effects may also occur where severe early reactions have led to impaired tissue recovery and/or development of infection.

The radiosensitivity of the cells of a number of normal tissues can be determined directly using in situ assays. Considerable variability in sensitivity is apparent and as with tumour cells, most of the difference appears to be in the shoulder region of the survival curve. The crudest functional assay for normal tissue damage is the determination of the dose of radiation given either to the whole body or to a specific organ that will cause lethality in 50 percent of the treated animals within a specified time (LD_{50}). The relationship between lethality and single radiation dose is usually sigmoidal in shape. Dose-response relationships for normal tissues are generally quite steep and well defined. For study of the response of individual organs, one widely used approach is to define a level of functional deficit and to determine the percentage of irradiated animals that express at least this level of damage following different radiation doses. Such results have been reported for specific functional deficits in many tissues (e.g. increased breathing rate in lung, reduced flexibility due to increased fibrosis in subcutaneous tissue, induction of paresis in forelimbs following spinal cord irradiation). This approach also results in sigmoidal dose response curves.

Increased cytokine and chemokine expression has been observed within hours after irradiation when there are no apparent functional or histopathological changes, and may recur and/or persist in cycles over many months. This cyclic expression has been documented most clearly in lung and brain tissue. Early increases in cytokine expression can occur after low doses of radiation (~1 Gy) but longer term changes have been observed after larger doses (5 to 25 Gy). The cytokines involved include pro- and anti-inflammatory factors such as tumour necrosis factor (TNF-α), interleukin 1 (IL-1α and IL-1β), and transforming growth factor (TGF-β). In specific tissues they may include other growth factors that are associated with collagen deposition, fibrosis, inflammation, and aberrant vascular growth. These inflammatory factors may induce production of damaging radicals such as reactive oxygen species independently of those caused directly by the radiation treatment. The interplay between these various factors (cell killing, cytokine production, vascular damage) in producing the overall tissue damage remains poorly understood and is likely to vary from one organ to another.

3.11.1. Acute tissue responses

Acute radiation responses occur mainly in renewal tissues and have been related to death of critical cell populations such as the stem cells in the crypts of the small intestine, in the bone marrow, or in the basal layer of the skin. Responses in these tissues depend on the cell

kinetics of the particular tissue but usually occur within 3 months of the start of radiotherapy. They are not usually limiting for fractionated radiotherapy because of the ability of the tissue to undergo rapid repopulation to regenerate the parenchymal cell population and in the case of skin because with high energy beams the dose to the skin surface is less than that at a depth below the basal layer. Radiation-induced cell death in normal tissues generally occurs when the cells attempt mitosis, thus the tissue tends to respond on a time scale similar to the normal rate of loss of functional cells in that tissue and the demand for proliferation of the supporting stem cells. Radiation-induced apoptosis has also been detected in many cells and tissues, such as lymphoid, thymic, and hematopoietic cells, spermatogonia, and intestinal crypts. In lymphoid and myeloid tissue a substantial fraction of the functional cells can die by apoptosis and, thus, this mode of death plays an important role in the temporal response of these tissues to irradiation. In the crypts of the small bowel there is a small fraction of stem cells that die by apoptosis, but the majority dies a mitosis-linked death and the significance of radiation-induced apoptosis is unclear. Endothelial cells in the vasculature supporting the crypts and villi of the small intestine of mice have also been reported to be prone to radiation-induced apoptosis, but these reports are controversial. Those cells were reported to be protected by treatment of the animal with basic fibroblast growth factor. This treatment also protected the animals against radiation-induced gastrointestinal injury, suggesting that dysfunction of the vasculature can reduce the ability of the crypts to regenerate. Radiation-induced apoptosis in endothelial cells occurs via activation of the ceramide pathway rather than as a direct result of DNA damage, thus inhibition of this pathway might protect the gastrointestinal tract against radiation damage.

Skin: Following irradiation of skin, there is early erythema within a few days of irradiation and this is believed to be related to the release of 5-hydroxytryptamine by mast cells, increasing vascular permeability. Similar mechanisms may lead to the early nausea and vomiting observed following irradiation of the intestine. Expression of further acute skin reactions (erythema, moist desquamation and ulceration) depends on the relative rates of cell loss and cell proliferation of the basal cells in the epidermis (these cells mature and differentiate to produced the keratinized layers of the skin) and desquamation of the outer skin layers. In human skin this occurs starting at about 2 to 3 weeks into a course of fractionated radiation therapy. The extent of these reactions and the length of time for recovery depend on the dose received and the volume (area) of skin irradiated, because early recovery depends on the number of surviving basal cells that are needed to repopulate the tissue. Erythema in human skin occurs at single doses greater that about 6 Gy, while moist desquamation and ulceration occur after single doses of 20 to 25 Gy. Increased cytokine levels have also been observed in skin and plasma following large doses of irradiation, although their exact role in the observed radiation effects is unclear.

Oral mucosa: Oral mucosa has a similar cellular organization to skin but the lifespan of the differentiated cells is shorter so there is more rapid response to irradiation. The mucosal reactions in the mouth are a major factor limiting the daily and weekly dose accumulation during fractionated radiotherapy of Head-and-Neck Squamous Cell Carcinoma (HNSCC). Many patients may develop spotted-confluent mucositis when doses of 60-70 Gy are delivered in 2 Gy fractions over 6-7 weeks. Similar effects can occur in the oesophagus starting at about 2 weeks into fractionated radiotherapy.

3.11.2. Late tissue responses

Late tissue responses occur in organs whose parenchymal cells normally divide infrequently and hence do not express mitosis-linked death until later times when called upon to divide.

They also occur in tissues that manifest early reactions, such as skin/subcutaneous tissue and intestine, but the nature of these reactions (e.g. mucosal atrophy, vascular damage, chronic inflammation, subcutaneous fibrosis and intestinal stenosis) is quite different from the early reactions in these tissues. Late responses (usually regarded as those which occur more than 3 months after treatment) usually limit the dose of radiation that can be delivered to a patient during radiotherapy. The nature and timing of late reactions depends on the tissue involved and can be expressed as diminished organ function, for example, radiation-induced nephropathy (symptoms of hypertension, increased creatinine and blood urea nitrogen levels). However, one common late reaction is the slow development of tissue fibrosis that occurs in many tissues (e.g., subcutaneous tissue, muscle, lung, gastrointestinal tract), often a number of years after radiation treatment. Radiation-induced fibrosis appears to be associated with the aberrant and prolonged expression of the growth factor TGF-β following irradiation. This growth factor can stimulate proliferation of fibroblasts and their differentiation into fibrocytes that produce collagen. Transforming growth factor-β also plays a major role in wound healing and the development of late radiation reactions has similarities to the healing of chronic wounds. Apoptosis has also been observed within hours after irradiation of a number of late responding normal tissues in rodents, such as the salivary glands, pulmonary and brain endothelial cells and spinal cord. For example, in rat spinal cord it has been reported that endothelial cell apoptosis following irradiation initiates the disruption of the blood/spinal cord barrier, which may be an early lesion leading on to the development of white matter necrosis and myelitis. Apoptotic endpoints, however, have often not correlated with clonogenic survival or functional or histopathological endpoints, and the relevance of apoptosis in radiation-induced late normal tissue damage remains to be established.

The lung is an important site of late radiation damage and is one of the more radiosensitive organs in the body. There are two types of reactions, pneumonitis that occurs 2 to 6 months after irradiation and fibrosis which usually occurs more than 1 year after irradiation. These reactions can cause increases in tissue density on lung scans and increases in breathing rate if severe. Measuring changes in breathing rate has been used extensively to assay the dose-response relationship for radiation-induced lung damage in rats and mice, particularly the development of pneumonitis. Studies in rodents have documented that there is a rapid induction of inflammatory cytokines in lung after irradiations, but the relationship between this induction and the later development of functional symptoms is unclear. Studies in lung cancer patients have related prolonged increases in TGF-β levels in plasma following radiotherapy to the likelihood of developing lung fibrosis. In rodents, genetic factors can influence the development of pneumonitis and fibrosis following lung irradiation, although these factors do not affect the radiosensitivity of lung cells directly. Genetic factors may help to explain interpatient differences in response to lung irradiation. The dose required to cause a functional impairment in lung depends on the volume of (functional) lung irradiated, with small volumes being able to tolerate quite large doses. This effect is due to the functional reserve of the lung because imaging with CT scans or plane X rays films demonstrates that the irradiated region has sustained severe damage and will develop fibrosis. Studies in rodents, using the dose required causing an increased breathing frequency in 50% of animals (ED_{50}) as an endpoint, have defined a relationship between ED_{50} and volume irradiated which is not linear with dose and which indicates that the base of the lung is more sensitive than the apex. The underlying mechanisms may relate to the functional reserve in different regions of the lung and/or to the extent of cytokine production following irradiation of different regions of the lung. There is also (limited) evidence for regional effects following irradiation of human lung.

The heart is often irradiated during thoracic irradiation. Acute pericarditis can occur at times longer than 1 year after irradiation and is associated with chest pain and shortness of breath. Severe cardiomyopathy is characterized by dense and diffuse fibrosis and in general is a later condition developing over many years. The volume of heart irradiated plays an important role in the incidence of this complication and doses of 45-50 Gy (in 2 Gy fractions) to 50% of the heart will cause about 10% incidence of this condition. There is some evidence that irradiation of different regions of the heart may cause different severity of symptoms. Recent animal studies have suggested that heart irradiation may also impact on the severity of symptoms associated with lung irradiation both at early and late times.

The kidney is another very radiosensitive late-responding organ. Radiation damage to the kidney develops slowly and results in nephropathy with arterial hypertension, increased proteinurea (e.g.blood urea nitrogen and creatinine), and anaemia if both kidneys are treated with doses in the range of 30 Gy in 2 Gy fractions. Again because there is functional reserve partial irradiation of kidney can be given to higher doses. In contrast to many tissue there seems to be little regenerative response in kidney with the result that extending the treatment time does not allow for a larger dose to be tolerated. Mechanisms of damage in the kidney may relate to damage to the individual tubules but there is evidence that disturbed function of the renin-angiotensin system is also involved. Drugs which block increased activity of this system (ACE inhibitors of AT-II blockers) have been found to provide some protection in animal models. The tolerance of kidney is a particular concern for total body irradiation prior to bone marrow transplantation as is liver tolerance. Liver has a large functional capacity so that its tolerance increases markedly if only part of the organ is exposed. The cell turnover rate in liver is quite slow so that liver function does not deteriorate for a number of months but this process is progressive and if the whole liver is irradiated, doses greater than 30-35 Gy (2 Gy fractions) can lead to fatal hepatitis.

In ***the central nervous system (CNS),*** radiation reactions mostly occur at 6 months or later. At the early times demyelination may occur in the white matter leading to somnolence (brain irradiation) or parathesia (spinal cord irradiation) but these early effects are usually reversible and do not necessarily predict for the development of more serious late brain necrosis or myelopathy. At later times (1-2 years) more permanent demyelination and necrosis of the white matter is seen but damage may also be observed in the grey matter associated with vascular lesions. The risk of late effects is very dependent on dose per fraction with lower fraction sizes reducing the risk. However, repair of sublethal radiation damage in CNS is slow relative to most other tissues with a component which appears to have a half life of about 4 hrs. This means that multiple fractions per day must be widely spaced to maximize repair.

3.11.3. Whole body irradiation

The response of animals to single dose whole body irradiation can be divided into three separate syndromes (hematological, gastrointestinal, and neurovascular) that manifest following different doses and at different times after irradiation. The ***neurovascular syndrome*** occurs following large doses of radiation (>20 Gy) and usually results in rapid death (hours to days) due to cardiovascular and neurological dysfunction. The ***gastrointestinal syndrome*** occurs after doses greater than about 8 to 12 Gy and in rodents doses at the upper end of this range usually result in death at about 1 week after irradiation due to severe damage to the mucosal lining of the gastrointestinal tract; this causes a loss of the protective barrier with consequent infection, loss of electrolytes and fluid imbalance. Intensive nursing with antibiotics, fluid, and electrolyte replacement can prevent early death from this syndrome in human victims of radiation accidents, but these patients may die later

due to damage to other organs. The ***hematopoietic syndrome*** occurs at doses in the range of 2 to 8 Gy in humans (3 to 10 Gy in rodents) and is caused by severe depletion of blood elements due to killing of precursor cells in the bone marrow. This syndrome causes death in rodents (at the higher dose levels) between about 12 to 30 days after irradiation and somewhat later in larger animals, including humans. Death can sometimes be prevented by bone marrow transplantation (BMT) and cytokine therapy (e.g., GM-CSF, G-CSF, stem cell factor) provided that the radiation dose is not too high (<10 Gy) when damage to other organs (e.g., gastrointestinal tract) may become lethal. There are substantial differences in the doses required to induce death from the hematopoietic syndrome (i.e., LD_{50} value) between different species of animals and even between different strains of the same species. The LD_{50} value for humans has been estimated at 4 to 7 Gy depending on the available level of supportive care (excluding BMT). Following doses greater than about 2 Gy, humans will develop early nausea and vomiting within hours of irradiation (prodromal syndrome), which may be controlled with 5-hydroxytryptamine antagonists.

3.11.4. Retreatment tolerance

Although tissues may repair damage and regenerate after irradiation, previously irradiated tissues may have a reduced tolerance for subsequent radiation treatments, indicating the presence of residual injury. For early responding tissues there is almost complete recovery in a few months so that a second high dose of radiation can be tolerated. For late-responding tissues the extent of residual injury depends on the level of the initial damage and is tissue dependent. There is substantial recovery in skin, mucosa, spinal cord, and lung over a period of 3 to 6 months, but kidney, heart, and bladder show little evidence of recovery. Clinical studies have demonstrated that retreatment to high doses with curative intent is possible depending on the tissues involved but usually entails increased risk of normal tissue damage.

3.11.5. Volume effects

As discussed above, the volume of a normal organ that is irradiated often plays a significant role in its sensitivity to irradiation. The effect of volume can be considered in the context of the functional subunits of an organ (e.g. in kidney, the tubules; in the lung, the alveoli) and whether the organ has a ***'parallel'*** functional structure (e.g. lung, kidney or liver), where the different function subunits perform the same function, or a ***'serial'*** functional structure (e.g. spinal cord) in which the functional subunits must work together in series for tissue function. Thus tolerance doses change markedly for lung, liver or kidney, if different volumes are irradiated but if a relative small length (~ 20 cm) of the whole cross section of the spinal cord is irradiated to 50-55 Gy (2 Gy fractions) myelopathy may be observed and the tolerance dose changes little as the volume is reduced until it gets below about 5 cm. Recent animal studies suggest that irradiation of part of the cross section of the cord can result in an increase in the tolerance dose. For skin or mucosa, volume is important because depletion of basal stem cells over a larger area of the surface results in a greater requirement for the surviving basal cells to proliferate and migrate to effectively repopulate the whole area prior to desquamation of the outer layers of the organ. If ulceration occurs this may predispose to infection and the development of consequential late effects. Modern radiotherapy using intensity modulation techniques (IMRT) can reduce the volume of normal tissue in the high dose volume, which can lead to reduced toxicity particularly in parallel organs but the improved high dose distribution is often gained at the expensive of giving a lower dose to a larger volume of normal tissue. The impact of this increased volume receiving a lower dose is currently unknown but has raised concerns about possible second malignancies.

3.11.6. Therapeutic ratio (or index)

The therapeutic ratio is ill-defined numerically but the concept is that of a comparison between tumour control and normal tissue complications (Figure 3.11). Tumour-control curves tend to be shallower than those for normal tissue response because of heterogeneity. The therapeutic ratio is often defined as the percentage of tumour cures that are obtained at a given level of normal tissue complications (i.e., by taking a vertical cut through the two curves at a dose that is clinically acceptable, e.g., at 5% complications after 5 years, to give the *TD*5/5 value). An approach more in keeping with the definition of other ratios, such as relative biological effectiveness (RBE) and oxygen enhancement ratio (OER), is to define the therapeutic ratio in terms of the ratio of radiation doses *D*n/*D*t required to produce a given percentage of complications and tumour control (usually 50%). It is then a measure of the horizontal displacement between the two curves. It remains imprecise, however, because it depends on the shape of the dose-response curves for tumour control and normal tissue complications. The curves shown in the figure depict a situation in which the therapeutic ratio is favorable (A) because the tumour-control curve is displaced to the left of that for normal tissue damage. The greater this displacement, the more radiocurable is the tumour. Because the tumour control curve is shallower than that for normal tissue damage, the therapeutic ratio tends to be favorable only for low and intermediate tumour-control levels. If the two curves are close together (B) or the curve for tumour control is displaced to the right of that for complications, the therapeutic ratio is unfavorable because a high level of complications must be accepted to achieve even a minimal level of tumour control.

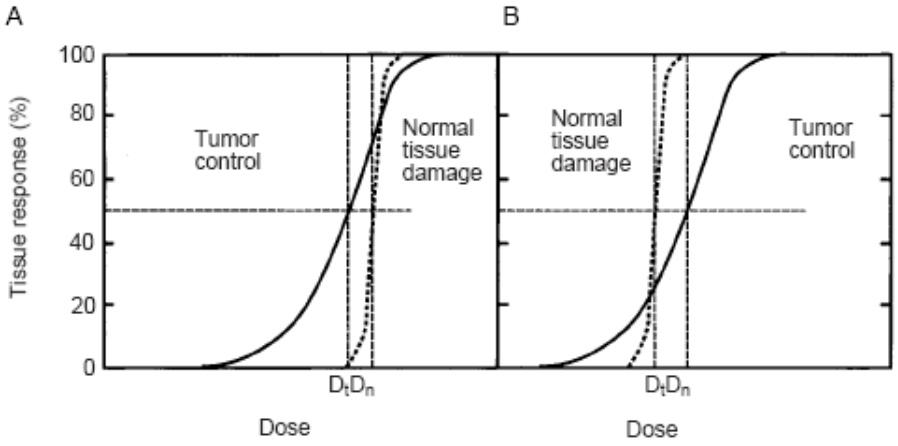

Fig. 3.11 Illustration of the concept of a therapeutic ratio in terms of dose-response relationships for tumour control and normal tissue damage. See the text for discussion of the two parts of the figure (Tannock et al., 2005).

3.12. Time-dose-fractionation

It is generally accepted that for conventional radiation therapy the overall patient outcome is improved by fractionating radiation treatments. Many of the underlying biological effects occurring during fractionated radiation treatment have been identified, and the improvement may be explained in terms of the biological response of tissue. The most important biological factors influencing the responses of tumours and normal tissues to fractionated treatment are often called the “four Rs”: repair, repopulation, redistribution, and reoxygenation. In recent years ‘radiosensitivity’ has been added to make 5 R’s, in order to allow for the differing radiosensitivity among normal cells, and among tumour cells in different individuals.

