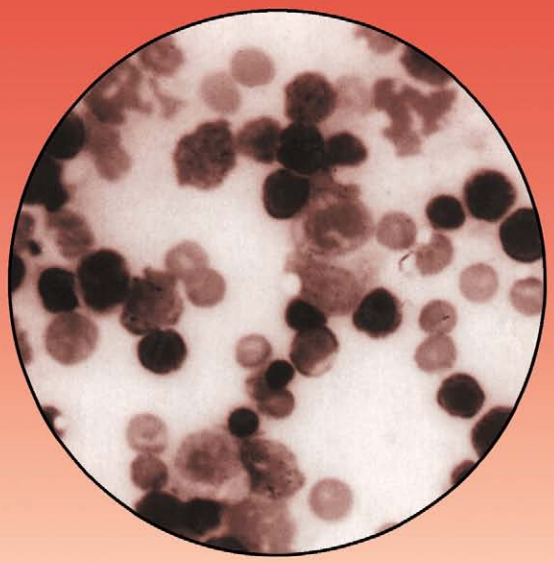
RADIATION TOXICOLOGY

BONE MARROW AND LEUKAEMIA



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To Professor Laszlo G.Lajtha, CBE, MD, DPhil, FRCP(E), FRCPath (1920–1995),

distinguished haematologist and radiation scientist, our mentor and close colleague

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Preface

The bone marrow is one of our essential organs: it provides us with our large daily requirement of red cells for carrying oxygen around the body via the blood, white cells for guarding against infections, and platelets for sealing any haemorrhages. The large production rate of cells is a consequence of a rapid tumour rate of precursor cell populations, and these latter can be injured by radiation, resulting in some very dramatic effects.

Low doses of irradiation can produce cell abnormalities which lead to leukaemia. High doses of radiation kill many cells and can reduce the incidence of leukaemia. Even higher doses produce much damage in the haemopoietic tissue and may be lethal to the individual unless measures are taken to improve recovery.

In this book we have asked experts in their fields to cover all these facets in some detail. This includes radiation dose levels and epidemiological studies of exposed populations, as well as treatment methods for accidentally exposed individuals or therapeutically irradiated patients. The first chapter is a general summary of all the aspects subsequently discussed in full. Chapter 2 links this summary with the later chapters by sketching the processes involved in blood production. It is hoped these two chapters, in addition to introducing the book, can stand alone to introduce the budding school scientist and interested layperson to a fascinating subject. The more detailed components are aimed at scientists and students working in haematology or in radiation studies, industrial health personnel and people involved in the teaching of these subjects.

We are grateful to Ann Kaye for her help with the editorial procedures here in Manchester, to the publishers for producing the book and to the Cancer Research Campaign (UK) for supporting our scientific endeavours.

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1.1
Introduction

It is a hundred years since the discovery of X-rays by Wilhelm Roentgen (1895) and nearly as long since the discovery of natural radioactivity of uranium by Henri Becquerel (1896). Pierre and Marie Curie came a little later in their discovery of the radioactivity of radium. Until recently, their name was honoured with the unit of radioactivity but a more soundly based physical definition on the basis of a nuclide's disintegration rate became necessary, and that honour reverted to Becquerel himself.

It is fair to say that, at that period in the history of radiation, these pioneer scientists did not recognise the potential dangers of their discoveries. Roentgen, for example, used to delight his friends by placing his hand in a beam of X-rays to expose the ghostly, skeletal form of his bone structure. In time, his hand suffered irreparable structural damage. Pierre Curie, his wife Marie and daughter Irene, all developed leukaemia from the constant 'delights' of the glow from radium. In fact, many of those pioneers died from some form of radiation disease, except Pierre—a road accident got him first.

In spite of Roentgen's little difficulty with his hands, X-rays were quickly recognised as a potential medical diagnostic aid and, even before the turn of the century, the British army was using a mobile X-ray unit to locate bullets and shrapnel in soldiers wounded at the battle of Omdurman in the Sudan. The physical bases of dose determination were not yet appreciated, and for several decades doses were estimated on a photographic exposure-match to a standardised, graded (black to white) colour chart and known as Holzknecht Pastiles. Holzknecht units, on this colorimetric scale, were doses in the neighbourhood of 1 Gy (see below). The ionising properties of X-rays were eventually recognised and doses became more reliably evaluated in terms of Roentgens (R) associated with the number of ion pairs produced. Similar ionising strengths from different sources and types of radiation did not always have the same biological effects, however, and measurement of dose was rationalised to recognise the amount of radiation energy absorbed in tissue. The dose was then quoted in 'rad' (numerically quite similar to Roentgens), but was redefined in 1985 in gray (Gy, where 1 Gy=100 rad) to honour the work of the British physicist L.H.Gray.

The need to extrapolate doses and effects between species and radiation qualities introduced further extensions to the vocabulary of radiation dosimetry. Relative biological effectiveness (RBE) and tissue weighting factors for , , , X and neutron irradiation had to be determined. Physically, 1 Gy is the unit of dose (=1 joule of energy absorbed per kilogram of the irradiated material), but in terms of biologically effective dose, the gray are converted into sievert (Sv). For common ener-gies of X- and -rays, gray and sievert are numerically the same. This conversion of units gave an opportunity to recognise the work of

Sievert, an eminent Swedish physicist. Table 1.1 illustrates the relationship between the historical (partly because they still crop up in the scientific literature from time to time) and the modern nomenclature and definitions. Throughout this book we will keep to gray and sievert where appropriate.

Radiation exposure thus has its dangers to the health and well being of its victims. Research in all its forms is aimed at understanding biological and biochemi-cal natures of these dangers, developing means of protection from them and creating guidelines for their safe handling. The authoritative body, the International Commission for Radiological Protection (ICRP) continuously studies, reviews and revises the international guidelines for the recommended maximum exposures of occupational radiation workers and also the general public. Much mystique, however, surrounds the whole subject and this has often created irrational fears in the general public. While none of us demurs at the use of X-rays in medical diag-nosis—the biggest single source of external radiation we are ever likely to receive— the thought of being within a hundred miles of a lump of plutonium, which is dangerous only if injected, or if incorporated directly via contact with an open wound or inhaled (its radiation will not even penetrate intact skin), fills the world with dread.

Some of the potential radiation doses likely to be received in a number of fam-iliar situations can make quite interesting reading (Table 1.2). Most striking is that exposure levels from transatlantic flights or having a sleeping partner are every bit as high as the fallout in Western Europe from the Chernobyl accident.