3.12.1. Repair

The shoulder on a survival curve after single radiation doses is indicative of the capacity of the cells to accumulate and repair radiation damage. If multiple doses are given with sufficient time between the fractions for repair to occur (4 to 24 hrs depending on the cells or tissue involved) the effective survival curves is straight on a semilogarithmic plot and has a shallower slope than the curve for big single doses. The effective slope depends on the size of the individual dose fractions, becoming shallower as the fraction size is reduced (Figure 3.12). This effect is also seen for irradiation of different tissues. The single dose survival curve for most cells has a finite initial slope apparently due to a (single-hit) non-repairable damage component (Figure 3.12), so there is a limit below which further reduction of the fraction size will no longer reduce the effective slope of the survival curve. At this limit, essentially all the repairable damage is being repaired between each fraction so that the cell killing is due almost entirely to non-repairable events. The fraction size at which this limit is reached is different for different cell populations depending on their repair capacity. When the size of the individual dose fractions is such that the survival is represented by the curvilinear shoulder region of the survival curve, as for most dose fractions used clinically, then repair will be maximal when equal-sized dose fractions are given. Repair kinetics have been estimated in a number of normal (rodent) tissues, and half-times for repair ranged from 0.5 hours in jejunum to 1 to 2 hours in skin, lung, and kidney. Thus, repair will be complete in most normal tissues after an interfraction interval of 6 to 8 hours. In the rodent spinal cord, it has been found that the effective repair halftime is greater than 2 hours (it appears to have two components with one component having a halftime of as much as 4 hrs), so repair is not complete even with an interfraction interval of 8 hours.

3.12.2. Repopulation

In both tumours and normal tissues, proliferation of surviving cells may occur *during the course* of fractionated treatment. Furthermore, as cellular damage and cell death occur during the course of the treatment, the tissue may respond with an increased rate of cell proliferation. The effect of this cell proliferation during treatment, known as repopulation or regeneration, will be to increase the number of cells during the course of the treatment and reduce the overall response to irradiation. This effect is most important in early-responding normal tissues (e.g., skin, gastrointestinal tract) or in tumours whose stem cells are capable of rapid proliferation; it will be of little consequence in late-responding, slowly proliferating tissues (e.g., kidney), which do not suffer much early cell death and hence do not produce an early proliferative response to the radiation treatment. Repopulation is important in reducing acute responses during prolonged treatments, such as those involving a period without irradiation (split-course treatment). Repopulation is likely to be more important toward the end of a course of treatment, when sufficient damage has accumulated (and cell death occurred) to induce a regenerative response. This appears to be true for tumours as well as for normal tissues. There is evidence that accelerated repopulation can occur in human tumours during the later part of a course of fractionated therapy. For HNSCC accelerated repopulation becomes apparent at 3 to 4 weeks after the start of the treatment. The data are consistent with an (accelerated) doubling time of about 4 days for the clonogenic tumour cells, compared to a median volume doubling time of about 2 to 4 months for unperturbed tumour growth. Repopulation of tumour cells during a conventional course of radiotherapy is believed to be an important factor influencing local tumour control in patients with head and neck or cervical cancer. It has been estimated that local control is reduced by approximately 0.5 percent for each day that overall treatment time is prolonged. Repopulation provides the biological

rationale for accelerating fractionated radiation therapy. Overall treatment time would be expected to be less important for slower-growing tumours such as prostate or breast cancer.

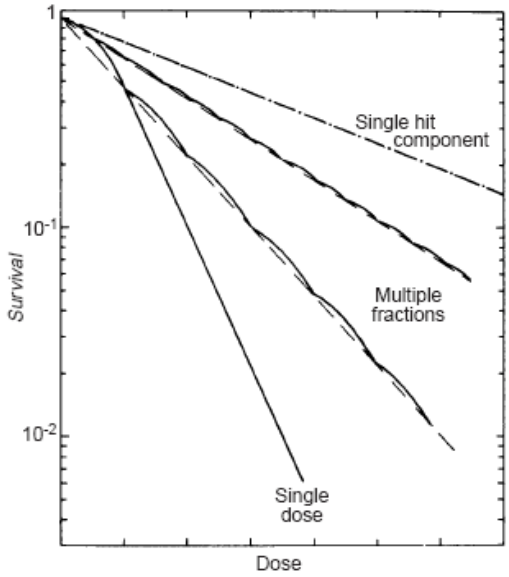

Fig. 3.12 The influence of fractionating the radiation treatment on the shape of cell survival curves. When repair occurs between the fractions, the shoulder of the survival curve is repeated for every fraction. The curve labeled "single-hit component" is discussed in the text. (Tannock et al., 2005)

3.12.3. Redistribution/recruitment

Variation in the radiosensitivity of cells in different phases of the cell cycle results in the cells in the more resistant phases being more likely to survive a dose of radiation. Two effects can make the cell population more sensitive to a subsequent dose of radiation. Some of the cells will be blocked in the G_2 phase of the cycle, which is usually a sensitive phase. Some of the surviving cells will redistribute into more sensitive parts of the cell cycle. Both effects will tend to make the whole population more sensitive to fractionated treatment as compared with a single dose. Because redistribution inevitably involves cell proliferation, the survival will also be influenced by repopulation, which reduces the effect of redistribution. Both redistribution and repopulation are important primarily in proliferating cell populations. Also, not all cell lines show large differences in radiosensitivity between cells in different cell cycle phases, and the effect of redistribution will be correspondingly less for these types of cells. In many normal tissues (and probably in some tumours), stem cells can be in a resting phase (G_0) but can be recruited into the cell cycle to repopulate the tissue. There is some evidence that cells in cycle are slightly more sensitive to radiation than G_0 cells, possibly because G_0 cells may repair more potentially lethal damage. Recruitment of resting cells into the proliferative cycle during the course of fractionated treatment, therefore, may tend to increase the sensitivity of the whole population. Neither recruitment nor redistribution would be expected to have much influence on late responses that occur predominantly as a result of injury to tissues in which the rate of proliferation is low.

3.12.4. Reoxygenation

The response of tumours to large single doses of radiation is dominated by the presence of hypoxic cells within them, even if only a very small fraction of the tumour stem cells are hypoxic. Immediately after a dose of radiation, the proportion of the *surviving* cells that is hypoxic will be elevated. However, with time, some of the surviving hypoxic cells may gain access to oxygen and hence become reoxygenated and more sensitive to a subsequent radiation treatment. Reoxygenation can result in a substantial increase in the sensitivity of tumours during fractionated treatment. Reoxygenation has been shown to occur in almost all rodent tumours that have been studied, but both the extent and timing of this reoxygenation are variable. Reoxygenation may result from increased or redistributed blood flow, reduced oxygen utilization by radiation-damaged cells, or rapid removal of radiation-damaged cells so that the hypoxic cells become closer to functional blood vessels. Measurements of the pO2 in human tumours (using Eppendorf oxygen electrodes) during fractionated radiotherapy have demonstrated improved oxygen status in some tumours, suggesting reoxygenation. However, these measurements do not distinguish between surviving cells and those already inactivated by the treatment. Although there is no direct evidence for reoxygenation of surviving hypoxic cells in human tumours, it is probable that it is a major reason why fractionating treatment leads to an improvement in therapeutic ratio (as compared to single large doses) in clinical radiotherapy. Evidence that the oxygen status of tumours can predict treatment outcome following radiation therapy suggests that reoxygenation is inadequate to eliminate the effects of hypoxia on treatment response for at least some tumours in man (e.g. HNSCC, cervix carcinoma).

3.12.5. Time and dose relationships

Repair and repopulation increase the total dose required to achieve a given level of biological damage (an isoeffect) in a course of fractionated radiation treatment. Redistribution and reoxygenation would be expected to reduce the total dose required for the isoeffect. Reoxygenation applies mostly to tumours (because they contain hypoxic cells), while repopulation and redistribution apply both to tumours and proliferating normal tissues. Repair is an important factor in the response of nearly all tissues. It is often difficult to dissect the influence of the individual factors but experimental studies suggest that repair of sublethal damage between fractions is more important than repopulation, certainly over the first few weeks of course of treatment. As the fractionated treatment is prolonged to longer times, the contribution of repopulation becomes greater.

The fact that the biological effect of radiation depends on the fractionation schedule has important implications for the planning of radiation therapy. To obtain the maximum dose to a tumour while minimizing dose to surrounding normal tissue, the radiation oncologist will often use a number of overlapping radiation beams. The dose at any given location will be calculated by summing the doses given by the various individual beams, and the dose distribution will be represented by a series of isodose curves (like contours on a map) joining points that are expected to receive equal percentages of the dose at a particular point (usually within the tumour). These isodose lines must be viewed with caution because the same total dose may not give the same biological effect if the doses delivered by the individual beams are of unequal size and they are not given in close temporal sequence. For example, equal-sized dose fractions allow for maximum repair; thus, if different beams are delivered on different days, the surrounding normal tissues that receive unequal contributions from different beams would have less optimal repair capacity than the tumour where the contributions from the different beams are equal. The biological effect would then be different

at different points on the same isodose line. This provides the radiobiological rationale for treating all fields daily when multiple fields are used to treat a tumour.

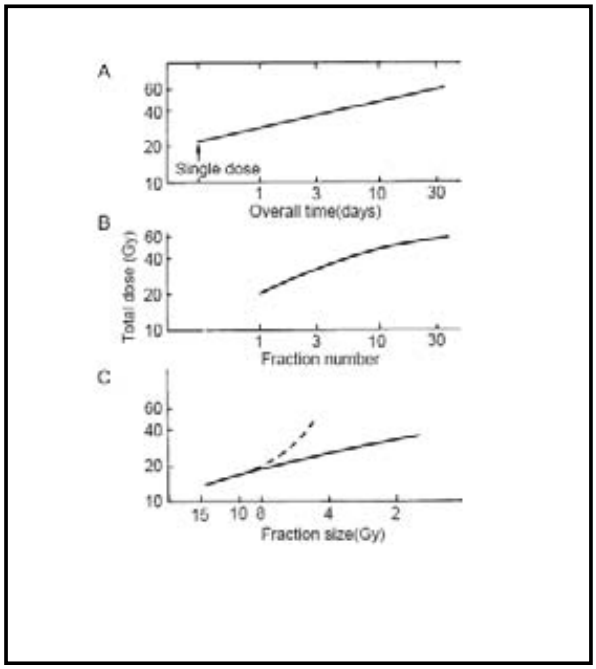

*Fig. 3.13 Isoeffect curves for fractionated treatments plotted in three different formats. (*A*) Line plotted by Strandqvist to define normal tissue tolerance and control of carcinoma of the skin and lip using the axes of total dose and overall treatment time. (*B*) Isoeffect curve for damage to pig skin plotted as total dose versus number of fractions. (*C*) Isoeffect curve for the crypt cells of the mouse intestine plotted as total dose versus fraction size using an inverted scale. The solid line is for fractions given 3 hours apart and the broken line for fractions given 24 hours apart (Tannock et al., 2005).*

3.12.6. Isoeffect curves

Different fractionation schedules that give the same level of biological effect can be presented in the form of an isoeffect curve. Isoeffect curves are generated by plotting the total radiation dose to give a certain biological effect against the overall treatment time, fraction number, or fraction size (Figure 3.13). Experimental studies performed mainly in rodents have established isoeffect curves for different normal tissues using endpoints of either early or late radiation damage. The isoeffect lines for late responses tend to be steeper than those for early responses (i.e. a larger increase in total dose is required to give the same level of late toxicity as the dose per fraction is reduced and the number of fractions increased). This implies a greater capacity for the repair of damage in tissues where it is expressed late than for damage in tissues where it is expressed early after radiation treatment. The reasons for this difference remain unknown. The observation that late-responding normal tissues demonstrate greater repair capacity than early responding normal tissues is a fundamental radiobiological principle underlying altered fractionation schedules using multiple daily fractions in clinical radiotherapy.

3.12.7. The linear quadratic equation and models for isoeffect

Most isoeffect relationship used clinically are based on the linear-quadratic (LQ) equation.

$$SF = N/N_0 = \exp -(\alpha D+\beta D^2)$$

In using the LQ model, it is assumed that each fraction has an equal effect, thus for a fractionated regime (n fractions of size d):

$$SF = [\exp -(\alpha d+\beta d^2)]^n \text{ or } -\ln SF = n(\alpha d+\beta d^2)$$

It is further assumed that if different fractionation regimes (e.g., n_1 fractions of size d_1 and n_2 fractions of size d_2) are isoeffective for a given tissue, they lead to the same surviving fraction (SF). Thus we have:

$$\text{Isoeffect } (E) = -\ln SF = n_1(\alpha d_1+\beta d_1^2) = n_2(\alpha d_2+\beta d_2^2)$$

which can be simplified to give:

$$n_1 d_1/n_2 d_2 = (\alpha+\beta d_2)/(\alpha+\beta d_1) = (\alpha/\beta +d_2)/(\alpha/\beta+d_1)$$

From this relationship and knowing the values of n_1, d_1, n_2, and d_2, the constant α/β can be determined for the particular tissue and used in the equation to predict other isoeffective treatment schedules. The parameter α/β has the units of dose (Gy) and is a measure of the shape of the survival curve. The parameter α defines the initial slope of the survival curve; the larger the value of α, the steeper the initial part of the curve. The parameter β defines the curvature of the survival curve and a large value of β implies more curvature. Thus, a large value of α/β implies a steep curve with little curvature (i.e., a small shoulder to the survival curve) and a small value of α/β implies a shallow curve with greater curvature (i.e., a large shoulder to the survival curve). Because the size of the shoulder of the survival curve is a measure of the repair capacity of the cells, a small value of α/β is consistent with greater repair capacity and a steep isoeffect curve, whereas a large value of α/β is consistent with lesser repair capacity and a shallow isoeffect curve.

Derived α/β values for different normal tissues in rodents suggest that late-responding tissues have values in the range 2 to 4 Gy (i.e., consistent with a steep isoeffect curve), while early-responding tissues have values in the range 8 to 12 Gy (i.e., consistent with a shallow isoeffect curve). The limited data available for human tissues suggest values in the same ranges. Most tumours appear to have α/β values similar to or greater than those for early-responding tissues, although recent data suggest that certain slow growing tumours (e.g. prostate cancer) may have lower α/β values between 1 and 3 Gy. There is no consideration of the effect of treatment time in the LQ model. In practice, this is a limitation that applies to early normal tissue responses, which occur in proliferative tissues (and tumours), rather than to late normal tissue responses, which generally occur in tissues that have slowly proliferating parenchymal cell populations, and for which the response to radiation is less influenced by the duration of fractionated treatment. In the LQ model, it is also assumed that there is complete repair between the fractions, and predictions from the model may lead to serious overdosing when the interfraction interval is too short or where repair of sublethal damage is slow (e.g. as in spinal cord).

3.12.8. Altered fractionation schedules

The higher capacity for repair of radiation damage in late-responding normal tissues (low α/β values) as compared with early-responding normal tissues and most tumours (high α/β values) can be exploited to obtain a therapeutic gain by reducing the fraction size below that used conventionally (from about 2 Gy to 1.5 Gy) and increasing the number of fractions. The increase in dose that can be tolerated at the isoeffective level of late normal tissue damage should be greater than that required to maintain the same level of tumour control (i.e., the tumour would receive a larger biologically effective dose and hence the control rate should be higher). The larger number of fractions required must be given more than once per day if the treatment time is not to be prolonged. Such a treatment protocol is termed ***hyperfractionation***. The intent of hyperfractionation is to reduce late effects while achieving the same or better tumour control and the same or slightly increased early effects. The time interval between the fractions must be sufficiently long to allow time for complete repair to occur. An increase in late morbidity would be expected when multiple fractions per day are given to fields that include the spinal cord, as has been observed in patients given three fractions per day; Continuous Hyperfractionated Accelerated Radiotherapy; (CHART regime- see below).

An increase in early normal tissue reactions would be expected with hyperfractionation because the larger α/β value for early-responding tissues implies a smaller change in the amount of repair as fraction size is reduced relative to that occurring in late-responding tissues. The increase in dose that can be tolerated can be estimated, but such calculations are limited by the low reliability of available estimates of α/β for human tissues. The rationale for hyperfractionation does not consider reoxygenation. Because there is no change in overall treatment time, it is assumed that reoxygenation will not be much different than for a conventional fractionation scheme. Clinical trials evaluating a larger total dose delivered by hyperfractionation have reported an increase in local control with no difference in late normal tissue damage. These results support the hypothesis that an increase of total dose can be achieved by hyperfractionation without increasing the probability of late complications.

Shortening of the overall treatment time might also improve the therapeutic ratio because it will reduce the time for repopulation to occur in the tumour during treatment. A similar effect might be achieved by blocking growth factors or their receptors, which are required for tumour cell proliferation. The tolerance of late-responding normal tissues should be little affected because cell proliferation is slow within them. Reduced treatment time is achieved by giving more than one fraction per day with standard dose fractions of 1.8 to 2.5 Gy given 6 to 8 hours apart to allow for repair, a strategy called accelerated fractionation. Randomized trials of accelerated fractionation compared to conventional fractionation for treatment of head and neck cancer have provided evidence supporting the importance of repopulation as a cause of treatment failure. A combined hyperfractionated accelerated radiation therapy (CHART) study (3 fractions per day for 12 days) gave a reduced dose in the experimental arm of the study but maintained the same tumour control level, with a slight reduction in late morbidity. A second study, which gave a similar total dose in both arms, reported increased tumour control in the accelerated fractionation arm, but there was also increased late toxicity. This increased toxicity was likely due to the short (4hr) interfraction interval, which was probably not sufficient to allow for complete repair between the fractions so that severe early reactions may have led to consequential late effects.

3.13. Predictive assays

3.13.1. Predicting the response of tumours

***Radiosensitivity*:** The knowledge of biological factors that influence the response of tissues and tumours to fractionated irradiation has led to interest in prediction of treatment outcome for individual patients based on assays that assess intrinsic radiation sensitivity of tumour and normal cells, the proliferative capacity of the tumour cells and the extent of tumour hypoxia. Even tumours of the same size and histopathological type are likely to vary in their proportion of stem cells. Thus, a dose-control curve for a group of human tumours will be a composite and the slope of the composite dose-control curve and will be less than that for the individual tumours. Fractionation of the radiation treatment and heterogeneity in the radiosensitivity of tumour stem cells will also result in a decrease in the slope of the dose-control curve. Thus, the slope of the dose-control curve derived from a clinical study is likely to be shallow due to tumour heterogeneity. It is therefore desirable to seek a way of assigning the tumours to more homogeneous groups, so that patients with differences in prognosis can be identified. This is a major motivation for attempts to develop predictive assays of radiosensitivity.

Studies of a wide range of cell lines derived from human tumours have shown intrinsic variations in radiation sensitivity (Table 4, Chapter 1). Survival curves can vary considerably even for cells of similar histopathological types and it is the shoulder of the curves that varies most widely. Even small differences in the shoulder region can be important because they are magnified during the multiple fractionated daily doses of 1.8 to 2 Gy given in clinical radiotherapy. Consider a tumour for which survival following a dose of 2 Gy is 0.8. Assuming that each fraction of a multiple-dose treatment is equally effective, and that there is no cell proliferation between dose fractions, the survival following thirty fractions of 2 Gy would be $(0.8)^{30} = 10^{-3}$. In contrast, for a tumour in which the survival following 2 Gy is 0.6, survival after 30 fractions would be $(0.6)^{30} = 2 \times 10^{-7}$. Thus, small differences in survival at low doses can translate into very large differences during a course of fractionated treatment. Estimates of the surviving fraction following a dose of 2 Gy for different human tumour cell lines growing in culture may be grouped according to histopathological type and compared with the likelihood that such tumours will be controlled by radiation treatment (Table 2.4,). There is a trend toward higher levels of survival at 2 Gy for the cells from tumour groups expected to be less radiocurable.