Hazards there are, however, and no matter how much we try to put exposure levels into perspective, care must be exercised to prevent overdosing in clinical use and to supply adequate protection to the radiographer who may otherwise be exposed throughout working life to low but repetitive doses of scattered irradiation. Most serious is the hazard of a catastrophe which is likely to distribute radioactive materials emitting all types of radiation over a wide area of the community. It was known, for example, that radiation from the dropping of atomic bombs on Japan in

Table 1.1 International system of radiation units (SI): relationship between old and new terminology

Quantity	New unit (symbol)	Other SI units	Old unit (symbol)	Conversion factor
Exposure	–	C kg^{-1}	roentgen (R)	1 C kg^{-1}~3876 R
Absorbed dose	gray (Gy)	J kg^{-1}	rad (rad)	1 Gy=100 rad
Dose equivalent	sievert (Sv)	J kg^{-1}	rem (rem)	1 Sv=100 rem
Activity	becquerel (Bq)	s^{-1}	curie (Ci)	1 Bq~2.7×10^{-11} Ci

Source: NRPB report NRPB-R171, 1984.

Table 1.2 Radiation background levels (annual)

Source	Dose (μ Sv)	Percentage of minimum level
Total	~1800	
Natural background	780	43
^{210}Po/Pb	160	10
^{226}Ra	120	8
Discharge fallout	2	0.1
Medical	925	52
Flying Atlantic (3 mrem)	30	1.5
Chernobyl fallout (western Europe) (3 mrem)	30	1.5

Source	Dose (μ Sv)	Percentage of minimum level
Body ^{40}K (2 mrem to partner)	20	1
(20 mrem to gonads)	200	

1945 would cause genetic damage and cancers, and follow-up studies of the effects continue to be an extensive source of data for risk analyses. For this reason, the nuclear industry is subject to the most stringent of safeguards, both for the protection of employees and for the prevention of accidental release of radioactivity. The amounts of release legally permitted from a nuclear power station in Britain are probably less than those naturally emitted from a conventional coal-fired power station. In addition, it is for certain, given the present awareness of the problems, that station operatives are not going to be keen to let safety standards slip. Nevertheless, accidents have happened, notably in Chernobyl in 1986, enormous releases in the Urals, Three Mile Island in the USA in 1979 and notoriously in a series of fires in the 1950s at Sellafield, UK. Many years later, there was speculation that cancers, particularly childhood leukaemias, in the area—the infamous Sellafield Cluster—may have been a direct result of the incidents there in the 1950s. Another possibility explored is that some cancers in the area might have been the result of inherited damage. Male operatives, exposed (probably in ignorance of the possible consequences) because of poor working practices may have passed on potential damage to their children. However, a recent, well publicised court case asked to pronounce on the balance of probabilities for this event, decided, after considering many specialist reports, against such an outcome.

Radiation, therefore, is a contentious issue leading to much misapprehension. It is, however, particularly important with respect to the blood-forming organs. For example, these organs set the primary limit on the amount of radiation which may be given to a patient undergoing cancer therapy—a person may die of blood failure before sufficient effective radiation treatment can be administered. It is the blood system that must be ablated before bone marrow transplantation therapy can be used. Sublethal damage may well lead to leukaemia. Parallel damage to the regulatory system for blood production may expose the blood-forming tissue to other toxins which alone may not induce neoplastic change. Since this regulatory system is closely linked with bone growth and development, tumours of the bone (osteosarcomas) are also a potential outcome.

Concentrating on blood-forming tissues, therefore, the following chapters present detailed accounts of radiation effects, induction and mechanisms of leukaemia development, epidemiological studies on the incidence of leukaemia, and finally ask what should and can be done if or when the worst should happen. We present below a summary of these thoughts. More generally, two publications are particularly useful. *Living with Radiation* (1989) is a well produced booklet that is designed to indicate the nature, sources and effects of radiation, and the means of protection against it. *Radiation and Life* by E.J.Hall (1984) is a delightfully readable perspective on the interaction of radiation and aspects of everyday events and develops its themes from the fascinating century-long history of the subject.

1.2 Bone Marrow

The basis of any investigations into blood changes or damage is the marrow of the bones: ‘red’ marrow in particular because this is the active blood-forming or haemopoietic tissue. In humans, at birth, the skeleton is filled with good red marrow, but by adulthood much of it, particularly in the long bones of the legs and arms, has been replaced by fatty ‘yellow’ marrow which does not participate in blood production. In small

rodents, the whole skeleton retains its haemopoietic activity throughout life and marrow from the large femoral bone is generally taken for experimentation, although sometimes the vertebrae or the tibiae are used.

In Chapter 2, it is calculated that an average man generates about 3×10^{11} blood cells per day. He achieves that by a large amount of cellular proliferation in the bone marrow cell population which has a defined developmental hierarchy of cell categories. This hierarchy starts with a stem cell which has the capacity both to regenerate itself and to differentiate into all the blood cell lineages. Of these, the red blood cells, the neutrophilic granulocytes, lymphocytes and platelets are the major groups (see Figures 2.1 and 2.2). Thus, the stem cell is considered multi- (or pluri-) potent in its developmental capacity. After a number of population-amplifying cell divisions, during which the probability of the cell remaining multipotent gradually falls, the cell sees a signal for differentiation which directs or commits it into one of the specific blood cell lineages. After further development, the cells become recognisable, within their specific lineages, as the developing blood cell lineages. Further proliferation amplifies the cell populations to their necessary sizes before releasing them into the circulation as mature functional blood cells. Most of these cells (lymphocytes and some monocytes excepted) have no capacity for further proliferation. Lymphocytes retain a small capacity for proliferation in order to develop immune competence when required at some later stage.

Haemopoiesis, during development from the embryonic stages to adult blood production, appears in sequence in the yolk sac, through the foetal liver and spleen to the bone marrow where, in the mouse, it appears at around birth. In the human, foetal haemopoiesis reaches the bones by about five months of gestation. Growth of the bone marrow as an organ continues to develop throughout growth of the individual but haemopoiesis in the liver and spleen quickly declines after birth. It has been thought possible that haemopoiesis could be particularly sensitive to radiation damage during this rapid expansion period.

The progression of the cell lineages is regulated, particularly at the commitment stage, by a series of controlling molecules known as growth factors. Most of these are growth stimulatory factors, e.g. granulocyte colony stimulating factor (G-CSF), granulocyte/macrophage (GM-CSF), interleukin-3 (IL-3), erythropoietin (EPO), though a few are inhibitory, e.g. transforming growth factor- (TGF-), macrophage inflammatory protein-1 (MIP-1), the tetrapeptide (AcSDKP) and the pentapep tide (pEEDCK). Their primary source of origin is the stromal microenvironment which acts also as a matrix on which haemopoietic cells develop. Conventionally, bone marrow has been viewed as a tissue without form or structure. This would certainly seem to be the case when examined microscopically, and, taken into a syringe, it readily deforms and collapses. In fact, an analysis of the 'position' of the various cell types in the marrow space shows that their geometrical and spatial organisation is equal to that of any other tissue, where the structures are obvious to the eye.