The concept that tumour response for an individual patient can be predicted has been tested using the survival following 2 Gy of radiation (or another parameter that reflects radiosensitivity at clinically relevant low doses) to predict for the outcome of fractionated radiotherapy treatment. Using a clonogenic assay for cells from primary human cervix Ca or HNSCC biopsies grown in soft agar it was found that patients with tumours containing radioresistant cells (SF2 > median) had significantly worse local control and survival than those with more tumours containing radiosensitive cells (SF2 < median). However, these results have been difficult to reproduce and the widespread application of such assays is limited by technical problems. Other potential limitations of such assays are: (1) they do not account for microenvironmental factors influencing radiosensitivity in vivo; (2) tumours may contain clonogenic subpopulations of different intrinsic radiosensitivity; (3) the assay may not be measuring the radiosensitivity of the stem cells in vivo. Other proposed measures of radiosensitivity (e.g DNA repair, micronucleus formation, levels of radiation induced apoptosis or reduced growth delay) have been similarly inefficient in predicting tumour response. It is hoped that the genomics/proteomics revolution will provide better signatures of radiation sensitivity.

***Proliferation*:** The potential doubling time (T_{pot}) is a measure of the rate at which new tumour cells are added to the tumour cell population and, as discussed above, values vary quite widely for human tumours. The median for HNSCC is in the range 4 to 5 days. The pretreatment T_{pot} has been suggested as a measure of the proliferative rate of the surviving tumour cells following radiotherapy and has been evaluated as a predictive assay. A trend for an adverse treatment outcome associated with short T_{pot} has been reported in patients with head and neck cancer and cervical cancer and T_{pot} was initially thought to predict for the repopulation potential of tumours during therapy. However, subsequent studies have not confirmed its utility, and the development of better assays of tumour cell proliferation during therapy is required.

***Hypoxia*:** Measurements of pO2 in human tumours using the Eppendorf oxygen electrode have revealed wide pO2 variations both within and between tumours. Results from clinical studies in cervix Ca HNSCC and NSCLC treated by radiotherapy or radiotherapy and chemotherapy indicate that hypoxic tumours (median pO2 value ~5 to 10 mm Hg) have a worse prognosis both in terms of disease-free and overall survival. Similar results have been obtained for soft tissue sarcoma in smaller studies. The data suggest that the hypoxia measurements can predict for distant metastases as well as local failure. Studies with intrinsic or extrinsic markers of hypoxia have also reported that increased levels of these markers have been associated with poorer treatment outcome in different tumour types but further studies are required to establish whether these markers will be reliable predictors of treatment outcome. The correlations between measured pO2 values, and/or between extrinsic and intrinsic markers have not been very consistent to date. Thus only measurements of tumour hypoxia using polarographic probes have demonstrated sufficient predictive power at present to be useful in planning cancer treatments, although such measurements have not been used clinically to modify treatment strategies.

3.13.2. Predicting normal tissue response

Patients receiving identical radiation treatments may experience differing levels of normal tissue injury; thus predictive assays might be useful in identifying those patients at greater risk of experiencing the side effects of radiotherapy. The enhanced radiosensitivity of patients with ataxia telangiectasia (AT) and Nijmegen breakage syndrome (NBS) supports a genetic contribution to individual variability in radiosensitivity. Studies of breast cancer patients have also shown individual correlation of acute and late skin reactions in one treatment field with those in a different treatment field. Several studies have quantitated the in vitro radiosensitivity of fibroblasts and peripheral lymphocytes as a potential predictive assay for normal tissue damage. These studies have shown variations in the radiosensitivity of fibroblasts from individual patients, but have been inconsistent in predicting late radiation fibrosis. While large differences in radiosensitivity, such as those observed in AT patients, are sufficient to cause discernable differences in late normal tissue effects, the differences in radiosensitivity of normal cells between most patients appear not be sufficient to override the effects of the other factors, such as cytokine induction and the response of the tissue stroma and vasculature, that also influence the development of normal tissue damage. Limited studies examining the expression of cytokines (e.g. TGF-β) following irradiation have also suggested that such measurements might be predictive for normal tissue toxicity in organs such as lung. However, these studies have not yet provide sufficient information to allow the development of a robust predictive assay. Current studies are investigating whether the evaluation of the expression of multiple genes in tumours using DNA microarrays or SNPs in specific genes might lead to better predictive assays.

3.14. Combined radiation and drug treatments

Therapeutic ratio (or index): Patients are treated frequently with drugs and radiation therapy. When two or more agents are combined to give an improvement in the therapeutic index, this implies that the increase in toxicity to critical normal tissues is less than the increase in damage to tumour cells. Because the dose limiting toxicity to normal tissues may vary for different drugs and for radiation, two agents may often be combined with only minimal reduction in doses as compared with those that would be used if either agent were given alone. Additive effects against a tumour with less than additive toxicity for normal tissue may then lead to a therapeutic advantage. Mechanisms by which different agents may give therapeutic benefit when used in combination have been classified as follows: (1) independent toxicity; (2) spatial cooperation, whereby disease that is missed by one agent (e.g., local radiotherapy) may be treated by another (e.g., chemotherapy); (3) protection of normal tissues; and (4) enhancement of tumour response.The above mechanisms suggest guidelines for choosing drugs that might be given in combination. Most drugs exert dose-limiting toxicity for the bone marrow, but this is not the case for vincristine (dose-limiting neurotoxicity), cisplatin (nephrotoxicity), or bleomycin (mucositis and lung toxicity). Thus many drugs can be given in combination with radiation without overlapping toxicity. Exceptions include doxorubicin and irradiation of the heart and bleomycin and irradiation of the lung. As new targeted drugs are introduced into cancer therapy it will be important to assess their toxicity when combined with radiation particularly for patients being given curative doses which generate normal tissue effects which are close to tolerance levels.

Synergy and additivity: Isobologram analysis: Claims are made frequently that two agents are synergistic, implying that the two agents given together are more effective than would be expected from their individual activities. Confusion has arisen because of disagreement as to what constitutes an expected level of effect (additivity) when two non-interacting agents are combined. Usually there is a range of possible additivity and an appropriate definition must take into account the dose-effect relationship for each agent used alone rather than a simple summation or multiplication of individual effects. The use of multiple agents may lead to an increase in the therapeutic index, but it is rare that a claim for synergy of effects against a single population of cells can be substantiated. The concepts of synergy and additivity between two agents can be understood by considering the level of cell survival after treatment of a single population of cells, either in a tumour or in a normal tissue. Isobologram analysis provides a method for defining the range of additivity (Figure 3.14). Dose-response curves are first generated for each agent used alone. These dose-response curves are then used to generate isoeffect plots (known as ***isobolograms***). These curves relate the dose of agent A to the dose of agent B that would be predicted, when used in combination, to give a constant level of biological effect (e.g., cell survival) for the assumptions of (1) independent damage and (2) overlapping damage. These curves define an envelope of additivity. If, when the two agents are given together, the doses required to give the same level of biological effect lie within the envelope, the interaction is said to be ***additive.*** If they lie between the lower isobologram and the axes (i.e., the combined effect is caused by lower doses of the two agents than predicted) the interaction is ***supra-additive*** or synergistic. If the required doses of the two agents in combination lie above the envelope of additivity (i.e., the effect is caused by higher doses than predicted), the interaction is ***sub-additive*** or antagonistic. Demonstration that two or more agents have a supra-additive or synergistic interaction has been used as a rationale for their inclusion in clinical protocols. This rationale is valid only if the interaction leads to a greater effect against the tumour as compared with that against limiting normal tissues (i.e., if it leads to an improvement in therapeutic index). It is theoretically possible that antagonistic agents (subadditive interaction) could improve therapeutic index provided that there was

greater antagonism of toxic effects for normal tissues as compared to toxicity for the tumour, or they have non-overlapping toxicities.

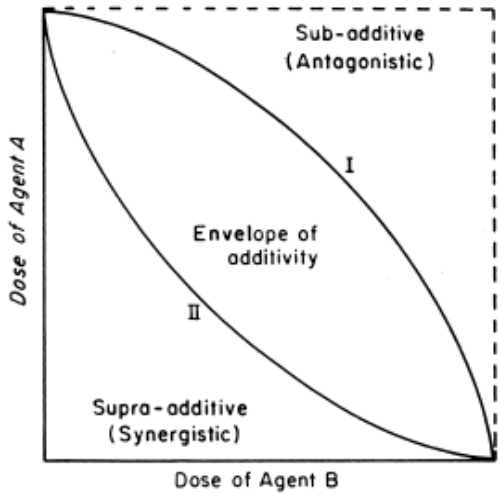

Fig. 3.14 Isobologram relating to the doses of two agents that would be expected to give a constant level of biologic effect when used together. It was generated from dose-response curves for each agent separately. Assumptions about overlap or nonoverlap of damage (Fig. 17.9) lead to the generation of two isobologram curves (I and II) that describe an envelope of additive interaction. Experimental data falling outside this envelope may indicate synergistic or antagonistic interactions, as shown. (Tannock et al., 2005)

Drugs and radiation: Many patients receive treatment with both drugs and radiation, and there is increasing evidence that concurrent treatment with radiation and drugs such as cisplatin leads to improvement in therapeutic index in a variety of cancer sites such as the head and neck and uterine cervix. Mechanisms of interaction between drugs and radiation at the cellular level may be evaluated from cell survival curves for radiation obtained in the presence or absence of the drug. Drugs may influence the radiation survival curve in at least three ways: (1) the curve may be displaced downward by the amount of cell kill caused by the drug alone; (2) the shoulder on the radiation survival curve may be lost, suggesting an inability to repair radiation damage in the presence of the drug; and (3) the slope of the exponential part of the radiation survival curve may be changed, indicating sensitization or protection by the drug. Most drugs influence survival curves according to the first two patterns; this corresponds to the limits of additivity, where sublethal damage may be independent or overlapping. The third pattern, leading to a change in slope of the dose response curve, defines agents that are radiation sensitizers or protectors. Sensitization of this type has been reported inconsistently for cisplatin and for prolonged exposure to 5-FU after radiation. Cisplatin and radiation is a widely used combination treatment treatment for a variety of tumours including HNSCC or Cervix Ca. Improvement in therapeutic index from use of drugs and radiation requires selective effects to increase damage to tumour cells as compared to those in normal tissues. One mechanism by which combined treatment with radiation and drugs leads to therapeutic advantage arises when radiation is used to provide effective treatment for sites of bulky disease (usually the primary tumour), and drugs are used to treat metastatic sites containing smaller numbers of cells. This spatial cooperation requires no interaction of the two modalities but involves different dose-limiting toxicities. The combined use of radiation and drugs might be used to obtain therapeutic advantage for treatment of a primary tumour if the combined effect of the treatment is greater on the tumour than the surrounding normal tissue. Currently there is limited information available about the

ability of drugs to increase the late effects of radiation on normal tissue, even though these late effects are most often dose limiting.

Genetic instability in tumours often leads to the presence of subclones, which coexist in the tumour with different levels of sensitivity to drugs and to radiation. When therapy is applied, any resistant cells that are present will have a selective survival advantage and will determine tumour response: thus, heterogeneity in therapeutic response may tend to make tumours more resistant to treatment than normal tissues. Combined treatment with radiation and drugs might then lead to improved therapeutic index if radiation can eradicate small populations of drug-resistant cells, or if drugs can eliminate populations that are relatively resistant to radiation therapy. This cooperative effect requires that mechanisms of resistance to the two therapeutic agents are independent. Mechanisms (other than hypoxia) that convey clinical resistance to radiotherapy remain poorly understood, but probably include enhanced ability to repair damage to DNA, increased levels of SH compounds such as glutathione (or of associated GST enzymes) that scavenge free radicals (especially in hypoxic cells), and decreased ability to undergo apoptosis. These mechanisms may also convey resistance to some anticancer drugs, whereas many other mechanisms of drug resistance are unlikely to cause resistance to radiation. Resistance to any given drug may be caused by multiple mechanisms so that a radiation-drug combination that provides therapeutic advantage for one tumour may not do so for another if different mechanisms of drug resistance are dominant. Effective use of combined treatment would be facilitated by rapid pretreatment assays that give insight into mechanisms of resistance prior to initiation of therapy. Proliferation of surviving cells during a course of fractionated radiation (i.e., repopulation) acts to increase the total number of cells that must be killed. Anti-cancer drugs given *during* the course of fractionated radiation (concurrent treatment) are in general more effective than combinations in which the treatments are given sequentially. Concurrent treatments might be expected to inhibit repopulation during fractionated radiotherapy. Combined treatment may then convey therapeutic advantage if the rate of repopulation is greater for the tumour cells than it is for normal tissues within the radiation field. Greater specificity would be expected for agents that inhibit specifically the proliferation of tumour cells; this might be achieved through use of hormonal agents (tamoxifen, antiandrogens) used concurrently with radiation for treatment of breast or prostate cancer.

Another possible strategy is to administer inhibitory growth factors (e.g. members of the TGF-β family) or agents that block receptors for stimulatory growth factors such as epidermal growth factor receptor (EGFR) if they are expressed selectively on tumour cells. Promising results are being achieved in clinical trials with the monoclonal antibody cetuximab, which inhibits signaling from the EGFR used together with radiation therapy. Repopulation during fractionated radiation therapy might also be influenced by prior treatment with neoadjuvant chemotherapy. Such chemotherapy may cause tumour shrinkage, followed by improved nutrition of surviving cells, with consequent stimulation of cell proliferation. If there is increased repopulation of surviving cells during the subsequent course of fractionated radiation therapy, any advantage from initial shrinkage of the tumour caused by chemotherapy may be lost or reversed because of the decreased net effectiveness of subsequent radiation treatment.

***Hypoxic cell sensitizers and cytotoxins*:** Another mechanism that has potential for exploitation through combined use of radiation and drugs depends on the presence of a hypoxic microenvironment within solid tumours. A hypoxic environment conveys resistance to radiation because cell killing is dependent in part on the presence of oxygen. One approach to reduce the influence of tumour hypoxia involves the use of drugs that mimic the

radiosensitizing properties of oxygen. These drugs, known as hypoxic-cell radiosensitizers, must diffuse to all parts of a tumour to be effective. A family of compounds, the nitromidazoles, has been found to contain members that can sensitize hypoxic cells both in vitro and in animal tumours. The most extensively studied of these compounds is misonidazole, which can sensitize hypoxic cells in vitro in a dose-dependent fashion and does not sensitize oxygenated cells. A large number of nitroimidazole sensitizers have been investigated, and nine have reached clinical evaluation. Overall, results from the trials using misonidazole have been disappointing, possibly because the dose of misonidazole was limited by a dose-dependent peripheral neuropathy. Studies using drugs that are less toxic, such as etanidazole and nimorazole, revealed conflicting results. Whereas nimorazole has been associated with improved tumour control in head and neck cancer, benefit was not demonstrated in two multicenter trials for head and neck cancer using etanidazole. Although most trials with nitroimidazoles have failed to demonstrate a significant benefit, a recent meta-analysis of results from over 7000 patients included in fifty randomized trials indicated a small but significant improvement in local control and survival, with most of the benefit attributed to an improved response in patients with head and neck cancer. The apparent lack of significant clinical benefit in most of the individual trials may be because only tumours that are severely hypoxic benefit from such treatment and prior measurements of the level of tumour hypoxia were not used to select patients entered into the trials.

Another approach to reducing the influence of hypoxia on the radiation response of tumours is to use (bioreductive) drugs that are toxic under hypoxic conditions. Complementary effects of radiation (against aerobic cells) and of drug (against hypoxic cells) might then increase the therapeutic ratio. The principal bioreductive drug of current clinical interest is tirapazamine, a benzotriazine di-N-oxide. Tirapazamine is cytotoxic to hypoxic cells because under hypoxia, it is metabolized to an oxidizing radical that produces DNA damage including double-strand breaks, probably by interacting with topoisomerases. In the presence of oxygen, the radical is converted (by oxidation) back to the parent compound. The drug also interacts with the chemotherapeutic agent cisplatin to increase its toxicity. Tirapazamine is being evaluated in clinical trials and has shown efficacy in a phase III trial with cisplatin in non–small-cell carcinoma of the lung. A clinical study in patients with cancers of the head and neck also demonstrated that tirapazamine, in combination with cisplatin and radiation therapy, was safe to administer, and resulted in disappearance of tumour hypoxia as assessed with F18 misonidazole positron emission tomography scans. A variety of other drugs which are specifically toxic to hypoxic cells are current at various stages of development and clinical study (e.g. AQ4N, which is a di-N-oxide prodrug that is reduced under conditions of low-oxygen tension to form the active species, AQ4, a DNA affinic, topoisomerase II poison).

***Radioprotection*:** An alternative approach to improve the therapeutic ratio is to protect normal tissue selectively from radiation damage. Many agents can protect against radiation damage to cells in culture. These include agents that can scavenge radiation-produced radicals, such as dimethyl sulfoxide (DMSO) or the superoxide dismutase enzymes (SODs), and those that can donate a hydrogen atom back to a radical site created on a macromolecule such as DNA, including the nonprotein sulfhydryls, glutathione, and cysteine. Because of the short lifetimes of radiation-induced radicals, these agents have to be present in the cell at the time of the irradiation. They are equally effective for tumour and normal cells in vitro; thus specificity in vivo depends largely on preferential uptake of such agents into the normal tissue. One agent that appears to fulfill this criterion is amifostine, a phosphorothioate compound that is converted into a sulfhydryl-containing compound in vivo by the action of alkaline phosphatases. Amifostine is localized selectively in normal tissues, probably because of poor penetration from tumour blood vessels and reduced levels of the activating enzyme, alkaline

phosphatase, in tumour cells relative to the activity on the membranes of normal cells. Therefore, it may offer selective protection against radiation (and a variety of drugs that damage cells by producing reactive intermediates which bind to sulfhydryl groups). There remain concerns, however, that this agent might also provide some protection of tumour cells. Amifostine was shown to protect a variety of normal tissues with variable, mostly small, protection of tumours in animal models and clinical studies in head and neck and lung cancers have shown substantial protection of normal tissue, including salivary gland, lung, and mucosa, without detectable change in tumour response.

Another strategy for radioprotection is the use of gene therapy with a viral vector designed to induce expression of manganese superoxide dismutase (MnSOD) that can block the action of reactive oxygen radicals. This approach is being used in the esophagus and lung by administering the vector topically or as an inhalant. It has been shown to provide protection for the lung and oral mucosa in rodents with no protection for the tumour growing in the lung. Presumably the viral vector can be effectively adsorbed through the mucosa or lung surface but cannot penetrate effectively into the tumour. Clinical trials of this strategy are in progress. A variation in this approach has suggested that the extent of subcutaneous fibrosis in patients may be reduced by direct injections of agents, such as SOD/α-tocopherol into the fibrotic region. A third developing approach is to block the development of late radiation effects with treatment given after the end of the radiation. The use of steroids after irradiation to prevent lung injury is an example, although this treatment appears to delay the development of symptoms rather than prevent them. Studies in rodents have demonstrated that expression of angiotension converting enzyme (ACE) is increased in lung and kidney at late times after irradiation. Agents which block ACE activity or agents which block directly the action of angiotensin II have been found to protect lung and kidney from the development of radiation induced fibrosis and nephropathy respectively. Only in kidney has it been demonstrated clearly that reduced functional damage can be sustained after the end of the drug treatment. Clinical studies are in progress investigating this approach.