The dynamics of blood cell production are regulated by the growth factors mentioned above and they act via a series of negative feedback loops: even stimulators act by negative feedback. (Positive feedback, i.e. some gives more, gives even more, *ad infinitum,* is not a control process.) While inhibitors can act directly on the cells they are controlling, the production of the stimulators must themselves be limited by the product they control (see Figure 2.11). Although there is considerable overlap in their range of effectiveness, such growth factors can induce quite dramatic changes in the amounts of differentiation, proliferation and proliferation amplification of specific cell lineages. It is here that the microanatomy of the tissue and its stroma play an important part because, for effective control of haemopoiesis, the right cell must be in the right place at the right time in order to respond to the appropriate, specific growth stimuli.

The complicated interrelationships of cellular development, lineage, growth factors and microenvironment are both the tissue's strength and its weakness. Organisation to do the right thing is its strength but the many points of interaction present scope for things to go wrong. Thus, for example,

uncontrolled proliferation may develop as leukaemia. This may come about as a direct result of damage, primarily to the haemopoietic stem cells, or possibly to the stromal microenvironment which normally provides the necessary range of growth factors. Radiation of all qualities has the potential to induce such damage.

1.3 Human Radiation Exposure

Humans may experience wide ranges of radiation doses from very low (natural background) to very high (medical application). Calculating doses is often retrospective (e.g. for accidental exposure) and always complicated by the correction factors necessary for different types and quality of radiation. Nevertheless, the highly developed science of radiation dosimetry now allows some degree of quantitation of dose to human tissues. The many naturally occurring radiation sources include cosmic rays, terrestrial -rays, potassium-40 in the body tissues and the -particle emitting radionuclides such as radium, thoron, polonium and radon.

A major problem associated with -emitters is their strong affinity for bone and its associated marrow, where they readily become incorporated. It is this property that promotes -irradiation as a potent source of bone cancer and leukaemia. Plutonium readily comes to mind in this context, but naturally arising polonium-210 along with radon-222 are considerably more important. Polonium-210 is not a bone-seeker but is interesting in that it occurs at the end of the uranium-238 decay chain with lead-210 as an intermediary. Ingested lead-210 is bone-seeking with a twenty-two year half-life and thereby supports the production of ^{210}Po in the marrow. By comparison, plutonium, which arises from fallout or through discharge from nuclear reprocessing, makes only a very small contribution.

Estimates of radiation levels are made from measurements of their retention on bone surfaces using a specialised form of autoradiography in which emitted -particles create etchable damage tracks in plastic detectors. Bone, however, undergoes considerable remodelling during growth, which removes activity from the surface into the marrow and this has important implications for calculating marrow dose. Teeth have proved a readily obtainable source of human material which may be assayed thus and used as an indicator of levels in bone proper.

This plastic autoradiographic technique has also been applied to -dosimetry in the human foetus where activity is concentrated mainly in the foetal skeleton and increases with gestational age. A disproportionately high level of activity in foetal teeth suggested that a significant fraction of the activity seen in 10 year old children's teeth is acquired prenatally. Attempts are now being made, using antibodies to haemopoietic stem cells, to map the distribution of cells potentially at risk in the foetus and relate this to the distribution of activity.

Teeth are also supplying valuable information on global differences. For example, activity in the teeth of Alaskan caribou relate to the capacity of lichens, their staple diet, to concentrate ^{210}Po. On a local scale, ^{210}Po in children's teeth has been related to proximity to major motorways.

Radon-222 is the only natural -radionuclide and the only example of natural ionising radiation to be firmly linked to cancer in man, i.e. lung cancer from radon exposure in uranium mines. On this basis it appears that radon exposure in the home is responsible for about 6 per cent of the UK lung cancer incidence and of such importance that maximum recommended airborne radon concentrations, above which remedial action must be taken to reduce exposure, have been introduced in the UK. As far as marrow radiation dose is concerned, it is particularly important that radon is sixteen times more soluble in fat than in blood and, while there is negligible fat in the newborn child, red marrow gradually becomes infiltrated with significant

quantities during growth. Since the spatial distribution of fat cells in the marrow is random, it follows that, despite the unique distribution of potential target cells, all will be equally at risk.

There are several specific high exposure groups; namely, the Japanese bomb survivors who received an average dose of 1 Sv; the American radium dial painters who, surprisingly, using a small brush would frequently lick the brush to a sharp point—and thereby ingest enormous amounts of radium resulting in massive bodily doses up to 200 Sv; radiology using thorotrast contrast medium giving 1.8 Sv y^{-1}; and uranium miners who typically received marrow-doses up to 117 mSv y^{-1}. Bomb fallout and thorotrast treatments have resulted in leukaemias, but osteosarcomas were the overwhelming result of radium ingestion.

In the low exposure groups, airline pilots receive 5–8 μSv h^{-1} from cosmic radiation; workers in the nuclear industries receive less than 3 mSv y^{-1} (only 10 per cent exceed 15 mSv y^{-1}); military weapons workers and radiographers get ~0.5 mSv y^{-1}. Chernobyl residents received marrow doses of less than about 1 mSv y^{-1} from caesium-137 and strontium-90. The major concern there was for iodine-131. Thyroid doses of up to 30 Sv in children under 1 year old have now been translated into a very significant increase in thyroid cancers.

From medical exposures to radiation, the dose range is very wide. A chest X-ray gives an average dose of 1.2 mSv; diagnostic radiopharmaceuticals deliver 1–100 mSv. By contrast, doses to regions of patients undergoing radiotherapy can reach 45 Gy and for localised doses, the scattered dose to red marrow can amount to 70 mSv per Gy delivered to the tumour. Although the Japanese bomb data revealed no excess in leukaemia mortality among children exposed during their foetal development, it is now accepted that obstetric X-ray screening during pregnancy does carry a slight risk and the practice has largely been discontinued.

The scale of radiation doses received by these various groups thus ranges from 10^{-6} to 10^{2} Sv and are put into their relative perspectives in Figure 3.23.

1.4
The Response of Bone Marrow to Low LET Irradiation

The bone marrow is the source of the mature functional cells found in the peripheral blood and the lymphatic organs. In the blood, the cells and their products, namely red cells, white cells and platelets, are fairly radioresistant except for circulating lymphocytes (see below). This means that with this important exception there are no dramatic changes immediately after irradiation in terms of cell number or cell function. However, with increasing time there are changes because of the sensitive nature of the precursor cells in the marrow, resulting in a lack of production of new mature cells to take the place of the existing cells when their life is finished.

Granulocytes (fighting bacterial infection) have a short half-life of around eight hours in man, and red cells have a long half-life of around four months. However, after high radiation doses the number of red cells may decline early because of many small radiation-induced haemorrhages which need platelets to seal the leakages.