Novel therapies: Multiple new drugs designed to target specific biochemical (signalling) pathways or specific processes in tissue (such as angiogenesis) that may be important for tumour growth and development are currently being developed and tested in clinical studies. While a number of such drugs are now used regularly for treatment of specific types of cancer (e.g. Gleevec for Chronic Myelogenous Leukemia or Gastro-intestinal Stromal tumours) most of these drugs have not been yet been extensively tested in combination with radiation. Partly this is because of the long times necessary to determine whether such agents will affect the development of the late toxicities which limit the doses delivered in curative radiation treatments. Furthermore few such drugs are tested for their toxicity to hypoxic cells despite our knowledge that most tumours contain a significant proportion of such cells. One interesting and potentially specific approach is the use of agents that are activated by radiation through the use of vectors that contain radiation-inducible promoters directly driving production of the toxic molecule (e.g TNF-α) or driving expression of enzymes which can convert prodrugs into toxic species (GDEPT - gene-directed pro-drug therapy). Such vectors should be activated only in the radiation field and would be expected to activate the prodrugs primarily in the irradiated volume. One current problem with such approaches is to get distribution of the vector into all the cells in the tumour, although this problem may be partially overcome if the toxic species produced can diffuse to neighbour cells and create a toxic bystander effect. Most of these strategies are currently at the experimental stage.

3.15. Clinical radiobiology of common cancers

There have been many biomathematical modeling studies of the response of common cancers such as in head and neck, lung, breast, cervix and prostate, as well as laboratory studies of particular biological parameters. In general, it has been shown that the dose-response slope for human cancer control is less steep than is the case for normal tissue complications and this is due largely to greater amounts of heterogeneity that varies between institutional series. Fractionation sensitivity (chararacterised by the reciprocal of the α/β ratio) is low for head and neck, lung and cervix tumours, higher for breast tumours, and very high for prostate tumours. The α/β ratio of around 1.5 Gy for prostate tumours has prompted various clinical trials to explore the potential benefits of using high doses per fraction. Regarding tumour clonogen repopulation during treatment, there is usually a lag phase of several weeks before repopulation effects become evident, equivalent to around 0.6 Gy per day using 2 Gy fractions for head and neck and lung tumours, about half that for cervix tumours, and around zero for prostate tumours. These differences are associated with the potential for repopulation, characterised by the potential doubling time of the tumour clonogens. Studies of tumour hypoxia have shown the presence and variability of hypoxia among tumours of the head and neck, cervix and prostate, suggesting that chemical modifiers of hypoxia could be used most efficaciously on the subset of patients that show high levels of tumour hypoxia.

3.16. Second cancers in radiotherapy patients

High doses directed to the cancer also result in some normal cells receiving low doses in the margins of the radiation beam. Low doses of radiation can induce mutations in cells that survive irradiation, and some of these mutated cells can lead to the production of second cancers. Hence there is a small risk of inducing a second cancer when curing a primary cancer. The issue of second cancers is becoming better recognised as treatments of primary cancers improve and patients survive longer, so that there is time available for development of any initiated malignancies. Second or higher-order cancers now account for 1 in 6 incident cancers reported to the US National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program. Second cancers can also arise in people who developed a primary cancer and were not irradiated, but who received other treatments such as surgery. These are second primary cancers, and they arise more frequently in patients who have certain genetic syndromes that predispose them to the development of various malignancies. Second cancers should be distinguished from secondary cancers, which are cancers that have arisen from malignant cells that have metastasised from existing primary cancers. It should be noted that the terminology of second or secondary is not always consistent in the scientific literature and sometimes the terms are used interchangeably.

The estimation of risk of radiation-induced cancer from radiotherapy of a first cancer can be derived from epidemiological studies of patients with common cancers that were cured with a similar probability when treated either with surgery or with radiotherapy, and these are mainly cancers of the cervix, prostate and testis. The risk of leukaemia following radiation is considerably smaller than after chemotherapy, usually in the order of 2-fold. Leukaemia risk is usually greatest about 5 to 9 years after radiotherapy exposure and then slowly declines. Radiation- related leukaemia risk is a function of dose to the active bone marrow, dose rate, and percentage of exposed marrow. The excess risk of leukaemia per unit of radiation dose is considerably larger after low doses than after high doses due to cell killing at higher doses. Thus, many studies in cancer patients have confirmed that high radiation doses to limited fields are associated with little or no increased risk of leukaemia. In contrast, exposure of larger volumes of bone marrow to radiotherapy may result in considerably higher risks as

shown in testis cancer patients treated with past radiation treatments to chest, abdominal, and pelvic fields, with resultant 11-fold risks of leukaemia. Low-dose total body irradiation [e.g. as used for the treatment of non–Hodgkin lymphoma in past years] has also been associated with high risks of leukaemia. Radiation has been associated with increased risks of acute myeloid leukemia (AML), chronic myelogenous leukaemia (CML), and acute lymphoblastic leukaemia (ALL). Only chronic lymphocytic leukemia (CLL) has not been linked with either prior radiotherapy or chemotherapy.

Breast cancer has emerged as the most common solid tumour among female survivors of Hodgkin lymphoma after treatment. Excess breast cancers, which are largely due to high-dose, large-field chest irradiation for Hodgkin lymphoma, are inversely correlated with age at treatment. The highest risks are observed among women treated for Hodgkin lymphoma at age less than 30 years, a finding that parallels the known sensitivity of the breast to ionizing radiation in the young. One large analytic, international investigation of Hodgkin lymphoma patients estimated long term risk according to radiation dose to the area in the breast where cancer was later diagnosed and that took into account chemotherapy- or radiotherapy-related ovarian damage. Statistical analyses were conducted to estimate the relative risk of breast cancer in terms of radiation dose to site of breast cancer and to the ovaries, cumulative dose of alkylating agent chemotherapy, and other risk factors. A radiation dose to the breast of more than 4 Gy was followed by a significantly increased 3.2-fold risk of breast cancer compared with women who received lower doses to the breast without alkylating agents. Risk of breast cancer increased with increasing radiation dose to reach 8-fold at >40 Gy, and excess radiotherapy-related breast cancers occurred for >25 years after exposure. The smaller radiotherapy fields and lower doses now used to treat Hodgkin lymphoma should eventually result in lower risks of breast cancer.

The interaction of chemotherapy with radiation or other risk factors in the development of solid tumours also needs consideration. For example, smoking multiplies the risk of either alkylating agent-associated or radiotherapy-associated lung cancer. In contrast, the effect of chemotherapy and radiation on lung cancer risk after Hodgkin lymphoma seems additive, as does the effect of cyclophosphamide and radiation on excess bladder cancers after non–Hodgkin lymphoma. Other relevant questions include the effect of the sequence and timing of exposures and interactions with other risk factors. Further, it will be important to understand whether relations between cytotoxic drugs, radiation, and solid tumour risk represent either an independent carcinogenic effect or radiosensitization by the chemotherapeutic agent, possibly by drug interference with the repair of radiation-induced DNA damage.

For cervix cancer, the data come from series of patients analysed from Scandinavia, USA, and Japan. In the regions of the body receiving high doses, the relative risk (RR) averaged over all 3 series for induced bladder cancer in cervix cancer patients surviving radiotherapy was about 1.6 (i.e. 60% greater) compared to the incidence in cervix cancer patients treated using non-radiation methods, and about 3.3 compared to the incidence of primary cancers in the general population. For induced rectal cancer the respective RR values were about 1.2 and about 1.5, and for induced colon cancer were about 1.0 and about 1.1. This indicates that in these cervix cancer patients there was a higher incidence of a second primary cancer in bladder and rectum than of a first primary cancer in these sites in the general population, and the use of radiotherapy increased the risk of a primary cancer in these sites.

For one large series of prostate cancer patients who received either radiotherapy it could be concluded that if a prostate cancer patient is to be treated with radiotherapy, the risk of developing a radiation-induced second cancer is approximately only 0.3%. Half of the 0.3%

risk of the radiation-induced cancers is in the low-dose regions such as for the lung. The other half of the risk is in the high-dose regions where radiotherapy frequently induces atrophy associated with chronic inflammation, which is a well-known pre-cancerous lesion. Over this and another large series, the most statistically significant increase in second cancers was in the bladder, where the RR was about 1.09 compared to the general population. Overall there was no significant difference in bladder RR for prostate cancer patients receiving radiotherapy versus other forms of treatment, and no significant elevation of risk regarding radiation induced rectal and colon cancer.

The accuracy of a calculated dose–response relationship for radiation-induced secondary cancer is limited by (1) the heterogeneity of the test population with respect to tumour characteristics (volume, grade, etc.) and dose distribution (total dose and dose per fraction) throughout the involved tissues, (2) accuracy of scoring outcome, (3) length of follow-up, and (4) validity of the degree of matching of the study to the reference populations. That is, the study and reference populations must be comparable in all respects for the computed risk to be a close approximation to the true risk. In fact, homogeneous test and reference populations are rarely available.

Direct observation of increased tumorigenic radiosensitivity in genetically cancer-prone humans has been made in cases of retinoblastoma, nevoid basal cell carcinoma (NBCC) syndrome, neurofibromatosis and Li-Fraumeni syndrome receiving radiotherapy for primary malignant disease. For these disorders, particularly for retinoblastoma and NBCC syndrome, there is evidence of increased risk of second, therapy-related cancer. There are also data showing that a number of cancer-prone genetic conditions are associated with chromosomal radiosensitivity, assessed in the G2 phase of the cell cycle. In addition to A-T and NBS which show marked increases, certain disorders associated with tumour suppressor gene mutation are also somewhat increased in chromosomal sensitivity, e.g. Li-Fraumeni syndrome, retinoblastoma and NBCC syndrome. Although still uncertain, it may be that in these disorders, the consequences of mutation of certain tumour suppressor genes for cell cycle control may provide an explanation for the effect. Radiosensitivity in a broad range of cancer-predisposing genetic disorders remains somewhat contentious, but recent work not only has achieved the discrimination of radiosensitivity in A-T heterozygotes but also raises the possibility that a significant fraction (as much as 40%) of unselected breast cancer patients are also characterised by increased chromosomal radiosensitivity. Hence the proportion of individuals in a population with increased susceptibility to cancer and to radiation-induced second cancer could be more extensive than currently thought. Other factors that may influence response to radiation exposure include radiation-related genomic instability (destabilisation of the genome), epigenetic phenomena (microenvironmental changes affecting cellular responses), and bystander effects (irradiated cells sending injurious signals to unirradiated neighbouring cells).

Intensity-modulated radiation therapy (IMRT) allows dose to be concentrated in the tumor volume while sparing normal tissues. This in turn allows the possibility to increase the dose and hence to increase the chance of curing the tumour. However, the downside to IMRT is the potential to increase the number of radiation-induced second cancers. The reasons for this potential are more monitor units and, therefore, a larger total-body dose because of leakage radiation and, because IMRT involves more fields, a bigger volume of normal tissue is exposed to lower radiation doses. Intensity-modulated radiation therapy may double the incidence of solid cancers in long term survivors. This outcome may be acceptable in older patients if balanced by an improvement in local tumor control and reduced acute toxicity. On the other hand, the incidence of second cancers is much higher in children, so that doubling it

may not be acceptable. IMRT represents a special case for children for three reasons. First, children are more sensitive to radiation-induced cancer than are adults. Second, radiation scattered from the treatment volume is more important in the small body of the child. Third, the question of genetic susceptibility arises because many childhood cancers involve a germline mutation.

3.17. Summary

Radiotherapy for cancer usually involves giving 25 to 40 individual dose fractions of about 2 Gy once daily, over a period of 5 to 8 weeks. These treatment schedules have been developed empirically and show a better therapeutic ratio than single doses because they give greater tumour control at tolerable levels of normal tissue damage. Improvements in therapeutic ratio have also been associated with the introduction of conformal and intensity modulated radiotherapy because these have allowed decreased normal tissue dose (and, hence, side effects) with dose escalation to tumour tissues. Other radiotherapy technologies that have been developed in order to improve the therapeutic ratio through physical means for some types of tumours include brachytherapy, conformal low-LET radiotherapy techniques, and high-LET irradiation., Studies with cells in culture and with animal models have identified biological factors (the "five Rs") that can influence response to fractionated treatment and hence may impact therapeutic ratio. These are radiosensitivity, repair of radiation damage, repopulation of damaged tissues by proliferation of surviving cells, redistribution of proliferating cells through the cell cycle, and reoxygenation of hypoxic cells. Repair and repopulation are the reasons why cells and tissues can tolerate a larger total dose when it is fractionated. They occur both in tumours and normal tissues, although repopulation has a minor effect on the late radiation damage that occurs in slowly proliferating normal tissues and is often dose limiting. Repopulation by tumour cells during the latter part of conventional (5- to 7-week) fractionated treatments may play an important role in increasing the dose required for tumour control. Reoxygenation in tumours contributes to the improved therapeutic ratio obtained with fractionated treatment. Both tumour and normal tissue responses to irradiation are complex. Radiation can kill individual tumour and normal cells directly and this can be expressed as mitosis-linked cell death or, in a few tissues, as early apoptosis. Particularly in normal tissues, there are also indirect effects, such as the induction of cytokines, which can influence early and late tissue effects. An example is the role of TGF-β in radiation-induced fibrosis. Tumour control requires the killing of all the tumour stem cells but there is heterogeneity in cellular radiosensitivity in tumours due to microenvironmental factors such as hypoxia and possibly also due to the development of resistant subpopulations as a result of genetic instability. In clinical radiotherapy, different fractionated schedules that give an equal level of normal tissue response or tumour control can be expressed in the form of an iso-effect relationship described by the parameters α and β of the linear-quadratic model. Late-responding tissues tend to have smaller α/β values than early-responding tissues, implying greater capacity for repair of damage that leads to late effects. The difference in the iso-effect relationships for early and late damage implies that reducing fraction size will reduce damage to late-responding tissues to a greater extent than to early-responding tissues or tumours. A therapeutic gain might therefore be achieved by using hyperfractionation, where treatment with smaller dose fractions is given several times per day. If this also reduces overall treatment time, this might also lead to a therapeutic gain if repopulation occurs more rapidly in the tumours than in the dose-limiting normal tissues. Other approaches to improving the therapeutic ratio have included attempts to reduce the resistance due to hypoxic cells in tumours, such as strategies to increase oxygen delivery to these cells or giving drugs capable of specific sensitization (and toxicity for) hypoxic cells, and predictive tests which are under further development.

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SUGGESTED PRACTICALS/TUTORIALS:

(a) DNA Laboratory techniques. Practical demonstrations of some of the techniques from the above lectures e.g. comet assay, micronuclei, flow cytometry (DNA analysis), gel electrophoresis.

(b) Survival curves in practice. Practical session on the shapes of survival curves, and their importance in various clinical scenarios.

(c) Analysis of scoring of normal tissue damage. LENT/SOMA vs RTOG/EORTC scoring systems HNSCC, Cervix Ca

(d) LQ model: BED, LQED; α/β ratio values

Fractionation calculations in practice

Physical dose distribution and biological response distribution

Combined brachy/teletherapy treatments; compensations for interruptions in treatment

Importance of treating all fields per day

Influence of radiation source decay with respect to repair half-time and dose effectiveness

Clinical impact of errors in dose delivery

(e) Critical reading of literature

4. EXTRA MODULE FOR RADIATION PROTECTION PERSONNEL

4.1. Introduction

The aim of Radiation Protection is to establish an appropriate level of protection for people and the environment against detrimental effects of radiation exposure without unduly limiting the desirable human actions that may be associated with such exposure. The first aim of radiation protection is to keep doses below the threshold value for tissue or organ reactions. These reactions are similar to the early and late side effects (morbidity) of radiotherapy in cancer patients, which occur only after high radiation doses and which show an increased severity with increasing radiation dose. They are not observed below a certain threshold dose. In the context of radiation protection these effects were previously called "deterministic radiation effects".

The main risks that radiation protection is concerned with are radiation-induced cancer and leukaemia, radiation-induced heritable damage and radiation-induced developmental damage to the developing embryo and foetus. The severity of both radiation-induced cancer and radiation-induced heritable diseases does not depend on radiation dose, but their frequency increases with increasing radiation dose. They are commonly called "stochastic radiation effects". Recent epidemiological and radiobiological data do, however, blur the clear distinction between both types of effects which are dealt with in a radiation protection context.

4.2. Radiation accidents and environmental radiation exposure

Accidents have happened infrequently in the history of radiology and nuclear research, and usually they have involved only small numbers of people. From those accidents, much has been learned about the health consequences and the appropriate medical management of radiation accidents. The Chernobyl accident in 1986 posed the greatest challenge to all radiation protection personnel involved in radiation accident management. The value of the experience gathered in previous accidents was shown after this event, and much more has been learned to be used in more recent accidents which involved even larger numbers of people such as in Brazil (abandoned radioactive source in Goiania), or which cause more severe bodily harm such as in Tokai-Mura, Japan.

4.2.1. Dose estimation

Radiation risks depend, above all, on the radiation dose received by the affected person(s). The risk increases with increasing radiation dose. Therefore, estimates of radiation risks have to be based on the careful evaluation of the individual radiation dose and the dose distribution in the body. Radiation exposure may come from external irradiation usually with γ-rays from radionuclides which may be natural or man-made, or it may come from internal irradiation, mostly with β-rays emitted by radionuclides from natural or man-made sources. It is in particular from naturally occurring radionuclides that also α-particles may be a problem in radiation protection from internal exposure.

External radiation doses of occupationally exposed people, i.e. radiation workers, are routinely measured with personal dosemeters which are usually either based on film dosimetry or thermoluminescent (TLD) dosemeters. These dosemeters are designed to measure the accumulated exposure over a period of usually one month at the body site where the dosemeter is worn but they also permit the measurement of the energy and penetration of the ionising radiation. Readings may be unreliable if exposure is very inhomogeneous. In

order not to underestimate radiation exposure it is important that the dosemeters are worn at a suitable site of the body, usually the chest. Internal contamination of radiation workers is most often investigated by measuring the particular radionuclides in the urine.

Whereas the determination of radiation doses of radiation workers is straightforward and follows a routine procedure, the determination of radiation doses in accident situations is much more complex and has to be especially designed to meet the individual scenarios. Retrospective determination of external and internal radiation doses after an accident has to be based on various measurements which then need to be fed into a complex model which takes account of the time-dependent changes of radioactive decay, transport of radioactivity in the environment and transfer in the human body.

Prospective determination of external and internal radiation doses are needed to define the permitted releases of radioactivity from planned nuclear installations during normal operation and, more importantly, to estimate the potential radiation exposures of the population during accidents as the basis for decisions on required countermeasures. These estimates are entirely based on model calculations which use several models in sequence: (1) the transport of radionuclides from the source (i.e. the site of the accident to the site of the population to be considered) is calculated using a metereological model of transport of the aerosols to which radionuclides are attached and their deposition (either dry (fall-out) or wet (wash-out)) to the ground. These models are based on metereological data and experiments and may be quite detailed including e.g. translocation of deposited radionuclides by rainfall into the sewer system or resuspension of radionuclides attached to dust. The radionuclides deposited on the ground lead to external irradiation with γ-rays (which often is the most important contribution to the total dose), or to internal irradiation through direct contamination of food; (2) the transfer of radionuclides deposited on the ground or in water (lakes or rivers) is determined with the use of radioecological models which describe the changes of activity concentration from one compartment to the next, e.g. from ground surface to plant roots, from there to the edible parts of the plants and from there to the actual food. The calculation of radiation doses requires also knowledge or estimates of food intake (how much and when); (3) the distribution of radioactivity which has been incorporated by eating (ingestion) or breathing (inhalation) is determined with the biokinetic model which relates the uptake with a dose factor which defines the committed dose per Bq incorporated radionuclide in the different organs of the body.

The determination of external radiation exposure immediately after accidental releases of radionuclides usually is relatively straightforward, and often can be performed on the basis of direct measurements, e.g. the dose rate at 1 m height above the ground from radionuclides deposited on the ground. On the other hand, the determination of internal radiation exposure usually requires many measurements in the food chain and complex modelling.

Retrospective dose estimation has to be performed for past exposures in order to estimate radiation risks. This has been done, e.g. for A-bomb survivors (see below), for populations exposed from the Chernobyl accident, for populations exposed in Siberia from the radioactive pollution of the Techa River, and from contamination of large areas from nuclear weapons test, e.g. in the Marshall Islands and near the Semipalatinsk test site. The radioecological methods have been developed in major international cooperative research projects and have reached a high degree of reliability. However, there is often the need to determine individual radiation doses which can best be performed by biological dosimetry techniques, e.g. in the liquidators after the Chernobyl accident. The best and most widely employed methods uses the assessment of unstable or stable chromosome aberrations.

The determination of radiation dose from accidental exposure in the first few weeks after the accident is commonly done by a combination of physical reconstruction of exposure scenarios and calculation of organ doses and total body doses as well as by biological dosimetry. The preferred method of biological dosimetry which has proven its value in many accidents is the determination of the frequency of unstable chromosome aberrations in stimulated blood lymphocytes. The method has been well standardised: phytohaemagglutinin is added to 5 – 10 ml heparinised blood to stimulate resting lymphocytes into proliferation. After incubation for 48 hours at 37 °C, cells entering mitosis are arrested in metaphase by adding colchicine. It is important to arrest cells in their first mitosis since many of the severe chromosome aberrations which are used as "dosemeters" are eliminated in the first cell division. As a general rule, the number of dicentric chromosomes is counted in 500 arrested metaphases. If there are 25 dicentrics among 500 metaphases, a total body dose of 0.3 Gy can be assumed. After a dose of 3 Gy, there is, on average, one dicentric chromosome to be found in each metaphase. After homogeneous total body irradiation, the number of dicentric chromosomes per cell follows a Poisson distribution. Marked deviations from a Poisson distribution are an indicator of very inhomogeneous dose distribution which may have consequences for the prognosis.

The determination of radiation doses from accidental exposures many months and years after irradiation is based on the measurement of stable chromosome aberrations, such as balanced translocations which can be visualised using fluorescence *in situ* hybridisation (FISH). Biological dosimetry based on cytogenetics requires time-consuming investigations by highly trained staff, and thus can usually not be performed on large numbers of accident victims. For this reasons, alternative methods which can be automated to some degree such as micronuclei in peripheral lymphocytes and glycophorin A (GPA) mutations in erythrocytes have been developed and used, e.g. in clean-up workers and affected populations of the Chernobyl accident. However, they have not found the same degree of general acceptance.

4.3. Diagnosis and medical management of radiation syndromes

4.3.1. LD-50 (Lethal dose 50)

The dramatic experience of the deaths and long term morbidity of thousands of people in Hiroshima and Nagasaki and the prospect of a nuclear war, initiated in the 1950s a large scale research programme into the acute radiation lethality of a wide range of mammalian animals ranging from mice to large animals such as goats and dogs. The death rates and the latency to death after different radiation doses given to the whole body were determined in laboratories around the world. The experiments with total body irradiation of mice in particular defined our understanding of the causes of death and of the lethal radiation doses. Lethality increased with increasing dose following a sigmoid dose response curve. Usually, radiosensitivity was defined in the steepest part of the dose response curve as the dose resulting in lethality in 50% of subjects (LD50) in a specified period after radiation exposure, most commonly in 30 days. Significant differences in the LD50/30 were found between different animals, there was a trend for decreasing LD50/30 with increasing body mass. Whereas in mice, the LD50/30 usually is about 7 Gy, in some large animals it is as small as 3 Gy.

From the experience of some radiation accidents and of some groups of atom bomb victims who did not suffer from extensive burns or wounds but still had received high radiation doses, the LD50 of humans within 60 days was estimated to be between 3 Gy and 4.5 Gy. However, gradually it has become clear that the lethality after total body irradiation depends more on factors such as co-morbidity and the quality of medical care than simply on radiation dose. An

ad-hoc committee of the Medical Research Council of the United Kingdom consequently dismissed the value of the concept of LD50 for man and rather defined three dose ranges with different prognosis: survival very likely, survival possible with adequate medical care, survival unlikely despite adequate medical care. For these prognostic categories, doses of ≤ 2 Gy, 2-8 Gy, ≥8 Gy were estimated.

4.3.2. Radiation syndromes

The experiments in mice demonstrated that there was a strong dependence of the latency to death on radiation dose: increasing the dose from 5 to 12 Gy, the survival time gradually decreased from about 2-3 weeks to about 4 days. Further increase of total body dose up to >30 Gy did not lead to further shortening of the latency to death in mice, however, even higher doses caused death within a few days and very high doses, even within hours. Three different radiation syndromes were associated with these three categories based on the latency to death: the haemopoietic syndrome after doses < 12 Gy, the gastrointestinal syndrome after doses of 12 to 30 Gy, and the cerebrovascular syndrome after even higher doses. The different latencies of the haemopoietic and the gastrointestinal syndrome were explained by the different cell turnover rates of the critical cell lineages in the tissues in which severe lethal hypoplasia occurred lead to death of the animal, i.e. the granulocyte cell production lineage in the bone marrow and the epithelial mucosal cell lineage in the small bowel. Death in the haemopoietic or bone marrow syndrome was associated with septic infection due to agranulocytosis, death in the gastrointestinal syndrome was associated with complete denudation of the small bowel surface leading to profuse diarrhoea and hypovolumic shock.

Since the 1970s, few additional experimental studies on radiation syndromes have been performed – although extensive research using similar methods has been directed at the development of total body irradiation with subsequent stem cell transfusion in the treatment of leukaemia and some other malignant diseases. Some results of this research certainly had also been used in the further refinement of treatment protocols of affected accident victims, yet the overall classification remained largely as first proposed by Bond et al. in 1965, nearly fifty years ago. However, the present understanding of the nature and the pathogenetic development of human radiation injury after whole body irradiation is less based on old animal experiments in mice and dogs but rather on careful clinical evaluation of human accident victims, from the Oak Ridge accident in 1958 to the Chernobyl disaster in 1986 and the Tokai-Mura accident in 1999. It became apparent that the simple classification of radiation syndromes based on latency and critical cell lineages is not appropriate to describe the complexity of the clinical features of human accident victims. In 2001, a manual of MEdical TREatment ProtocOLs METREPOL was published by a European consortium of expert scientists, as the results of a comprehensive evaluation of all existing data on radiation accident victims (referenced in Chapter 1).

4.3.3. Medical management of radiation accidents

The METREPOL system of radiation accident management defined response criteria for four separate organ systems each of which is involved together in the development of signs and symptoms of health damage after accidental radiation exposure. However, depending on the special accident scenarios, in different accidents each one may take the leading role in defining symptoms and may need special attention for medical management. These four organ systems are the neurovascular system, the haematopoietic system, the cutaneous system, and the gastrointestinal system. The basic idea behind this concept is to unravel the complexity of the acute radiation syndrome. The first step is to divide it into more accessible elements, i.e.

those clinical signs and symptoms that characterise the extent of damage to the four early reacting organ systems under concern (N, H, C, G) and defining their severity in four grades (mild = spontaneous recovery certain; moderate = recovery with possible deficit; severe = recovery possible with intensive medical care but probable deficit; fatal).

4.3.3.1. The neurovascular syndrome (N)

Irradiation may cause both cerebrovascular disorders and nervous tissue injury. Although electrophysiological studies after total body irradiation with doses >6Gy have demonstrated significant changes at the synaptic level in brain tissue consistent with a state of increased brain excitability, the clinical symptoms are most likely linked to cerebral oedema with an increase in intracranial pressure. Along with early oedema, acute inflammatory reactions occur as well as decrease of the blood-brain barrier. The onset and duration of the different phases of the neurovascular syndrome depend on radiation dose. Symptoms such as nausea, vomiting and anorexia characterise the prodromal phase. Although the symptoms are expressed by the gastrointestinal system, the control site is located in the brain. After high radiation doses, the severity of symptoms gradually increases and results in a fatigue syndrome, associated often by hypotension and dizziness. With increasing severity of the neurovascular syndrom, survivors have a high risk of developing late effects, in particular impairment of cognitive functions and neurological deficits. Grade N1 is defined by late onset of mild prodromal symptoms and symptoms of mild fatigue which may persist for several weeks. Anti-emetic treatment on an outpatient basis is usually sufficient. N2 is defined by episodes of vomiting in the prodromal phase and moderate fatigue lasting several weeks. These patients need anti-emetic treatment and regular clinical monitoring in hospitals. N3 is defined by severe nausea and vomiting within the first hour after exposure lasting for about 2 days. Symptoms recur after a symptom-free interval and persist for about 2 weeks, leading to electrolyte imbalance. Patients also suffer from headaches and severe fatigue syndrome, hypotension and fever. Hospitalization of these patients is obligatory, medical management has to include intravenous glucocorticoids, electrolyte and fluid replacement and analgesics. N4 is characterised by rapid incapacitation by severe nausea, vomiting, headaches, fever, erythema and drowsiness within the first hour after exposure. Recovery is unlikely and mostly primary symptoms continue intermittently. Only sufficient fluid and electrolyte replacement, analgesic medication and the application of intravenous glucocorticoids and mannitol infusion to reduce intracranial pressure, will increase the patient´s chance of survival.

4.3.3.2. The haematopoietic syndrome (H)

Signs and symptoms of the haematopoietic syndrome are directly related to reduction of concentration of specific cells types in the blood. Radiation-induced cytopenia is strongly related to dose. The impact of acute radiation exposure on the physiology of normal haematopoiesis and the balance of cell production in the bone marrow and cell loss after the cell lineage specific life span has been thoroughly investigated after exposure in humans and animals. Radiation does not decrease life span or function of blood cells but it blocks in a dose dependent way the production of new cells.

Normal human erythrocytes have a life span of about 120 days. Therefore, even after complete cessation of all erythropoiesis, the decline of red blood cell concentration is less than 1% per day and anaemia is not a clinical problem unless there is additional damage which causes increased loss such as haemorrhage from wounds or thrombopenia, or haemolysis which is frequently observed after severe burns. Radiation damage to erythropoiesis can be monitored by measuring the concentration of reticulocytes in the blood. Granulocytopenia is the main cause of critical health effects after total body irradiation

leading to increased risk of systemic bacterial infections (sepsis). Granulopoietic cells originate from bone marrow stem cells which undergo proliferation and differentiation into the mature granulocytes of the peripheral blood. The transit time for cells from the myeloblast to the first non-dividing cell, the metamyelocyte in the bone marrow is about 6 days, and another 3–4 days are required. Granulocytes disappear from the blood randomly with a half time of 6-7 hours. Their overall life span is approximately 30 hours. Thrombocytes (also called platelets) are produced by megakaryocytes. The total transit time from the most immature megakaryocytes in the bone marrow to the release of platelets is 8-10 days, and the life span of platelets in the blood is on average 8-10 days.

The radiation-induced cytopenia of the blood cells is the consequence of inhibition of cell production in the proliferative precursor cells. Regeneration, however, depends on the survival of a sufficient number of bone marrow stem cells. Stem cells in the circulation can be assessed by characteristic surface markers. However, grading of the haematopoietic syndromes is based on the response patterns of blood cells as described in Figure 1 (see Fliedner reference, page 24). H1 is defined as cell counts at just below the lower end of the normal range, and no specific treatment is necessary because spontaneous regeneration will occur. H2 is defined as a lymphocyte count on day 2 of between 500 and 1500 / µl, by transient granulocytosis within the first few days, followed by a decrease to the lower end of the normal level until day 10, followed by a second, abortive rise. Then the clinically important granulocytopenia occurs between days 12 and 20 with a value < 1000 granulocytes / µl which may result in general infection in some patients. Regeneration starts after day 30. Platelets decrease gradually during the first three weeks to a value around 50,000/µl which, in some patients may cause haemorrhage, particularly into the bowels. Only those patients who develop infection or haemorrhage need to be treated with antibiotics or platelet transfusion. H3 is defined by a rapid decrease of lymphocytes to 250-500/µl and by transient granulocytosis during days 1-3. This is followed after day 5 by a steady decrease to a plateau of about 500/µl around days 10–15 with regeneration starting around day 30. Platelets decrease steadily to a nadir in the third week which may be well below 50,000/µl. Treatment options are similar as for H2 patients, however, stimulation of haematopoiesis with growth factors should be considered as soon as possible. Platelet tranfusions should be given to maintain stable values in the blood of >20,000/µl. Treatment with granulocyte colony stimulating factor (G-CSF) is commonly performed in medical oncology to treat drug-induced cytopenia with daily subcutaneous injection of 10µg/kg for 2 weeks. Based on experimental data, an addition of a single intravenous injection of 5µg/kg thrombopoietin may increase the effectiveness of growth factor treatment. A clinical response of growth factor treatment should be apparent 10 – 14 days after initiation of treatment, and prolonged treatment is not indicated. H4 is defined by very rapid decrease of all blood cells to very low values; lymphocytes to < 250/µl on day 2, granulocytes to < 500/µl at the end of the first week after exposure, and platelets to 0 at day 10. The only real option for therapeutic intervention into an otherwise lethal progress of the haematopoietic syndrome H4 is stem cell transplantation. The procedure of stem cell transplantation is presently used most frequently for the treatment of leukaemias. If no HLA **(**human leukocyte antigen) identical sibling is available, the best option might be umbilical cord blood stem cells.

4.3.3.3. The cutaneous syndrome (C)

The experience from the Chernobyl accident showed for the first time that radiation damage to the skin from beta particles emitted by radionuclides deposited on the skin could be a major clinical problem, and in the special case of the firefighters of Chernobyl might have been the main cause of death from grade 4 cutaneous syndrome. Signs and symptoms of the cutaneous

syndrome are the consequence of radiation damage to the proliferating and the stem cells in the epidermis and a pronounced inflammatory response in the dermis. They follow a distinct time pattern which is determined by the proliferative organisation of the epidermis.

C1 is defined by an early transient erythema (reddening) of the skin which subsides within 36 hours. A second wave of erythema appears 5 days after exposure, 3 to 4 weeks after exposure the skin will appear dry due to the loss of sebaceous glands. This may be associated with mild pain, discomfort and itching. Treatment is symptomatic with anti-inflammatory lotions or powder. C2 is defined by erythema progressing to oedema and blistering 5 to 10 days after exposure, covering no more than 10% of the body surface. Transient loss of hair may develop at around 14 days after exposure. Treatment with topical glucocorticoids, linoleic acid creams and systemic antihistamines is usually required. C3 is defined by the same signs and symptoms but covering 10 to 40% of the body surface. There is a risk of deeper ulceration which is influenced and complicated by the concomitant haematopoietic syndrome. Systemic treatment with glucocorticoids and analgesics should be used. C4 is defined by the same signs and symptoms but covering >40% of the body surface and involving deeper underlying tissues of the skin and subcutis. Intensive care treatment is essential to deal with the multitude of symptoms such as pain, infection, and necrosis, but even if the patient survives, long term skin damage is likely to persist.

4.3.3.4. The gastrointestinal syndrome (G)

Symptoms related to the gastrointestinal radiation syndrome are the prodromal symptoms which are secondary to the neurovascular changes describe above, such as nausea, vomiting and anorexia. Symptoms in the manifest gastrointestinal syndrome, which usually starts in the second week after radiation exposure, are mainly abdominal cramps and diarrhoea. After high radiation doses, the loss of the mucosal covering of the bowels, which if associated with thrombocytopenia, may also lead to bloody diarrhoea and to entry of enteric pathogenic and non-pathogenic bacteria. Since cell turnover is fastest in the small bowel, the signs and symptoms of radiation damage occur earlier in the small bowel than in the large bowel. Experimental studies have demonstrated that in addition to the damage to the proliferating mucosal cells, symptoms of the gastrointestinal syndrome are very much affected by functional changes in the neural and immune cells in the bowel wall. This is particularly obvious in the stomach in which, even after low doses of 1 to 2 Gy, functional changes such as decreased gastric motility, decreased production of gastric juice and inflammation (gastritis) have been observed.

G1 is defined by a few episodes of altered stool consistency and frequency with associated abdominal pain. Treatment is usually not necessary. G2 is defined by changes in frequency and consistency and blood in stool together with abdominal cramps. Spontaneous recovery is certain however treatment of diarrhoea with Loperamide is indicated. G3 is defined by a higher frequency of these events, with several episodes per day over several days and weeks. Spontaneous recovery is likely but may be incomplete with recurring episodes of diarrhoea alternating with constipation. To prevent electrolyte imbalance, the individuals should be carefully monitored and replacement therapy given. In addition, antibiotics, anti-inflammatory drugs and analgesics may be necessary as indicated by the clinical symptoms. G4 is defined by rapid onset of diarrhoea which may be explosive. This is more due to functional disturbances than to mucosal damage. Frequent episodes of severe diarrhoea will lead to severe fluid and electrolyte imbalance and will be accompanied by severe painful abdominal cramps. Septicaemia is also very likely due to the simultaneously occurring granulocytopenia.

Treatment is purely symptomatic with the main emphasis on fluid and electrolyte replacement, systemic antibiotics and analgesics.

This description of the signs and symptoms of the four radiation-induced acute syndromes, their diagnosis, classification into severity grades and their treatment is very brief and should only give an impression of the complexity of the pathogenesis, symptomatology and treatment options. The Manual on the Acute Radiation Syndrome prepared by the Concerted Action METREPOL under T.M. Fliedner gives a full account of all aspects of the medical management of radiation accidents (Fliedner et al., 2001).

The Tokai-Mura accident in Japan in 1999 demonstrated that acute exposure to very high radiation doses leads to a new type of radiation syndrome which is well described by the term Multi-Organ Involvement. In Tokai-Mura, a criticality accident happened due to poor working practice when 3 workers poured uranium fuel from a bucket into a larger vessel where a critical mass was formed leading to non-uniform radiation exposure of the three workers with mean body doses in the lethal range. The haematological grading was H4. Bone marrow stem cell transplantation using umbilical cord cells in one patient led to transient restoration of haematopoiesis after 10 days which was complete after 50 days, yet the patient died 210 days after the accident. Failure of several organs was diagnosed. However, most critical was the nearly complete loss of immunological responsiveness leading to the activation of cytomegalovirus infection (which was successfully treated with gancyclovir). Reactivation of radiation burns of the skin and loss of mucosal barriers together with a multitude of other delayed damage finally caused the death of this patient despite the heroic effort to keep him alive at all cost.

4.3.4. Methods of triage for treatment after a radiation accident

In major accidents such as the Chernobyl accident, the decision on the need for treatment and prognosis of the individual accident victim cannot be based on dose estimates which are time consuming, uncertain and with little impact on the medical response. Rather, these decisions have to be based on clinical criteria which are simple, early and permit the reliable identification of accident victims who do not need special treatment. It is more important not to miss any victim who may need treatment than to identify only those who will certainly need treatment. Such criteria have been established for many decades and they proved their usefulness particularly in the acute aftermath of the Chernobyl accident, when the members of the rescue teams had to be assessed as to who would need which treatment and when (Table 4.1)**.**

TABLE 4.1 TRIAGE CRITERIA USED AFTER THE CHERNOBYL ACCIDENT

Severity	Vomiting time	Lymphocytes day 3 per µl	Hair loss within 2 weeks	Cytogenetic radiation dose	Lethality including skin burns
Mild	no	>600	no	< 2Gy	0/105
Intermediate	after 1-2 h	300-600	no	2 – 4 Gy	0/53
Severe	after 30-60 min	100-300	yes	4 – 6 Gy	6/23
Very severe	immediate	<100	yes	6 – 16 Gy	19/22

4.4. Radiation carcinogenesis

4.4.1. Mechanisms of carcinogenesis

The development of cancer in tissues is assumed to be a multi-stage process that can be sub-divided into four phases: neoplastic initiation, promotion, conversion and progression. These subdivisions are likely to be over-simplifications and, in different tissues, they may vary. Yet the subdivision into different phases provides a suitable framework for identification of the specific molecular and cellular changes involved.