Lymphocytes in the blood and in lymphatic tissue show a spectrum of radiosensitivities, but many are very sensitive and die within hours of irradiation, without dividing to express genetic damage in the form of lethal chromosome injury. The acute form of cell death has been called ‘apoptosis’ since 1972, earlier it was referred to as ‘interphase’ cell death. The high sensitivity of lymphocytes and their rapid death can be used as an indicator of the dose received by individuals after a radiation accident.

Small animals are more tolerant of whole-body irradiation than large animals such as man. The $LD_{50/30}$ (death of 50 per cent of irradiated animals within 30 days) ranges from around 15 Gy for the former down to 3–4 Gy for the latter. One likely reason for this difference is that small animals may be able to tolerate higher levels of depletion of their marrow precursor cells than larger animals. In man, doses up to around 3 Gy can be tolerated if good medical care is provided, and above this dose there is an increasing probability that the individual will die as a result of marrow failure (see later). It helps if some of the marrow has been shielded from the exposure, and even shielding part of one limb can have a dramatic effect on survival. Also, it helps if the irradiation is of low energy and hence only lightly penetrates the body. Even with reasonable penetrating radiation, the $LD_{50/30}$ can be 20 per cent higher when irradiation is given from one direction compared with two directions to even out the dose. With very low energy X-rays there is an additional dose at bone surfaces due to the interactions with the higher atomic number elements compared with those in soft tissue.

As mentioned above, the haemopoietic precursor cells in the marrow are fairly radiosensitive compared with precursor cells in other types of tissue. The reason for this is not known; possibly there is a contribution to cell death from apoptosis. Precursor cells in the dog have been reported to be especially radiosensitive compared with those in other species, and again the reason for this is not known.Certain genetic disorders have been reported to predispose individuals towards high radiosensitivities. This includes a certain deficiency in the immune system (*scid*— severe combined immunodeficiency) which also causes a defect in DNA repair in mice and those few humans with this syndrome as well as patients suffering from ataxia telangiectasia (AT). The latter is a recessive disorder affecting a very small percentage of the population. In both *scid* and AT syndromes cells of the body are two to three times more sensitive than normal. Cells exhibiting changes in the apoptosis pathway may also show a different radiosensitivity.

Recovery of the marrow from damage occurs fairly rapidly. This happens through a cascade of growth factor production largely from the marrow-supporting stroma. There is a period of proliferation and later differentiation both to replenish the stem cell population and to provide the differentiated mature cells necessary for survival of the animal.

The stroma nurtures the marrow and provides growth regulatory molecules which maintain homeostasis, i.e. the normal steady-state. The stroma is relatively quiescent, with little cell division occurring. It is resistant to irradiation, and high doses of radiation can actually increase its production of growth factors for haemopoiesis. Then the gradual expression of genetic damage at cell division occurs over a long time period, making stromal recovery a fairly slow process. This is important for residual marrow damage which may persist for many years after irradiation. Residual injury is characterised by a near-normal complement of functional cells but a reduced stem cell population which is cycling more rapidly. In addition there are probably more transit-cell divisions to increase the output of mature cells. The reduced stem cell population has the effect of producing a more severe reaction to second courses of treatment.

Protracted irradiation, either from ingested radionuclides or from fractionated acute exposures, is in general less efficient than high single acute exposures, particularly when delivered over very protracted periods when repopulation of precursor cells can occur.

1.5
High LET Irradiation

High LET implies a high rate of energy transfer along the radiation tracks in the tissue. Thus, its range of activity is very short, damage is very localised and is characteristically produced by heavy particles such as -particles and neutrons. Alpha-particles (and neutrons) provide a more menacing danger as they are

associated with both the nuclear industry and weaponry. Indeed, the continuing analyses of the 1945 atomic bomb survivors, the intensive study of the apparent leukaemia clusters around Sellafield and Dounreay in Great Britain, and the Chernobyl accident in the Ukraine, testify to these concerns. To what extent are they justified?

They are justified to the extent that any uncontrolled, unprotected radiation is a potential major hazard. Detailed surveys of groups of people exposed by way of employment or medical application demonstrate a host of long-term problems (see Chapter 11). In the bone and haemopoietic tissues, bone cancers appear to be more common than leukaemia but there is some evidence that potential leukaemogenesis may sometimes be masked by the earlier onset of osteosarcoma and there is clear evidence that plutonium and radium can produce a small incidence of leukaemia under experimental conditions.

Analysis of the effects of -particle emitters in haemopoietic tissue is complicated by the short range of their penetration into tissue which has a non-random distribution of cells. Almost exclusively they have a high affinity for bone tissue so the primary target of this radiation is the bone and a narrow rim of marrow close to the bone. This results in changes in the spatial organisation of the tissue and a potential origin of disorganised cell production which could foreseeably manifest itself as leukaemia. The supposed leukaemia clusters, however, were detected in young children, implying damage during development of the blood-forming tissues. While no evidence for leukaemia induction in experimental systems has been found, it is clear experimentally that long-term malformation of haemopoietic tissue may result from contamination of the mother during pregnancy. This is because during foetal development, the liver is the major blood-forming organ. It is also the major organ for concentrating plutonium crossing the placenta from the contaminated mother. Calculations in Chapter 5 on the uptake, transfer and retention of plutonium-239 injected into pregnant mice at different stages of gestation or into the young offspring demonstrated a 2500-fold difference in the radiation levels in the liver (500-fold in the bone marrow) between contamination via the mother at embryonic stages compared with direct contamination at weaning. From the degree of damage to haemopoiesis, it is clear that early embryonic haemopoiesis is considerably more sensitive to -particle irradiation than adult haemopoiesis.

Interpretation of these effects is complicated by the involvement of the stromal tissue which provides the regulatory microenvironment for efficient haemopoiesis. It is clearly damaged by plutonium-239 and radium-224 injected in adult mice. In all probability this limits the growth of the stem cell population and it is not clear that there is any direct kill of haemopoietic stem cells. Similarly, with most cases of contamination during foetal and neonatal development, major damage to the developing stromal tissue appears to be the main factor causing long-term damage to haemopoiesis. Indeed, cross-transplantation experiments between offspring from contaminated mothers to normal recipients and vice versa revealed that contamination at mid-term gestation resulted in damage only to that microenvironment. This again questions whether leukaemogenesis is a result of damage to haemopoiesis or a potential consequence of ill-regulated haemopoiesis.

How effective is -particle irradiation compared with low LET X- or -radiation? Direct irradiation of the stem cells indicates a relative biological efficiency (RBE) of 2–4. Official government agencies use a relative quality factor of 20 for -particles. When considering a whole tissue response *in vivo,* however, it rapidly became clear that even the higher value of 20 could not match up biological effects from - and - irradiation. From the amount of plutonium-239 incorporation at mid-term gestation, an average radiation dose of 10–14 mGy (a very small dose experimentally) to the foetal liver was calculated. A 20-fold higher - ray dose had no effect on the long-term development of haemopoietic tissue structure. In fact an RBE of 300 was required to match the -particle damage showing that foetal haemopoiesis is extremely sensitive to this form of high LET treatment.

Neutrons and pions have received attention regarding their potential use in radiotherapy. However, radiotherapeutic assessments showed that, in spite of their anticipated favourable radiation qualities, they were not significantly more effective for therapy purposes that X- or -rays. Experimentally, neutrons did cause more long-term marrow damage and, like -particles, they resulted in a long-term stimulated stem cell proliferation so that blood output could be maintained. This could suggest they are potentially as dangerous.

The long delay in appearance of leukaemia and then in only a very small proportion of animals suggests one is not dealing with a single event. The prolonged stimulation of stem cell proliferation required to maintain blood output renders these cells potentially more vulnerable to a secondary leukaemogenic insult which may be encountered later in life. This later event may be more radiation, virus infection, mutagenic drugs, etc. Chromosomal aberrations have been encountered and an abnormally delayed form of chromosome damage has appeared following -irradiation (see Chapter 8). Thus, high LET radiation may be associated with leukaemogenesis but the mechanisms of induction lie unexplained. The connection with human studies is suggested but of this, more will emerge below.

1.6 Experimental Approaches for Therapeutic Treatment of Radiation-induced Haemopoietic Injury

Individuals who have received a few gray of irradiation are at risk from marrow deficiencies and they need medical treatment. In the past, the treatment has included antibiotics and the provision of fluids, electrolytes and blood products such as fresh platelets. Marrow transplants have been performed in severe cases. Experiments with dogs showed that modest clinical support after irradiation would result in the animals tolerating around 30 per cent more radiation dose.

Stimulation of haemopoiesis by immunomodulators and more recently specific growth factors, has been investigated. The use of immunomodulators is based on the concept that such agents as endotoxin (lipopolysaccharides) or bacterial wall components are capable of significantly increasing non-specific host defence. This acts as a broad spectrum prophylaxis against the challenge of infection as well as stimulating haemopoietic recovery. The best such agents increase the tolerable radiation dose by about 20 per cent when given twenty hours prior to irradiation. The use of these agents is being largely superseded by the development of cytokine therapy.

Cytokines, or growth factors, are agents that promote the growth of cells. There is available a wide range of factors produced by recombinant technology using bacteria, and these molecules act on specific cell types in the various branches of the marrow cell lineages. In addition to promoting cell proliferation, some of these agents regulate differentiation, can suppress apoptosis and hence increase survival of critical cells after irradiation, and they can also promote the function of mature cells. Current research is aimed at discovering new growth factors, characterising the functions and interactions of single and combinations of factors, and designing protocols which will optimise the effects of the various factors in recovery situations.

Much work has been done with GM-CSF (promoting recovery of granulocytes and macrophages) and G-CSF (granulocytes alone). Both agents not only stimulate the growth of their target cell populations but also enhance the phagocytic activity of granulocytes. In addition, stimulated neutrophils produce other factors which help in host defence and inflammation reactions. In dogs, treatment with G-CSF plus clinical support was able to double the tolerable dose of radiation. In some instances delayed administration of these factors, a week after irradiation, is also effective in these model animal systems.

Platelet production is also important in radiation injury to prevent or heal haemorrhages. Several growth factors have been identified as important in this megakaryocyte lineage, including IL-3. The latter stimulates primitive (less differentiated) progenitor cells, and has also been variously reported to assist in platelet production. Other cytokines have also been found to assist in this way. Recently, thrombopoietin has been discovered which is lineage-specific for the production of megakaryocytes and platelets. This specificity is likely to lead to many investigations of its therapeutic potential.

Combinations of cytokines are being investigated, including the use of fusion proteins carrying two factors, e.g. GM-CSF and IL-3 (called PIXY321), and clinical trials are under way. Synthetic cytokines are also being studied, which have particularly advantageous properties, e.g. high affinity for receptors, no enhanced inflammatory activity. Progenitor cells extracted from peripheral blood have been shown to provide long-term reconstitution of ablated marrow. This is an attractive procedure prior to cytotoxic therapy. After whole-body accidental irradiation, T-cell extraction is required to reduce graft versus host disease when using cells from a related donor.

Expansion of haemopoietic progenitor cell numbers in culture is an attractive way of enriching the cell populations responsible for recovery. This is under continued investigation using different growth factors for expansion of particular cell populations. Progenitor cells derived from umbilical cord blood are also being studied. These cells can be stored for future use, have potentially fewer infectious agents than in adults, have greater quality and less potential for graft versus host disease, and they can provide a source of rare phenotypes.

These recent advances may well provide the clinician with an alternative as well as an adjunct to bone marrow or peripheral blood cell transplantation.

1.7
Clinical Approaches to Treatment of Radiation-induced Haemopoietic Injury

Basic treatments of radiation-induced haemopoietic injury include the prevention and treatment of infections, maintenance of nutrition and fluid balance, and administration of blood products, e.g. platelets, to provide transient support. With severely damaged marrow, new trends for treatment include the use of well-matched transplanted donor cells, transplantation using selected and amplified stages of haemopoietic progenitor cells, and new transplant regimes using grafts depleted of T-lymphocytes in order to reduce the risk of graft versus host disease. In addition, growth factors are being used increasingly. Apart from GM-CSF, G-CSF and IL-3, there are, for example, other interleukins, stem-cell factor, fusion proteins consisting of two growth factors, e.g. PIXY321 comprising IL-3 and GM-CSF, erythropoietin, and very recently thrombopoietin has been discovered and is being investigated.

Radiation-induced haemopoietic injury occurs in the medical use of irradiation for bone marrow diseases, and also in accidental cases of irradiation. Many radiation accidents have occurred over the years. These include scientists irradiated when working in nuclear reactor centres, or near radiation-generators. Also, there have been exposures to natural -irradiation from radiotherapy-type sources. A major accident occurred with the nuclear reactor in Chernobyl, Ukraine, in 1986: 117 individuals were irradiated to doses up to 9 Gy and above.

Various treatments were used for these individuals. Some of the nuclear reactor scientists received bone marrow transplants from matched individuals, but their efficacy was difficult to evaluate. Some of the Chernobyl victims were given GM-CSF, and six of them were given a foetal liver transplant (this is rich in stem cells). Thirteen of them who received doses above 6 Gy had a bone marrow transplant from matched

donors at 4–16 days after irradiation. Few of these individuals survived, and the death of four of them could be related to the transplantation.