Neoplastic initiation leads to the irreversible potential of normal cells for neoplastic development by creating unlimited proliferative capacity. There is good evidence that this event results from one or more mutations in a single cell which is the basis of the clonal evolution of the cancer. Further neoplastic development of initiated cells depends on promotional events which involves intercellular communication, e.g. by growth factors, hormones or environmental agents. This results in the proliferation of the initiated pre-neoplastic cells in a semi-autonomous manner. During the process of conversion of the pre-neoplastic cells into fully malignant cells, additional mutations in other genes are accumulated, probably facilitated by increasing loss of genomic stability. The subsequent progression into an invasive cancer depends on still more mutations in the unstable genome.

Two classes of cancer-associated genes have been identified. Proto-oncogenes are normal genes involved in growth regulation. Mutations e.g. by the translocation of a promoter, may result in an increased rate of proliferation. Proto-oncogene mutations to oncogenes are thus classified as gain-of-function mutations. Tumour suppressor genes are genes that are involved in growth regulation of normal cells and that prevent excessive cell proliferation. The critical mutation in these genes are loss-of-function mutations which may be the result of partial or complete loss of the gene structure, e.g. by deletions. Since radiation-induced DNA damage preferentially causes deletions, it is generally assumed that the inactivating mutation of tumour suppressor genes is the most probably mechanism of the induction of cancer by radiation.

Since there is good evidence that many if not most cancers are the clonal descendants of a single neoplastic cell and, furthermore, that a single double strand break may, although with an extremely low probability, cause a deletion in a specific DNA sequence, e.g. of a tumour suppressor gene, it has been argued that, in principle, a single mutational event in a critical gene in a single target cell *in vivo* can create the potential for neoplastic development. Thus, a single radiation track traversing the nucleus of an appropriate target cell has a finite probability, albeit very small of generating the specific damage of DNA that results in the initiating mutation. This argument would strengthen the hypothesis that the risk of radiation induced cancer increases progressively with increasing dose with no threshold.

Although these basic facts are generally accepted, the conclusion that they necessarily exclude the possibility of a dose threshold has been debated extensively. So far, no agreement has been reached about the role of the influence of a range of other biological mechanisms on the dependence of radiation-induced cancer rates on dose at very low doses such as are the object of radiation protection regulations. These mechanisms range from low-dose hypersensitivity which may eliminate specifically cells harbouring DNA damage after very low radiation doses and non-targeted radiation effects such as radiation-induced genomic instability, to effects related to intercellular communication including bystander effects and immunological surveillance mechanisms. The report of the Académie Nationale de Médicine in Paris stressed that “cell responses are based on a complex network of intra- and intercellular signalling, and

may be expressed in several ways, including the repair of damage, apoptosis, delayed death or prolonged quiescence of initiated cells. The modalities of the response are adapted to the context and vary according to the dose, fractionation, dose rate, LET, cellular redox state, cell status before irradiation, the presence of signals emitted from neighbouring cells and, possibly, of other toxic agents." The respective roles of direct radiation-induced initiating, transforming mutations in normal target cells on the one hand, and of the complex mechanisms which may respond to those initial processes as listed above, continue to be the subject of continuing radiobiological research. This is done in the hope that better understanding of these processes may permit a science-based judgement on the existence or not of a dose threshold below which the risk of radiation-induced cancer is zero. ICRP 2007, § 65 draws the conclusion from this controversy that biological and epidemiological information that would unambiguously verify the Linear Non-Threshold (LNT) model is unlikely to be forthcoming. "Because of this uncertainty … it is not appropriate … to calculate the hypothetical number of cases of cancer or heritable diseases that might be associated with very small radiation doses received by large numbers of people over very long periods of time."

4.4.2. Epidemiological evidence for radiation carcinogenesis

The assessment of radiation risks in exposed populations can use either of two epidemiological methods which differ considerably in their workload, in the information they can provide and in their duration and costs. Cohort studies define, usually soon after exposure, a cohort of often many thousand people who were exposed to different radiation doses. Individual or group doses have to be determined for all members of the study cohort. Health effects are subsequently collected as the cohort is ageing for as long as possible, ideally life-long. The best example of a cohort study of radiation effects is the Life Span Study (LSS) of the Japanese atomic bomb survivors.

Case-control studies define patients, usually several hundred, who suffer from the disease to be investigated with regard to the role radiation exposure might have played in its causation. These patients are called "cases". For each case, 1 to 5 patients are selected who have a different disease but matched as closely as possible to the individual case, e.g. with regard to age, sex, socioeconomic status. For each case and each control, a comprehensive exposure history is collected in a structured interview which includes information on radiation exposure but also on competing risk factors such as smoking but also occupation. In addition, various measurements may also have to be performed, e.g. of individual radiation exposure. The radiation risk is estimated by comparing the radiation doses of the cases with those of the controls. The best examples of case-control studies in radiation epidemiology are the radon-in-homes studies. Cohort studies permit the evaluation of different risks from the same exposure such as cancer, cardiovascular diseases, stroke etc. in the Life Span Study. The identification of other risk factors is usually difficult and may require nested case-control studies. On the other hand, case-control studies permit the identification of different risk factors involved in the causation of the same disease.

4.4.3. The A-bomb survivor Life-span Study, cancer mortality and cancer incidence

The dramatic experience of the people of Hiroshima and Nagasaki in 1945 was the initiator for a proposal by the National Academy of Sciences of the USA to develop a programme for life-long follow-up of all A-bomb survivors. This programme, started in 1949 by the US Atomic Bomb Casualty Commission (ABCC) and, since 1975, continued by US-Japanese co-operation in the Radiation Effects Research Foundation (RERF), is arguably the largest, most

comprehensive and most detailed epidemiological study ever performed – and it has been decided that even now, more than 60 years after exposure, follow-up will continue. The results of this study are the most important source of information on which the rules and regulations of radiation protection are based. No other epidemiological study has comparable influence. Animal experiments and *in vitro* studies may provide mechanistic information but the RERF studies are the "gold standard" against which all other epidemiological and radiobiological studies on the long term effects of radiations on man have to be judged. The reason for this outstanding role is that in this study a large normal and healthy population of all ages and both sexes who have been exposed to a wide range of radiation doses to all organs of the body. Most important, however, is that through a massive effort, the radiation doses to all critical organs of each member of the cohort has been individually assessed by various methods of retrospective dosimetry.

The Life Span Study is comprised of 120,321 people including about 54,000 atomic bomb survivors who were within 2.5 km of the hypocentre at the time of bomb explosions and about 40,000 survivors who were between 2.5 and 10 km of the hypocentre. The latter were selected by matching them to the cohort closer to the hypocentre which includes everybody who responded to the population census of 1950. In addition, about 26,580 individuals are included who were either temporarily not in Hiroshima or Nagasaki at the time of the bomb explosions or further away from the hypocentre than 10 km. On 1.1.1999, 52% of the study population was still alive, and this includes in particular >85% of the nearly 50,000 individuals who were children or adolescents (<20 years of age) in August 1945. For >90% of the total study population, detailed information was collected by Japanese interviewers in the early 1950s on their exact location at the moment of bomb explosion, not only with regard to the place where they were but also whether they were in a house or outside, the exact structure of the house and the place and position inside the house to permit precise evaluation of shielding parameters. Whereas in the early dose assessments, data from test explosions with regard to kerma in free air were the basis of analysis grossly corrected for shielding in the houses, later analyses (called the DS86) were based on Monte Carlo calculations of track passage from the source in the exploding bomb through the air (particularly taking into account the pronounced attenuation of neutrons by the high humidity), through the building structures of the houses to the body of the individual, finally calculating mean organ doses for different critical organs. In 2002, a refined new dosimetric system (DS02) was published which also took into account the results of various measurements of the products of interaction of neutrons with materials such as bronze statues. With each new dosimetric system the contribution of neutrons to the total dose became less. However, differences between the DS86 and DS02 are small and have little influence on risk estimates.

The most important and most significant long term health damage observed in the LSS of the A-bomb survivors is a dose dependent increased mortality from cancer. The latest analysis of the mortality pattern until 1997 was reported by Preston and colleagues in 2003. Among the 44,771 deceased members of the life span cohort with detailed dosimetric information available, there were 9,335 deaths from solid cancers and 582 deaths from leukaemia. By analysing the relationship with radiation exposure, it was concluded that until 1997 approximately 440 solid-cancer deaths (i.e. approximately 4%) and nearly 100 leukaemia deaths (i.e. approximately 15%) could be attributed to the radiation exposure from the bomb in 1945. Significant relationships with radiation exposure were found for the following types of malignant disease (in decreasing probability of cancer mortality): stomach, colon, lung, leukaemia, breast, oesophagus, bladder, ovary, liver. Since, at the time of the last evaluation of data, nearly 50% of the cohort were still alive, it is not possible to make well-founded statements on the life-time risk of dying from radiation-induced cancer for people who were

young at the time of exposure. The mortality data have the great advantage of nearly 100% coverage due to the unique "koseki" system of registration in Japan (the vital status data and other important information including death certificates of Japanese are held at the same place of registration wherever the individual lives and dies). However, these advantages may be outweighed by the greater precision of cancer diagnosis and the inclusion of non-fatal cancer diseases which are possible in epidemiological studies on cancer incidence. For this reason, cancer registries were established in 1957 and managed by RERF in Hiroshima city and Nagasaki prefecture. Since most cancer patients in Japan are treated in the large hospitals, few tumours are missed in this analysis. The RERF cohorts are routinely linked with the cancer registries to identify cohort members. However, study members who are treated for cancer outside the catchment areas of the cancer registries are not included in the analysis.

In 1994 the first analysis of cancer incidence data between 1958 and 1987 was published. The recent publication of cancer incidence data 1958 to 1998 by Preston et al (2003) is the most comprehensive and detailed analysis of the late carcinogenic effects of atomic bomb radiation and will remain, for the foreseeable future, the gold standard of the assessment of cancer risks after irradiation. The number of cancer cases increased since the last analysis by 50% to 17,448 cases of solid cancer over a period of forty years, nearly 90% of which were verified by histology, endoscopy or surgery. These data permit comprehensive evaluation of radiation risks for fatal and non-fatal malignancies and risks associated with histological types. More importantly, the number of cases was high enough to investigate in great detail temporal patterns, gender differences, birth cohort patterns and age at exposure patterns. The main results are briefly as follows. Of the 17,448 cancer cases observed in this study, 7,851 occurred in individuals who had received a dose of > 0.005 Gy and thus were considered exposed. 853 of these, i.e.11% were attributable to the radiation exposure, but of the 645 cancer observed in individuals exposed to more than 1 Gy, 307, i.e. 48% were attributable to radiation exposure. For a person aged 70 who was exposed to the radiations from the bomb at the age of 30, the excess relative risk (ERR) per Gy was 0.47 for all cancers combined. This ERR was 0.58 for females and thus much higher than for men, for whom it was 0.35. There was strong evidence for a linear increase of excess cancer incidence with increasing dose. 156 of the estimated excess cancers occurred among individuals in the low to moderate dose range of 5 – 200 mGy.

The large data basis of nearly 10,000 cancer cases in non-exposed study members permitted a thorough analysis of the dependence of age-specific cancer rates on birth cohort. These trends complicate the interpretation of the effects of age at exposure on the excess risk, particularly for the ERR (excess relative risk). This became particularly obvious in the analysis of time trends of breast cancer. The apparent strong dependence of the relative risk of radiation-induced breast cancer in the Life Span Study turned out to be due nearly entirely to the birth cohort effect, since baseline breast cancer rates increased dramatically in more recent birth cohorts, yet excess absolute risk still showed a significant age-at-exposure effect. Similarly, the increase in excess relative risk of lung cancer with increasing age at exposure is largely a consequence of the large smoking-related birth cohort effect on lung cancer baseline rates. The major conclusions of the 2007 study on cancer incidence in the Life Span Study are that overall cancer incidence was well described by a no-threshold linear dose response relationship down to doses of < 0.2 Gy. The longer follow-up of the new study demonstrated that the oesophagus and the bladder are particular radiosensitive with regard to radiation-induced cancer which has, however, already been taken into account adequately in the tissue weighting factors based on the older mortality data. The new recommendations of the International Commission on Radiological Protection (ICRP) in 2007 are based, for the first

time, on the cancer incidence data described above whereas until now, recommendations by ICRP were always based on cancer mortality data.

4.4.4. The Chernobyl accident

The Chernobyl accident in 1986 was the most severe accident in the civil use of nuclear energy, so far. It was caused by careless manipulation of safety systems in a nuclear power plant which lead to a core melt-down resulting in the release of a large proportion of accumulated fission products over a period of 10 days until the accident was brought under control. Many thousands of people were evacuated from the near-by town of Pripjat and more people were relocated later. The radioactive cloud changed direction several times during the long period of release and distributed radioactivity, in particular caesium and iodine all over Europe as far as England, Finland and also to Turkey. Several hundred acute emergency personnel were exposed when they worked to contain the accident e.g. by extinguishing the fire on the roof of the turbine hall which threatened to affect the adjacent other reactor building. The severity of exposure was determined using the triage criteria shown in table 1. The most severely affected were treated in Moscow, the others in Kiev. In some of the most severely affected, bone marrow transplantation was attempted but the benefit of this heroic treatment was not convincing. Of the 134 confirmed exposed emergency workers, 28 died in 1986 from acute radiation syndrome, most of them having multi-organ involvement. There were particular problems posed by extensive radiation damage to the skin from smoke particles from the burning graphite which were loaded with beta-rays emitting radionuclides, and these became attached to the wet clothing of the fire-fighters.

In the aftermath of the accident, many thousands of people who were spread all over the former Soviet Union, rescue workers, called liquidators, as well as relocated people who had lived in the contaminated regions close to the reactor, were concerned about possible health damage from the radiation they had been exposed to during and after the accident. It was impossible to set up a comprehensive research programme such as after the Hiroshima and Nagasaki bomb explosions which covered all affected people. However, several epidemiological studies have been initiated and continue to provide important information on health consequences which complement the information gathered from the Life Span Study, in particular the studies performed in the liquidator registers held in Russia, Estonia, Ukraine and Belarus. Even more important, however, is the comprehensive programme of monitoring and treating thyroid cancer in the general populations of Belarus and Ukraine. The results of these studies have been summarised recently by the World Health Organisation (WHO).

Among the 192,000 Russian emergency workers under study, individual radiation doses have been determined for 72,000. The mean dose was approximately 0.1 Gy from external irradiation, and internal radiation doses are assumed not to contribute much to the total dose. There was no increase in overall or cancer specific mortality compared to the general population up to 1998, although more recent data point to a possible increase in the incidence rates of leukaemia. The latest estimate of WHO in 2006 suggested that 4.6% of all fatalities that occurred during 1 year after the accident can be attributed, either directly or indirectly, to radiation exposure associated diseases, among them 2.3% to radiation-induced cancer, 2% to cardiovascular diseases and 0.3% to radiation-induced leukaemia. The liquidator studies are certain to provide much important information in the future on radiation risks from low dose rate radiation exposure.

The most important results of the studies on the populations exposed to radiation from the accident, however, concern the massive epidemic of thyroid cancer among the young which, until 2002, had affected nearly 5000 people who were under 17 in 1986. The data could be

well-fitted to a no-threshold linear dose response relationship with an eight-fold increase of risk after 1 Gy thyroid dose. The highest rate was in those who were children under 4 years of age at the time of the accident. In young adults, the risk may be lower by nearly one order of magnitude. More information is being collected in an on-going cohort study on >25,000 subjects with individual dose estimates who are regularly screened for thyroid disease every two years. There is still considerable uncertainty on details of the shape of the dose response relationship at different ages, and in particular how long the increased risk of thyroid cancer will remain high and whether it may actually follow a relative risk model which would mean that the numbers might continue to increase until 2040. Therefore, it is imperative that these epidemiological studies which are unique in providing reliable estimates on the radiation risks posed by one of the most important fission products released also during normal operations of nuclear reactors in the most radiosensitive organ of the body, i.e. the thyroid of very young children. Great effort went into the estimation of individual radiation doses to the thyroid in the children of Belarus and Ukraine. The most important contribution to those doses came from iodine-131 in milk from cows which were grazing on contaminated meadows. Several weeks after the accident, nearly 350,000 people were assessed for radioactivity in the neck region in order to estimate possible uptake of iodine-131 in the thyroids. From these point measurements of dose rate at the time, total thyroid dose can be estimated but only by making a range of assumptions on the kinetics of intake and the possible influence of thyroid blocking by stable iodine which has been distributed, although too late to have the expected effect. For those individuals who were not directly measured, estimates of thyroid doses were made based on radio-ecological models of iodine deposition, milk consumption etc, with individual factors being included in the calculations based on interviews and measurements of ground contamination of Cs. However, the deposition of caesium did not follow closely the deposition of iodine since maximal release took place at different times. Extensive studies have been performed on the uncertainties associated with the various methods of dose estimation, and the thyroid doses of those directly measured by external gamma-ray monitors appear to be more reliable than those derived using ecological methods, but still a relative standard deviation of a factor of 2 has to be assumed.

Most of the thyroid cancers diagnosed in patients who had been exposed in childhood were papillary cancers. Extensive international pathology review programmes were established to validate each diagnosis. Moreover, a large programme to investigate molecular changes in those cancers to look for fingerprint mutations which would be specific for radiation-induced papillary cancer of the thyroid. The Chernobyl thyroid cancer cases provide a unique opportunity for such a study since >90% of cancers occurring in those born between 1980 and 1986 were radiation-induced whereas < 10% of those occurring in those born after 1987 were radiation-induced. Yet, so far no convincing difference in the frequency of different molecular changes, in particular ret-PTC (rearranged in transformation/ Papillary Thyroid Carcinomas) translocations have been observed if data are corrected for age at diagnosis. The largest group of 741 patients with thyroid cancer who were children at the time of the accident was treated in Minsk. Most were treated by total thyroidectomy (426), the others by less radical surgery. 464 received treatment with iodine-131 for residual cancer or distant metastases. Recurrences were diagnosed in 27% of the cases. So far, few of the patients died from thyroid cancer or treatment related complications, the overall prognosis of these people who are young adults now, appears good.

4.4.5. Patients treated for benign diseases

Up to the 1960s, more patients were treated with radiotherapy for non-cancer diseases than for cancer. The most successful indications were painful degenerative joint disorders such as

osteoarthritis, frozen shoulder, tennis elbow, autoimmune diseases such as ankylosing spondylitis, Dupuytren contracture, endocrine orbitopathy related to hyperthyroidism, and bacterial infections such as mastitis or sweat gland abscesses. Radiation doses were only less than 10 % of the doses typically given to treat cancer, and results were usually fast and persistent. Most of these treatments are regarded as obsolete today, mainly because pharmacological treatment options are available which are more convenient to doctor and patient and, more importantly, since it became increasingly obvious that some of these treatments were associated with a significantly increased risk of later induction of leukaemia and cancer.