Transfusion of blood cells and blood products, e.g. platelets, has been used in several accident situations with favourable results. This is usually employed in the low-dose range where transient assistance is required to allow cell repopulation from surviving endogenous progenitor cells.

GM-CSF has been used in several accident situations, and IL-3 on two occasions. The GM-CSF treatments have produced clear increases in granulocyte numbers but their real efficacy regarding survival has been difficult to judge.

With accident cases it is necessary to estimate the exposure level, the parts of the body exposed, and any concomitant injury from other agents (e.g. heat, blast). Physical dosimetry can be done using activation of elements such as phosphorus-32 in hair and nails, and sodium-24 in the blood, as well as luminescence from free radicals in hard materials. Biological dosimetry can be performed using blood lymphocyte counts, chromosome injury in lymphocytes, sperm counts and neurophysiological disturbances.

The level of blood lymphocytes reaches a nadir after about one day and this level is related to the dose. A more accurate method is to score chromosome aberrations in short-term cultures of cells from the blood. The dose-response relationship for these aberrations is well characterised for different types of radiation, e.g. -rays, and neutrons. An easier method is to score micronuclei (a specific type of chromosome aberration) which are readily recognisable and which are amenable to automation, i.e. counting on a large scale for accurate quantitation.

With accidents there are often complications from combined injuries which have to be carefully considered and treated, e.g. heat, blast, stress. Late effects in the marrow can also be a problem, with prolonged hypoplasia indicating stromal injury which is difficult to manage. Medically-irradiated patients have to be considered separately from the accident cases. They receive much more homogenous irradiations but they are generally suffering from disease. Also, they are prepared for their irradiation, both from the psychological and physical aspects.

Total-body irradiation is still used prior to bone marrow cell transplantation for the treatment of leukaemia. Reasonably high doses of irradiation are given, typically 12 Gy in six doses over several days. These patients sometimes have their lungs shielded to prevent later pulmonary injury appearing. Also, they may have chemical treatments to prevent graft versus host disease developing. The latter is due to incompatibility between graft and host which provokes an immune reaction. Transplants from siblings are best but long-term survival can be achieved with a reasonable success rate using unrelated donor marrow.

Opportunistic infections can also be a problem after these high doses of irradiation. Bacterial infections can be treated, but fungal infections are more difficult to eradicate. Viral infections are the main risk of infection in the first weeks after irradiation. Anti-viral drugs such as acyclovir have proved to be effective in this context. However, there is no effective therapy for cytomegalovirus which is the most common opportunistic pathogen detected and which is a major cause of morbidity and mortality.

New developments in transplant procedures include the positive selection of immature haemopoietic cells, which have been used in clinical trials with some success. This also helps to prevent graft versus host disease in non-matched marrow transplants. Growth factors, as detailed above, are also under rapid development to assist in marrow regeneration. In addition, selection, culture and amplification of progenitor cells prior to infusion is being investigated in detail. Major improvements in outcome for patients and other individuals receiving total-body irradiation are likely to be achieved by these new procedures.

1.8
The Pathogenesis of Leukaemia

Having established that ionising radiation can cause leukaemia in experimental situations (we look at the evidence for leukaemogenesis in humans later), what are the events which lead to its development? To a large extent, there are probably at least as many as there are different types of leukaemia. Basically, two categories of genetic change in cells can be defined.

1 The *gain-of-function mutation* alters the activity of genes involved in mediating responses to growth regulatory signals. Such genes, the *oncogenes* encode growth factors, receptors and interfere with normal intracellular signalling processes.
2 The *loss-of-function mutation* reduces the activity of *tumour-suppressor genes* responsible for inhibition of proliferation and differentiation.

Leukaemic cells suppress and replace normal haemopoiesis and, unlike most tumours, are not localised. They proliferate diffusely throughout the marrow and infiltrated tissues. The leukaemias fall into four broad categories depending on whether the disease is acute or chronic and on whether the major component is myeloid or lymphoid. A comprehensive morphological classification, known as the French-American-British (FAB) system which defines the many subcategories is now internationally accepted. Myelodysplastic syndromes, giving ineffective haemopoiesis and which are often preleukaemic are also classified on the FAB system. As far as radiation may be an initial cause of leukaemia, the Japanese bomb survivors provide the most cogent information. There, it seems, acute myeloid leukaemia (AML) was the most common. Chronic myeloid leukaemia (CML) increased with a shift to a younger age group. T-cell leukaemia was higher but, complicated by the association with a leukaemia virus (HTLV-1) and chronic lymphoid leukaemia (CLL), with no apparent increase, has come to be regarded as not normally being radiation inducible.

Very little is understood of the detailed events that contribute to leukaemogenesis. It is generally accepted that the transition is a multistep process involving both genetic and epigenetic changes that ultimately result in the emergence of a dominant malignant clone. The first important clue to the process of leukaemogenesis was the observation that it is clonal proliferation and, with rare exception, the progeny of a single cell (in CML and most AMLs) which is a haemopoietic stem cell. For lymphoid leukaemias, transformation appears to arise in rather more mature cells.

Current understanding of normal mechanisms of haemopoiesis allows some speculation of how a dominant leukaemic clone may emerge. Initial stem cell damage resulting in an abnormal response to normal growth regulatory mechanisms, by subtle alteration of a component of the signalling pathways, may be such that their probability of proliferation is increased. Still controlled, albeit imperfectly, the shortened intervals between cell proliferation cycles allow less opportunity for genetic housekeeping. The abnormal stem cells accumulate replication errors and enter clonal expansion. Still initially recognised as stem cells by the regulatory system, inhibitory factors increase, suppressing the normal stem cell population and permitting emergence of the dominant leukaemic clone. This scenario is reinforced by the finding that MIP-1 , a normal inhibitory regulator, has no effect on the proliferative status of CML progenitor cells.

Most leukaemias seem to have an abnormal genetic element. The best understood and characterised is the Philadelphia (Ph) chromosome, a translocation of the *bcl* and *abl* genes between chromosomes 9 and 22, which arises in virtually all CMLs. Chromosomal instability is a fundamental characteristic of CML, and it is generally believed that acquired genetic abnormalities are critical events leading to accelerated growth

and blast crisis. The molecular basis of CML is not well understood, but it seems that the oncogenes *c-myc* and *ras* and loss of function of the tumour suppressor gene, TP53, are probably involved.

The acute leukaemias are more variable in their anomalies. Mutations of the *ras* oncogenes are most commonly detected, but *c-myc, c-fms, c-fes* and *c-fos* have also been implicated. Aberrant p53 has been detected, but usually in association with multiple chromosome abnormalities.

In some experimental models, induced autocrine stimulation by aberrant growth factor secretion or growth factor receptor function, may be implicated. Some AMLs can secrete GM-CSF, G-CSF, M-CSF and other cytokines which may seem to render them independent of outside sources. It is uncertain, however, to what extent this arises in normal conditions.

Experimental radiation-induced leukaemias, like the human diseases, are clonal. They can be classified using the FAB criteria and more than 80 per cent of the myeloid leukaemias in CBA-H mice given 3 Gy X-irradiation showed deletions of chromosome 2 which may, therefore, contain genes encoding a tumour suppressor gene. Since abnormalities were detectable within days of the radiation, chromosome 2 breakage may represent an initiating event. An unstable Y-chromosome also appears to be involved and increasingly human sex-chromosome abnormalities are being linked to leukaemias.

This chromosome instability invites speculation that such a phenomenon may be important in leukaemogenesis. Alpha-particle emitters will induce leukaemia in contaminated mice. In parallel with this, -particle irradiation of haemopoietic stem cells has recently been shown to induce an instability which manifests itself as chromosome damage only at a later stage and after several division cycles. Much work remains for this and other genetic lesions before they can be definitively linked to the leukaemogenic process.

1.9
Genetic Effects of Ionising Radiation with respect to Leukaemia

There is no doubt that ionising radiation (IR) can cause leukaemia: there is evidence (see Chapter 11) from the Japanese survivors of the atomic bombs, the ankylosing spondylitis patients receiving X-ray therapy, and foetuses receiving diagnostic X-rays. It is likely that some individuals are more susceptible than others to radiation-induced leukaemia, depending on their genetic make-up. The heritable component of IR damage which might predispose offspring to leukaemia induction is unknown and very controversial.

Leukaemogenesis is a multistep process, consisting of initiation, promotion and progression. Initiation is the crucial first event: it is DNA damage which leads to chromosomal abnormalities. These abnormalities can be translocations (transfer of genetic material from one chromosome to another) or deletions of parts of chromosomes. The second event is promotion, which gives the initiated cell an advantage over its normal neighbours. This may be in terms of delayed apoptosis, increased proliferative capacity or an increased probability of producing more self-renewing cells at each division rather than differentiating. The third event is progression, which provides promoted cells with secondary genetic changes and further proliferative/renewal advantages.

Initial genetic events can be deletions in tumour suppressor genes. These genes are growth regulatory genes whose loss of function can lead to malignancy. Usually this requires loss of function of both copies of the gene, indicating that they are recessive (not dominant) genes. Hence two ‘hits’ are required for the effect to occur. The identity of leukaemic tumour-suppressor genes and their temporal significance in leukaemogenesis is still uncertain.

There is virtually no dominant or sex-linked single gene disorder that leads directly to leukaemia. Single gene disorders can be dominant, recessive and X-linked. In dominant disorders, members of each generation

are affected. Recessive diseases usually occur only among siblings, not parents, and X-linked diseases affect the male offspring of recessive female carriers. In addition, the expression of these disorders can be modified by 'imprinting', whereby one of the parental alleles of a gene is inactivated. This influences the expression of the other allele, and the degree of 'penetrance' expresses the differences between the expected and observed incidence of a certain genetic disease. It is possible that imprinting could affect the susceptibility to radiation-induced leukaemia, but there is no evidence for this at present. It has been suggested that fragile sites in chromosomes may also be especially susceptible to radiation damage, but there is little evidence for this.

Mutations in both single and multiple genes can contribute to the development of hereditary disease. A predisposing gene can be defined as a gene mutation which is both necessary and sufficient to cause disease. A susceptibility gene can be defined as a gene mutation which increases the chance of a disease being triggered and it usually acts in concert with other genes. In many cases it is difficult to be sure of the distinction between these two types of mutation in the development of disease.

Regarding the hereditary basis of human leukaemia, epidemiological case control and cohort studies have reported an increased frequency of leukaemias among relatives of leukaemia cases, particularly siblings. The risks are highest in patients with CLL, and lower in CML. There is a higher rate of leukaemia among identical twins, which is explained by the fact that the leukaemia cells, although monoclonal, are transferred between both individuals.

Regarding chromosome abnormalities associated with an increased risk of leukaemia, the best documented example is Down's syndrome. The leukaemia risk is about twenty-fold higher than in the normal population. The reason for this is unknown, but there does also appear to be abnormal bone marrow function.

There are four major groups of inherited single gene disorders which predispose to leukaemia. These are the bone marrow failure disorders (e.g. Fanconi anaemia), the genetic instability diseases, (e.g. Ataxia telangiectasia, Bloom's syndrome), various primary immunodeficiencies and the inherited cancer predisposing syndromes (e.g. neurofibromatosis and the Li Fraumeni syndrome). The first three of these have defective haemopoiesis which may lower the tolerance of bone marrow cells to radiation stress.

1.10
Experimental Radiation Leukaemogenesis

The unfortunate demise of many of those early radiation pioneers from leukaemia and the potential for a larger-scale problem in the post-war atomic/nuclear era prompted experimental radiation leukaemogenesis models. By the 1950s, radiation-induced thymic lymphosarcoma and lymphoid and myeloid leukaemias had been produced in mice. As an aid to understanding the process of leukaemogenesis, however, the induction methods were limited because the latent period was long— the first myeloid leukaemias appeared about 225 days after irradiation—the incidence window was wide—the leukaemias continued to appear throughout the rest of a fairly normal lifespan and in dogs it ranged from 300 to 2000 days—and the incidence was small (~25 per cent). Thus, apart from trying to formulate the incidence levels (in order to facilitate risk assessments), considerable effort went into improving experimental models: reducing the latent period, tightening the window of incidence and increasing the strike rate.

In mice, myeloid leukaemia is the most common leukaemia. It is associated with widespread infiltration, particularly in the spleen and liver. The leukaemic marrow is dominated by excessive numbers of blasts and young, immature granulocytic cells. Immature cells appear in the peripheral blood. In some strains, thymic lymphomas develop. Chronic lymphocytic leukaemia is more usually associated with chemical

leukaemogenesis rather than following irradiation. Most of these leukaemias can be transplanted and passaged through many generations, thus showing they have a stem cell function capable of maintaining the clone.

Much effort has gone into defining radiation-dose/leukaemogenic-response relationships. For low LET (X-rays), incidence of myeloid leukaemia *(Y)* was mainly dependent on the square of the dose *(D)* implying the interaction of two targets damaged by the radiation, but modified because at higher doses, cell-killing reduced the yield. Thus the yield was determined as:

$$Y=aD^2e^{-bD}$$

where *a* and *b* are constants. Myeloid leukaemias in women treated with radiation for invasive cancer of the uterine cervix followed the same pattern. It is interesting that young mice seem considerably less susceptible to radiation-induced leukaemia than are adult mice.