Court-Brown and Doll in 1957 analysed the mortality of 14,554 patients who had been irradiated for ankylosing spondylitis between 1935 and 1954 at any one of 87 radiotherapy centres in Great Britain and Northern Ireland. Among the 1,582 recorded deaths, the most striking finding was a tenfold increase in death from leukaemia, 52 patients, compared to the 5 who had to be expected in these patients by comparison with the general population. This excess occurred from the first years up to about 15 years after irradiation. Later follow-up studies confirmed these conclusions. Besides the Life Span Study of the Japanese atomic bomb survivors, this study remains the most important source of information about radiation-induced leukaemia. In a later study irradiated patients were compared to ankylosing spondylitis patients who were managed conservatively without radiotherapy and the radiation risk estimates were confirmed. An increased risk of leukaemia has also been found in epidemiological studies of other groups of patients treated with radiotherapy for benign diseases such as nearly 10,000 women treated between 1925 and 1965 with intrauterine radium or external X rays for bleeding from the uterus compared to women with comparable clinical diagnosis but not irradiated (64 leukaemia deaths compared to 37 expected deaths). Other patients found to have a twofold increased leukaemia risk were treated for peptic ulcer or tinea capitis. In contrast to most other radiation-induced malignancies, radiation-induced leukaemia may become manifest already after a few years, however risk remains increased for at least 25 years after irradiation. The maximum risk depends on the age at irradiation. Children are more sensitive by about a factor of two and have a shorter latency time than adults. The latency of the different leukaemia subtypes shows significant differences with chronic myeloid leukaemia (CML) having the shortest mean latency of approximately five years.

Post-partum mastitis has been one of the most successful indications for low-dose radiotherapy in the past. If irradiated in its early stages, one or two 0.5 Gy fractions would abolish the inflammation within a day or two, no abscess would develop, no antibiotics or surgery would be required and breast feeding could be resumed quickly. Yet in most countries, this indication for radiotherapy has been completely abandoned as the extraordinary radiosensitivity of the breast of young women with regard to cancer induction became apparent. The most important evidence for this comes from the epidemiological analysis of just these patients. Shore et al studied 601 American women who had been irradiated between 1940 and 1957 for acute post-partum mastitis with doses ranging from 0.6 to 11.5 Gy, the median dose being approximately 3.5 Gy which is very much higher than doses that had been given in central Europe for this condition. After a mean follow-up of 30 years, they observed 56 women with breast cancer whereas according to the observation in the patients' sisters, only 32 would have been expected. The dose response curve, however, was indicative of a proportional increase of cancer risk with dose. Mattson et al. studied 1216 Swedish women who between 1925 and 1954 received radiotherapy for acute or chronic mastitis or for fibroadenomatosis with doses ranging from <1 cGy to 50 Gy, the mean dose being 5.8 Gy. They were compared with 1874 women of similar age who had the same diagnosis but were

not irradiated. 198 irradiated women developed breast cancer compared with 101 unirradiated women, and the standard incidence ratio was 3.3. The incidence rate ratio decreased starting after about 25 years but was still increased 40 years after irradiation. 1182 breasts received <2Gy and developed 35 breast cancers - this was not a significant increase but there was a clear dose response relationship within this group of patients. Even irradiation of very young girls, in particular for haemiangioma, increases the risk of developing breast cancer later in life. There was a linear dose response relationship, and 12 % of all breast cancers (8 cases) in this group were attributable to irradiation.

4.4.6. Radon exposure of hard rock miners or in homes

The publication of a report in 1879 on lung disease among the miners in Schneeberg in Saxony was a milestone in the history of occupational medicine. The report was written by a young doctor and a mining engineer who were working in a small town in the ore mountains (Erzgebirge) in Eastern Germany where silver and semi-precious metal such as cobalt and bismuth had been mined since the Middle Ages. It had been known for centuries that the miners there tended to die early from a lung disease called the "Schneeberg mountain illness". By careful medical observation, thorough post-mortem examination, comprehensive study of conditions in the mines and the development of improved working conditions, Härting and Hesse in 1879 showed that (1) the mountain illness was "lymphosarcoma" of the lung, today called small cell lung cancer, (2) only miners working underground developed the disease, usually after about 20 years working underground, (3) every miner who did not die prematurely from other diseases or accidents, died from lung cancer, (4) the induction of lung cancer in the mines is related to the exposure of the lung to toxic substances in the dusty air in the mines which contained e.g. arsenic, (5) cleaning the air by the introduction of wet drilling and forced ventilation reduced the lung cancer rate significantly within 10 years. This was the first study to describe the carcinogenic effects of radiation, but published more than 15 years before the detection of radioactivity by Becquerel in 1896.

The disease was soon ascribed to the exposure of the miners to radon, but only in the 1950s was it recognised that the cause of radon-induced lung cancer were the radioactive decay products of the radon gas which are heavy metal atoms such as lead and bismuth, and which attach to aerosols in the mine air and are inhaled and deposited on the bronchial epithelium causing irradiation of the epithelial stem cells with α-particles. Nearly 100 years after the first description of lung cancer among miners, several large cohort studies of uranium miners, from the USA (Colorado Plateau), Czech Republic (Jachymov, close to Schneeberg) in particular, confirmed the findings in Schneeberg. Radon progeny potential alpha energy exposure was defined as the product of radon concentration corrected for the decay product equilibrium in the mine and exposure time. This measure of exposure was given the name working level months (WLM). Based on detailed modelling investigations which also took into account the size distribution of the aerosols which determines the site of aerosol deposition, radiation doses in Gy have been calculated from these data. With increasing radiation dose a proportional increase of the risk of lung cancer has been determined. Contrary to the old data from Schneeberg which reported the effects of extremely high radon concentrations, all types of lung cancer have been found to be increased in the uranium miner studies although small cell lung cancer showed the highest risk factor. The most important finding was, however, that there is a clear supra-additive interaction between exposure to radon and cigarette smoking. This increased risk of lung cancer from radon has also been found in other hard rock mines with increased radon concentration and is probably an occupational hazard e.g. in tunnel builders etc. Yet, the most important conclusion form these

studies is the possibility of lung cancer risk for the general population from exposure to radon and its decay products in houses.

Extensive measuring programmes have demonstrated that radon concentrations vary by orders of magnitude between houses in the same country and, in some parts with special geological features, radon concentrations can reach values which genuinely cause concern. For this reason, in several European countries and in China large case cohort studies on the contribution of radon exposure to the lung cancer risk have been performed. In the German study which is the largest single study (Wichmann et al. 2005), nearly 3000 cases of lung cancer and 4,200 controls were investigated. In addition to a comprehensive interrogation by a structured questionnaire about demographic characteristics, residence history, life-time active and passive smoking and occupational history, radon concentration measurements were performed in the current homes and in the previous homes, going back 5 to 35 years using alpha-track detectors. As expected the most important risk factor for lung cancer was cigarette smoking: >95 % of the lung cancer patients were current smokers or ex-smokers while among the controls this number was little more than 60%. There was a strong relationship between the number of packs smoked and relative risk, reaching 46 fold increase in relative risk for heavy smokers (>20 cigarettes per day for more than 30 years). Despite this strong influence of smoking, a clear dependence of relative risk on radon concentration in the homes was observed, as was the case in most other studies e.g. in Finland, Sweden and the United Kingdom. The overall excess lung cancer risk at a radon concentration of 100 Bq/m³ was 10%. The excess risk from radon was found for smokers as well as for non-smokers, and risks interacted in a multiplicative way. A similar result had been found in a meta-analysis of older studies and, what is even more important, this risk value is very close to the predicted risk based on the analysis of the miner data.

4.5. Heritable radiation effects

In the public perception, the most dangerous long term risk of environmental, occupational of medical radiation exposure may be the risk of heritable damage to children and future generations. Up to the 1970s, rules and dose limits in radiation protection were mainly concerned with heritable radiation effects. Radiation exposure of the general population from diagnostic procedures in medicine and from atmospheric atom bomb test explosions were generally recorded and reported as genetically significant radiation doses which were calculated as radiation doses to the gonads corrected for the age and sex dependent probability of having children. Since then, a complete re-evaluation of the risks of heritable radiation damage has taken place.

No significant increase in heritable diseases was found in a study on 70,000 children of Japanese A-bomb survivors whose parent had received a conjoint radiation dose to their gonads of approximately 0.15 Gy on average. Moreover, no dose dependent increase in the frequency of biochemical mutations was found. The fact that each person is genetically unique and different from even his brothers and sisters makes the direct determination of heritable radiation effects of low radiation doses in man virtually impossible. Such studies just lack the required sensitivity and are prone to all sorts of bias. In addition to the Japanese A-bomb survivor studies, a few studies have been performed on children of radiotherapy patients that confirm this conclusion. For this reason, in contrast to the risk of radiation-induced cancer and radiation-induced developmental damage, radiation-induced heritable damage cannot be determined by epidemiological research but has to be based on the results of animal experiments. The present concepts of the risks radiation-induced heritable diseases combine experimental data on the dose dependence of mutation rates in the various stages of

germ cell development with epidemiological data on the spontaneous frequency of naturally-occurring genetic diseases, with different patterns of inheritance, and with mathematical models developed in population genetics research to describe the equilibrium between mutation and selection and the dynamics of mutant genes in populations.

Mutation rates after irradiation of highly inbred strains of mice have been determined in large breeding experiments which involved millions of mice. The most important of those studies used the seven-locus method. By extensive inbreeding and crossbreeding, mouse strains were developed which are homozygous for seven different genes with recessive Mendelian inheritance. Each of these genes produces, in the homozygous genotype situation phenotypes that are characterised by changes in features which can be identified easily even in the early weeks of life of the affected animal. The experiment is based on the irradiation of wild-type animals and subsequent mating of the irradiated animals with homozyous partners. If no mutation is induced in the irradiated animal, all progeny of the mating show the phenotype of wild-type mice. However, if a mutation in a germ cell is induced, the phenotype typical for the respective mutation will be visible in the progeny which inherits this mutation along with the mutation from the other parent. The results of the experiment with low dose rate irradiation are given in Table 4.2.

TABLE 4.2 MUTATION RATES AFTER LOW DOSE RATE IRRADIATION IN THE SEVEN LOCUS TEST

Dose	Number of offspring	Number of mutations	Mutation rate per locus per million gametes
0.9 Gy	-	-	8
0.9 Gy	59,810	6	13
3 Gy	108,026	25	33
6 Gy	59,711	23	55
8.6 Gy	24,281	12	71

These results suggest an increase in the mean mutation rate per locus which is proportional to dose. The spontaneous mutation rate of approximately 1 in 100,000 is doubled by approximately 1 Gy. This is called the doubling dose at low dose rates. Other experimental models have also been used to determine the mutation rates after irradiation such as skeletal disorders with dominant inheritance, cataracts with dominant inheritance, recessive lethal mutations, dominant lethal mutations, mutations coding for enzymes and chromosome translocations. The spontaneous mutation rate per gene is similar in mice and men. There is no good reason to assume that in humans, the doubling dose may differ significantly from that in mice. However, the mutation doubling dose alone does not give any useful information on the risk of heritable disease. The relationship between and increase of mutation rate and increase of frequency of heritable diseases is complex and depends on the specific phenotype and its influence on the health of the affected person, on the pattern of inheritance and the interaction of the mutated gene with other factors in multifactorial diseases. Therefore, the mouse doubling dose is combined with information derived from human population genetics to estimate the risk of heritable disease in the progeny of irradiated people.

Heritable diseases may occur as direct result of a mutation in a single gene (single gene disorders). Inheritance of these diseases follows the rules established by Mendel, and may be autosomal dominant, autosomal recessive or sex-linked recessive. For these "Mendelian" diseases, there is a straightforward relationship between mutation and disease and the pattern of transmission is simple and predictable. Data from human population genetics give an overall frequency of Mendelian diseases in the population of 2.4% (1.5% autosomal dominant, e.g. Huntington´s disease, 0.75% autosomal recessive e.g. phenylketonuria, and 0.15% sex-linked recessive, e.g. haemophilia). A new mutation in a gene with dominant inheritance following the Mendel rules of single gene inheritance will directly lead to the respective phenotype. In most instances this mutation will be passed on to later generations, if the impact on the health of the affected is low, in particular in the first few decades of life. It may be transmitted through many generations since its influence on the Darwinian selection will be small. It will be eliminated in the first generation if it causes death before the affected had any chance to have children. This balance between mutation and selection is the basis of population genetics theory. This relationship is described by the mutational component which mathematically describes the ratio of increase in mutation rate to increase in disease rate. For the known heritable diseases with dominant inheritance the mean mutational component is 0.3, which means that, on average, new dominant mutations stay in the population for 3 generations. For recessive mutations, the mutational component is close to 0. In addition to single gene disorders which have a frequency at birth of 3.3 %, approximately 6% of live births are affected by a congenital abnormality with some genetic component and 65% of the population will develop, later in life, chronic disease with some genetic component as well, although environmental factors play a much bigger role. These are called multifactorial diseases and comprise common diseases such as diabetes, essential hypertension and coronary heart disease. This complexity of heritable diseases is incorporated in the present method of estimating the heritable risk among the progeny of irradiated people.

The equation to calculate genetic risk combines population genetic data in humans and radiation genetic data in mice as follows:

$$\text{Risk} = \text{Prevalence} \times 1/\text{Doubling Dose} \times \text{Mutation Component} \times \text{PRCF}$$

Risk is the probability that an offspring of the exposed person will develop heritable disease of one of the groups described above (Mendelian or Multifactorial). The prevalence data are given above. For protracted irradiation, the accumulated dose in the gonads before conception is divided by 1. The mutation component is a factor which describes the relationship between the increase in the mutation rate and the rate of additional disease. Even for dominant diseases this factor is not 1, since the majority of existing mutations are inherited from parents and grandparents, often through many generations. A cautious estimate suggests that doubling of the rate of new dominant mutations will cause only a 30% increase of diseases with dominant inheritance in the first generation and 15% in the second generation. The same value of the Mutation Component is allocated to sex-linked recessive diseases. Since the development of single gene disease with recessive inheritance requires mutations in both alleles of the same gene, the relationship between a mutation and disease is very remote and the mutation component is therefore assumed to be close to zero. For multifactorial diseases, the relationship between mutation and disease is also not very close, and presently the mutation component is assumed to be 0.01.

The potential recoverability correction factor (PRCF) has been introduced to account for the fact that the molecular structure of radiation-induced mutations differs markedly from the molecular structure of "spontaneous" mutations, in that most spontaneous mutations are point

mutations with a single base pair altered or a minute deletion whereas radiation-induced mutations are mostly large deletions, often affecting whole genes. Depending on the gene affected these mutations may not be compatible with inter-uterine development and most will lead to premature termination of pregnancy. A cautious estimate is that 30% of radiation-induced mutations are not leading to intrauterine death and thus may be "recovered" at birth. Thus, the value of PRCF suggested today is 0.15 to 0.3.

Using this equation for estimating the risk of heritable diseases of a young man who had been exposed to a radiation dose of 1 Gy from radiotherapy e.g. of pelvic lymph nodes in Hodgkin´s disease, the risk of radiation-induced dominant and sex-linked heritable disease would be:

$$\text{Risk} = 0.0165 \times 1 \times 0.3 \times 0.3 \times 0.5$$

The last factor of 0.5 has been introduced to account for the fact that only the father was irradiated. The result is a risk of less than 0.1%. The risk of multifactorial disease is similar at < 0.1% and the risk for radiation-induced recessive disease among the children is essentially zero.

In addition to single gene disorders and multifactorial diseases, another class of heritable damage which is rare in the general population with a spontaneous frequency of 0.2%, but which may be particularly important after radiation exposure to the gonads, is developmental injury. Such developmental defects may affect multiple organs. Recent investigations of the potential molecular manifestations of genetic damage induced in the germ cells of irradiated individuals and transmitted to the progeny suggested that the genetic consequences of radiation exposure are mainly related to micro-deletions, i.e. deletions of multiple, functionally unrelated, yet physically contiguous genes that are compatible with survival of the individual receiving them. Such micro-deletions are known to cause multi-system congenital abnormalities which share some common features such as mental retardation, growth retardation, and various malformations. Unlike the majority of congenital abnormalities which are typical multi-factorial disorders, these abnormalities would show the same inheritance pattern as autosomal single gene diseases. These diseases are rare. The estimation of the risk of multi-systems congenital abnormalities resulting from radiation-induced micro-deletions does not use the "doubling dose method" but rather uses experimental data on radiation-induced skeletal mutations and cataract mutations with dominant inheritance in mice for which such a molecular mechanism of mutation induction is likely. The estimated risk for multi-system developmental abnormalities is similar as for heritable diseases caused by single gene mutations (approximately 0.1% per Gy or Sv to one parent).

The risks of radiation-induced heritable diseases have been estimated indirectly on the basis of mouse data on induced mutation rates, so far. Yet, the progress of molecular radiation genetics and closer understanding of the molecular basis of the different heritable diseases in humans offer the prospect to determine the effects of radiation exposure of humans on the risk of heritable diseases in the progeny directly. The assumption underlying all previous risk estimates was that radiation would equally cause all different classes of heritable diseases. Yet it has become clear in recent years that radiation-induced heritable diseases are most likely to be genomic disorders rather than single gene disorders. Genomic disorders are multi-system developmental abnormalities, most often associated with mental retardation and other neurological defects, and are caused by deletions or gene duplications. These occur spontaneously, predominantly by non-allelic homologous recombination in meiosis, in

particular in oocytes. Similar effects are likely to occur as a consequence of repair of radiation-induced DNA double strand breaks in specific stages of meiosis, leading to genomic changes which might be specific for radiation induction permitting their direct identification against the huge background of heritable diseases caused by spontaneous mutations.

4.6. Effects on the developing embryo

More than 1,500 children born between September 1945 and March 1946 in Hiroshima and Nagasaki were investigated at regular intervals between 1948 and 1964 to study the effects of radiation doses between 0.01 and >1 Gy on intra-uterine development at different stages of pregnancy. This study remains, until today the only reliable source of information on the radiosensitivity of the unborn human.

Experimental studies in mice demonstrated a wide range of characteristic malformations such as spina bifida, exencephaly or bone malformation of the extremities at doses well below 1 Gy. The type of malformations showed a very strict dependence on the stage of pregnancy. A difference of 12 hours often resulted in very different types of malformations and in different organs. Yet, in the children of Hiroshima and Nagasaki no such malformations were found increased in a dose dependent way. However, there were 18 children who presented with micro-cephaly and severe mental retardation. The mothers of 15 affected children had been exposed to radiations from the bomb explosions at close distance from the hypocentre when they were in week 8 to 15 of pregnancy, while 3 affected children were exposed in later stages of pregnancy. Findings of dystopic grey matter by MRI investigations (magnetic resonance imaging) of some of those severely retarded people are in accordance with the results of experimental studies on the effects of radiation doses < 1Gy given to pregnant mice in late pregnancy. These experiments demonstrated that migration and maturation of immature neural cells during the development of the forebrain was severely disturbed. The result of this disturbance of migration was severe disorganisation of the structure of the synaptic network in the brain.

The development of the mammalian brain is very radiosensitive over long periods of pregnancy due to the prolonged duration of cell formation and maturation processes. Throughout the process of brain development, damage and compensation processes occur side by side leading to a very complex pattern of radiation response. The most particular effect of radiation on brain development in the period of enhanced sensitivity is during cortical cell formation which is associated with extensive migration of cells formed in the ventricular regions towards the surface which determines the later organisation of the neuronal network. Even radiation doses as low as 0.1 Gy cause significant disorientation of the cytoarchitecture and the neuronal network. Neurophysiological alterations appear to be related to those structural defects caused by radiation doses of <0.2 Gy which affect neuronal cell migration, branching, apoptosis of individual cells causing failure of anchoring of neuronal synapses and axon process formation. In animal experiments, radiation doses of <0.3 Gy given in the period of enhanced radiosensitivity i.e. in the period of corticogenesis, may lead to neurofunctional damage such as changes in the electroencephalogram (EEG) patterns, behavioural changes, deficiency in learning and memory and seizures. Similar radiation effects are likely to occur in the human brain, although the plasticity and capacity for compensation is so pronounced that clinical damage may not be obvious unless radiation doses exceed a certain threshold.

Damage to intrauterine development was found in none of the experimental studies in mice after doses < 0.1 Gy. Also in the studies of the Hiroshima children there was evidence for a threshold dose of >0.1 Gy. At higher doses, the risk of severe mental retardation increases to a value of 40% at 1 Gy. In later stages of pregnancy, the threshold dose may be higher. At the

age of 10, all children who were exposed *in utero* to radiation from the A-bomb explosions had an IQ test. Also the school performance at the same age was analysed. There was statistical evidence for a dose dependent decrease of the mean IQs as well as the mean school performance scores of those children who were exposed in weeks 8 to 15 and 16 to 25 to doses >0.1 Gy. No decrease of intellectual development was recorded if irradiation had occurred before week 8 or after week 25, even if radiation doses were >0.5 Gy.

Embryos in the pre-implantation stage are very radiosensitive. However, the radiation damage inevitably will lead to death of the embryo and early abortion. Those embryos, however, which survive develop normally. In human embryos in the first few weeks after implantation during the period of major organogenesis, a comparable all-or-nothing effect is likely i.e. either an early, spontaneous abortion or normal development. The results of these studies as well as of some follow-up studies and anecdotal reports after medical exposures demonstrate the high radiosensitivity of the developing embryo and foetus, in particular during the time of brain development. The findings of a probable threshold of 0.1 Gy will influence the advice to be given to pregnant women after a diagnostic radiology procedure. In particular after abdominal CT investigations, careful analysis of radiation doses in the uterus has to be performed. A recommendation of termination of pregnancy because of possible radiation injury is very unlikely in most cases of women exposed in diagnostic radiology procedures either because radiation did not occur in weeks 8 to 15 or because radiation doses to the uterus from most radiological procedures was well below 0.1 Gy.

4.7. The system for radiation protection

Given the effects that radiation exposure can lead to, there is clear need to have a system that affords appropriate levels of radiation protection in situations where radiation is being used or is present.

There is much guidance on what is required for radiation protection, and in particular there are 3 important international players. First, the United Nations Scientific Committee on the Effects of Atomic Radiations (UNSCEAR) closely monitors the progress of radiation research and publishes extensive, critical and authoritative reviews at regular intervals on the sources and levels of global radiation exposure, and on the biological effects of ionising radiation. The International Commission on Radiological Protection (ICRP), an independent charity, issues recommendations on radiation protection, based primarily on the scientific foundation provided by the UNSCEAR reports. The advice of ICRP is aimed principally at authorities, bodies and individuals that have responsibility for radiological protection but it does not provide regulatory texts. In essence the ICRP develops policy. Finally, the International Atomic Energy Agency (IAEA), a member of the UN family, uses the ICRP recommendations as the basis for developing regulatory-style radiation protection requirements. These IAEA Safety Standards, comprised of Safety Fundamentals, Safety Requirements and Safety Guides, provide the basis for the regulation of radiation protection in many countries of the world, especially in the so-called developing world. The IAEA also assists its Member States in the application of these Safety Standards.

This section on the system of radiological protection is only a brief overview, with particular emphasis on the risks of health effects arising from low dose exposures, and how doses from low dose exposures are assessed from a radiation protection perspective. For more detail on radiation protection, reference should be made to the many publications of the ICRP and the IAEA, among others.

In its 2007 Recommendations, the ICRP introduced 3 types of exposure situations to cover all conceivable circumstances. *"Planned exposure situations"* are situations involving the deliberate introduction and operation of sources. Planned exposure situations may give rise both to exposures that are anticipated to occur (normal exposures) and to exposures that are not anticipated to occur (potential exposures). *"Emergency exposure situations"* are situations that may occur during the operation of a planned situation, or from a malicious act, or from any other unexpected situation, and require urgent action in order to avoid or reduce undesirable consequences. *"Existing exposure situations"* are exposure situations that already exist when a decision on control has to be taken, including prolonged exposure situations after emergencies. The ICRP also distinguishes between 3 categories of exposures: occupational exposures, incurred by workers in the course of their work; medical exposures, incurred by patients for the purpose of diagnosis or treatment; and public exposure, incurred by members of the public from sources in any planned exposure situation, existing exposure situation, or emergency exposure situation.

Radiation protection needs to address all exposure situations and all categories of exposure. The ICRP has long espoused three principles of radiation protection – the principles of justification, of optimisation and of application of dose limits. The principle of justification aims to ensure that any decision that alters the radiation exposure situation should do more good than harm. The principle of optimisation of protection is that the likelihood of incurring exposures, the number of people exposed, and the magnitude of their individual doses should all be kept as low as reasonably achievable, taking into account economic and societal factors. These first two principles apply to all exposure situations – planned, existing and emergency. The third principle of application of dose limits applies only to planned exposure situations, and in effect is a "safety net" applied after the first two principles have been implemented. Dose limitation means that the total dose to any individual from all planned exposure situations other than medical exposure to patients should not exceed the appropriate limits specified by the ICRP.

4.7.1. The derivation of risk coefficients and organ weighting factors from epidemiological data

Radiation exposure can lead to many harmful health effects. Such effects were classified by ICRP in 1990 into deterministic and stochastic effects, and both categories of effect are considered by the ICRP in establishing dose limits in their recommendations. Radiological protection aims at avoiding deterministic effects (also called “tissue reactions” in the new ICRP recommendations in 2007) by setting dose limits below the threshold at which they occur. Stochastic effects (called “cancer/heritable effects” in the new ICRP recommendations) are believed to occur even at the lowest doses and therefore have to be taken into account whatever is the radiation dose. With this understanding, the dose limits cannot prevent such stochastic effects, but in stead aim to reduce their likelihood to acceptably low levels. The term “detriment” was introduced by the ICRP as a measure of the harmful health effects to individuals or descendants of the exposed individual that could occur as a result of radiation exposure at low doses. In general, the detriment in a population is defined as the mathematical expectation of the induction of cancer and hereditary damage caused by an exposure to radiation. Detriment is a complex concept combining the probability, severity and time of expression of radiation harm.

Detriment is assessed by the calculation of the effective dose which takes into account the total risk attributable to the exposure of all tissues irradiated. The fundamental dosimetric quantity in radiological protection is the absorbed dose. The detriment depends not only on

the absorbed dose but also on the type and energy of the radiation causing the dose, in particular the LET of the radiation. This is taken into account by weighting the absorbed dose by a factor related to the quality of radiation. The radiation weighting factor is selected for the type and energy of the radiation incident on the body of, in case of sources within the body, emitted by the source. The weighted absorbed dose is the product of the absorbed dose averaged over the respective tissue or organ and is called "equivalent dose". The "effective dose" is derived by weighting the equivalent doses of the different tissues and organs by a factor which represents the sensitivity of the respective tissues and organs to stochastic effects, primarily radiation-induced cancer. Thus, the detriment for all radiations is expressed as effective dose E which is measured in sieverts (Sv). The values of the radiation weighting factors for a specified type and energy of radiation has been selected to be representative for values of the relative biological effectiveness (RBE) in inducing stochastic effects at low radiation doses. The radiation weighting factors recommended by ICRP in 2007 are shown in Table 4.3.

TABLE 4.3 RADIATION WEIGHTING FACTORS (ICRP 2007)

Radiation type		Radiation weighting factor
Photons and electrons, all energies		1
Protons and charged pions		2
Alpha particles, fission fragments, heavy ions		20
Neutrons*	$E_n < 1$ MeV	$2.5 + 18.2\, e^{[-\ln En]2/6}$
	$1 \text{ MeV} \leq E_n \leq 50$ MeV	$5.0 + 17.0\, e^{[-\ln 2En]2/6}$
	$E_n > 50$ MeV	$2.5 + 3.25\, e^{[-\ln 0.04En]2/6}$

*) these values have replaced the following step values that were recommended in 1990: < 10 keV, 5; 10 keV to 100 keV, 10; 100 keV to 2 MeV, 20; 2 MeV to 20 MeV, 10; >20 MeV, 5.

The tissue weighting factors were intended to ensure that a weighted tissue equivalent dose would produce the same degree of detriment irrespective of the tissue or organ involved. They are representative values averaged over age at exposure and sex and, besides the risk for fatal cancer in the respective organs, also make allowance for different losses of life span, for the morbidity resulting from the induction of non-fatal cancers and for the risk of serious hereditary diseases in the first two generations of descendants of the irradiated individual. On the other hand, any possible health damage arising from developmental damage *in utero* such as severe mental retardation is not included in the tissue weighting factors.

The period of observation of exposed populations does not extend to a full life time in any of the major epidemiological studies. Therefore, it is necessary to project the probability of cancer induction or mortality from the period of observation to the full lifetime of the exposed population. This is done using two alternative projection models: (1) the absolute or additive risk model predicts a constant excess of induced cancers throughout a life-time unrelated to the spontaneous cancer rate, (2) the relative or multiplicative model predicts that the excess of induced cancers increases as a constant multiple of the age-dependent spontaneous rate. By averaging the results of the dependence of risk on age, sex, projection model and their influence on the base-line cancer risks in different populations e.g. those of Japan, United

States of America, United Kingdom and China, the relative probabilities of cancer after irradiation of different for a nominal world population of all ages were derived. These form the basis for the tissue weighting factors, taking also into account the expected number of years of life lost due to the different types of radiation-induced cancer, which is highest for leukaemia and breast cancer (since both have a high mortality and early onset) while lung, stomach and colon have a lower value due to their appearance later in life, despite having similar lethality.

In 2007, ICRP recalculated its nominal risk, taking into account in particular the latest development in genetic risk estimation and the results of the cancer incidence data from the Japanese Life Span Study. From the cancer incidence data, sex-averaged nominal risk coefficients for cancer were calculated which are adjusted for lethality and quality of life. On the basis of these calculations, ICRP proposed nominal risk coefficients for detriment-adjusted cancer risk as 5.5% per Sv for the whole population and 4.1% per Sv for adult workers. For hereditary effects, the detriment adjusted nominal risk in the whole population is estimated at 0.2% per Sv, and in adult workers as 0.1% per Sv (Table 4.4). The most significant change from 1990 is the 6 – 8 fold reduction in the nominal risk coefficient for hereditary effects as a result of the considerations described above.

TABLE 4.4 DETRIMENT ADJUSTED NOMINAL RISK COEFFICIENTS (% PER SV) FOR CANCER AND HEREDITARY EFFECTS (ICRP, 2007).

Exposed population	Cancer		Hereditary effects		Total	
	2007	1990	2007	1990	2007	1990
Whole	5.5	6.0	0.2	1.3	6.0	7.3
Workers	4.1	4.8	0.1	0.8	4.0	5.6

Based on these values ICRP in 2007 confirmed that their previous overall fatal risk coefficient of 5% per Sv continues to be appropriate for radiation protection puposes. While the overall fatal risk coefficient has not changed compared to 1990, the value of some of tissue weighting factors was significantly altered as shown in Table 4.5**.**

TABLE 4.5 RECOMMENDED TISSUE WEIGHTING FACTORS

Tissue	ICRP 2007	ICRP 1990
Bone marrow, colon, lung, stomach,	0.12	0.12
Breast	0.12	0.05
Remainder *)	0.12	0.05
Gonads	0.08	0.2
Bladder, oesophagus, liver, thyroid	0.04	0.05
Bone surface, skin	0.01	0.01
Salivary glands, brain	0.01	part of remainder*)

*Remainder tissues: adrenals, gall bladder, heart, kidneys, lymphatic nodes, muscle, oral mucosa, pancreas, prostate, small intestine, spleen, thymus, uterus, extrathoracic region. The

tissue weighting factor for the remainder tissues applies to the weighted mean doses of the 13 listed organs which are added up to be multiplied by the tissue weighting factor of 0.12.

At radiation doses below 100 mSv in a year, the increase in the incidence of stochastic effects is assumed by ICRP to occur with a small probability, and in proportion to the increase in radiation dose over the background dose. Use of this so-called linear, non-threshold (LNT) model is considered by ICRP to be the best practical approach to managing risk from radiation exposure and a prudent basis for radiological protection.

Effective dose and tissue weighting factors are to be used for prospective dose assessment for planning and optimisation of protection of the general population or for working populations but not for calculating risks for individuals or specific populations such as young women. As stated in ICRP 2007, § 157: "effective dose is intended for use as a protection quantity on the basis of reference values and therefore is not recommended for epidemiological evaluations, not should it be used for detailed specific retrospective investigations of human exposure and risk. This is especially important in cases of individual doses exceeding dose limits".

4.7.2. Dose limits

Implementation of the third ICRP principle of application of dose limits for planned exposure situations (excluding medical exposures) requires the setting of values for the dose limits. As said above, the values of the dose limits should ensure the avoidance of deterministic effects or reduce the risk of stochastic effects to acceptable levels, as relevant. Despite the changes in the nominal risk coefficients underpinning the dose limits, as discussed above, the values given in ICRP 103 are the same as those in their previous recommendations given in ICRP 60. It should be noted that new data on the radiosensitivity of the eye ares expected to become available, and these will be reviewed by the ICRP in terms of any change needed to the dose limit for the lens of the eye. While dose limits are expressed in terms of dose quantities and are part of the system of radiation protection, their heritage is in radiation biology and radiation effects.

For occupational or public exposure, arising from planned exposure situations, an individual may be exposed by several sources, so an assessment of the total exposure has to be attempted which includes all sources causing exposure to the individual. The doses from this total exposure are compared with the appropriate dose limits. Table 4.6 shows the dose limits for planned exposure situations to the public and to radiation workers.

TABLE 4.6 DOSE LIMITS FOR PLANNED EXPOSURE SITUATIONS (ICRP 2007)

Type of limit	Occupational	Public
Effective dose	20 mSv per year *)	1 mSv per year **)
Annual equivalent dose		
In lens of the eye (under revision)	150 mSv	15 mSv
Skin ***)	500 mSv	50 mSv
Hands and feet	500 mSv	--

*) the 20 mSv value applies to the average value over a period of 5 years with the additional provision that in any one year the dose should not exceed 50 mSv. Moreover, once a woman has notified pregnancy to the employer, her exposure for the remainder of the pregnancy should not exceed 1 mSv since the embryo/foetus is considered the same as the public.

**) the 1 mSv value applies to the average value over a period of 5 years.

***) skin dose average over 1 cm² area of skin

4.7.3. Risk-benefit considerations in breast cancer screening using mammography

In medical uses of radiation, all exposures should be, as a result of medical indications, for the patient's diagnosis or treatment. This means that both the risk and the benefit apply to the same person. Moreover, radiation risks are usually negligible compared to the benefit of the individual. In any case, radiation protection for the patient is afforded through the application of the principles of justification and optimisation.

The situation is very different in radiological screening programmes for early diagnosis of specific diseases such as cancer. In any screening programme, for each person who will be identified by the screening exposure at an early stage of disease and who might therefore have a better change of cure, there will be hundreds or thousands of healthy people exposed to radiation who do not have the disease and who will not benefit directly from the radiation exposure. The most important example is mammography screening for early breast cancer too small to be found using clinical examination.

Breast cancer is the second leading cause of cancer death for all women and the leading cause of death in women between the ages of 30 and 55 years. Age standardised breast cancer rates vary markedly between countries, the lowest rates are reported from China and Japan (20 to 35 per 100,000), the highest rates in white American and some European women (75-100 per 100,000). The probability of cure depends critically on the risk of the primary cancer having already spread to lymph nodes and distant sites, which is directly related to the size of the primary breast cancer. If the primary cancer is smaller than 1 cm, the risk of distant metastasis which would preclude cure is less than 10%, while if the primary is larger than 4 cm the risk of distant metastasis would be over 50%. Therefore, the smaller the primary tumour at diagnosis the greater is the chance of cure. Clinical methods such as palpation are not reliable detecting tumours smaller than 1 cm while mammography is very capable of doing this. Therefore, it is expected that regular investigation of a breast with mammography will detect cancers at a much smaller size than regular palpation, and by this way increase the chances of cure and decreasing the risk of premature death from breast cancer. Yet, in each mammographic investigation with state-of-the-art methods, the breast is exposed to a dose of 4 mGy which varies much between women depending on the size of the breast. Since the breast is one of the most sensitive organs of the body with regard to radiation-induced cancer, the radiation doses from mammography have to be kept as low as compatible with the diagnostic aims and be justified by the expected decrease in breast cancer mortality. Elevated breast cancer risk following radiation exposure has been demonstrated both in the Life Span Study of A-bomb survivors as well as in different cohorts of medically exposed women.

Among the 29,700 women of the Life Span cohort of the A-bomb survivors who had received a dose of >5 mGy to the breast, 173 died from breast cancer by 1997. Analysis of the dose dependence of risk provided strong evidence for a linear dose response relationship and a strong dependence on age at exposure with decrease of risk with increasing age, the risk becoming insignificant if exposure occurred at ages over 50. Both the relative risk model and the absolute risk model fitted the data equally well. Altogether 24% of the 173 cases were attributable to the radiation exposure, the excess relative risk per Gy was 0.79 (with 90% confidence limits of 0.29 to 1.5), and the excess absolute risk was 1.6 per 10,000 person-years. The most important epidemiological data from medical radiation exposure of the breast come from studies in tuberculosis (TB) patients treated with pneumothorax under

fluoroscopic control and from women given radiotherapy for bacterial infection of the breast after giving birth (postpartum mastitis). In the biggest TB study, there were 349 breast cancer deaths in 13,078 women (compared to 237 deaths expected), and in the most important mastitis study, there were 210 breast cancers in 3,034 women. A pooled analysis of these and several other studies showed differences between studies. However, all of them confirmed that radiation-induced breast cancer occurs at ages similar to those at which breast cancers are seen in the absence of exposure, that the excess risk increases linearly with radiation dose, and that age at exposure and attained age both have a great influence on the risk of radiation-induced breast cancer.

However, there are also other risks of mammography screening which may be even more important than radiation-induced breast cancer such as: (1) false negative mammograms which may give inappropriate reassurance, and diagnosis of breast cancer and treatment may be delayed. Up to 25% of invasive breast cancers are not detected by mammography in 40 – 49 year old women compared to only 10% in women older than 60, (2) false positive mammograms, more common in younger women, cause unnecessary interventions such as biopsies and psychological stress, (3) overtreatment e.g. of ductal carcinoma in situ, which is frequently diagnosed by mammography especially in younger women, but some ductal carcinoma will not progress to invasive cancer. Since the radiation risks of breast cancer induction are lower after age 50 years and the rate of false negatives as well as false positives is reduced as well in older women, and in particular, since the incidence rate in women over 50 is much higher than in younger women, it has been recommended to perform mammography screening for breast cancer only in women aged 50 and older. In several large epidemiological studies, in particular in Sweden, a reduction of mortality from breast cancer due to mammography screening of over 20% was determined. Depending on the assumptions of radiation risks, improvement of therapeutic outcome and breast cancer incidence, different ratios of benefit versus risk have been calculated but for women over 50, all estimates have been very positive with a benefit to risk ratio ranging from 20 to >100. These results were the basis of mammography screening programmes on a national basis in many countries.

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