For high LET radiation either with neutrons or from incorporated -particle emitters, the yield *(Y)* of either myeloid leukaemias or thymic lymphomas was directly proportional to the dose *(D)* though still modified by that high-dose, cell sterilising effect. Thus:

$$Y=aDe^{-bD}$$

where *a* and *b* are constants.

In many practical situations, radiation may be delivered in protracted fractions or at a continuous low dose-rate. Dose rate appeared to make no difference in patients treated for ankylosing spondylitis. In mice, however, a low dose-rate was less effective than a single acute dose. This type of response seems common in murine studies and is consistent with the results from an extensive study on women with invasive cervical cancer. The shape of the dose-response curve was thus similar for low and high dose-rates, but this formula was fitted by different values for the constants *a* and *b*.

For experimental purposes, i.e. to study the process and mechanisms of leukaemogenesis, it is clearly desirable to reduce the latent period and incidence window and increase the total yield. Many attempts to this end have been made with some, though not outstanding, success. The major approach has been to use an additional stress treatment. Prolonged anaemic stress, for example, increased the yield to 100 per cent in irradiated and bled animals compared with 5–40 per cent of irradiated-only animals. Steroids increased the incidence from 20 to 50 per cent. Incidence can also be affected by the combined administration of haemopoietic growth factors, in particular CSF-1, and a synergistic effect of radiation and ethylnitrosourea (a chemical carcinogen) was also observed.

Studies of radiation on haemopoiesis have indicated (Chapters 4 and 5) that not all responses are as a result of direct effects on the stem cell, but the results of effects on regulatory control by the microenvironment. Fresh leukaemic cells do not survive well in culture, but growth-factor-dependent, non-leukaemic cell lines grown on irradiated stromal layers can become factor-independent in their growth and become leukaemic on retransplantation into animals. This emphasises the importance of not restricting studies solely to the haemopoietic cell populations but of always considering their behaviour in the light of damage to their microenvironment.

Potentially leukaemic cells can be detected by transplantation into sublethally irradiated animals where they are allowed to express their leukaemic potential. This technique has been used to address the problem of the importance of initial versus late events in radiation carcinogenesis. Passaging cells in this way gave strong support for the idea that a common radiation-induced critical event is followed by selection of a much rarer secondary event which will give rise to the transformed state. Following transplantation, leukaemia develops with a much narrower window of appearance. Not only that, if transplanted sufficiently late after the initial insult (radiation) the incidence is considerably higher than would have arisen if the leukaemia were left to develop in the primary irradiated mice.

This transplantation method, therefore, is successful in both increasing incidence and tightening the incidence window and should provide a good model for more detailed study of the mechanisms of experimental radiation-induced leukaemia.

1.11
Human Studies in Radiation Leukaemogenesis

Tying human leukaemogenesis with radiation exposure relies heavily on epidemiological studies. Hence, the atomic bomb survivors, the uranium miners and people exposed for clinical reasons have been valuable sources of data for assessing risk factors. Currently, radiation leukaemogenesis is focused on the shape of the leukaemia dose-response curve and the variation in risk with age at irradiation and with dose rate. Radiotherapy, giving up to 60 Sv at the tumour site, provides high-dose studies while recent workers (who have been subject to continuous monitoring for safety purposes) in the nuclear industry provide low-dose studies (~0.05 Sv). The Life Span Study (LSS) of the Japanese atomic bomb survivors, however, has provided the bulk of the data.

The Life Span Study is unique in radiation epidemiology: it not only has a large number of subjects (around 120000) but these are also of both sexes, of all ages and cover a wide range of exposure doses. Leukaemia cases appeared within a few years of the bombings and recent reclassification using the FAB system has allowed leukaemia subtypes to be studied. Leukaemia accounts for only 3 per cent of the total cancer cases observed, but if one eliminates the cases which might have been expected without the bombs, 15 per cent of the excess cancers were leukaemia. Leukaemia, therefore, is more easily induced by radiation than other cancers.

Assessing relative risk, however, is not straightforward. Account must be taken of disease rates in unexposed populations and of age and sex differences. Even then, it appeared that the risk of stomach cancer should be almost twice that of leukaemia. Leukaemia, however, is rare in Japan and it turns out that the excess relative risk (at a dose of 1 Sv) is much greater (around ten times greater) for leukaemia than for stomach cancer.

The atomic bombs emitted both - and neutron radiation and survivors were estimated to received doses up to about 4 Gy giving an average excess absolute risk of 2.7 cases per 10^4 person-year per Sv. The pattern of risk, however, differed between males and females and varied with age at exposure. Overall, excess relative risk was maximal during the first ten years after exposure and a value (at 1 Sv) of 3.9 was greater than the corresponding estimate for any of the solid tumours. Breakdown of the various types of leukaemia showed that acute myeloid was the most common. AML, CML and ALL (total 84 per cent) all indicated radiation dose-response relationships, whereas T-cell leukaemia (around 10 per cent) did not, but was endemic and commonly associated with the leukaemia virus, HTLV-1. CLL is extremely rare in Japan anyway.

While this LSS dataset is important and used to set radiation protection standards, results may not transfer directly to other populations. Also, radiation was instantaneous and virtually uniform over the body. Other studies were therefore required to supplement the LSS findings. The main alternatives for high-dose radiation are patients receiving radiotherapy. Another study showed that 150000 and 110000 women with cancer of the uterine cervix or corpus, respectively, suffered a two-fold increase in risk of leukaemia (other than CLL) from which it was concluded that CLL is probably not radiation linked. Variation of incidence with time compared well with that for the LSS studies. In the USA, maximum relative risk following radiotherapy ranged from 2.3 for uterine cancer to 11.0 for ankylosing spondylitis cases.

Unlike the bomb survivors who received uniform whole body doses, these clinical treatments consisted of large localised exposures which required that high-dose sterilisation should be taken into account, as was predicted and found necessary in the experimental dose-response studies.

Occupational exposures often amount to low doses over a protracted period of time. Extrapolation from the high dose, acute exposures is, therefore, suspect. Nevertheless, using the dose-response relationships suggested experimentally, a study of 96000 workers in the nuclear industry appeared to have an estimated relative risk of leukaemia (not CLL) at 2.2 per Sv; this is consistent with data for the Japanese bomb survivors.

Diagnostic X-rays also have potential risk but studies have been limited owing to the lack of good dosimetric estimates. They do become particularly important *in utero* because of the potentially greater sensitivity of the foetus. Consequently, many case-control studies of both singletons and twins have consistently found the relative risk of leukaemia (and other childhood cancers) to be about 1.4. However, it should be stressed that of 1263 children exposed *in utero* in the Japanese bombings, no case of childhood leukaemia was observed.

As with the animal studies, small excesses of leukaemia have been demonstrated following injection of radium-224 or thorotrast (used in patients with tuberculosis or ankylosing spondylitis) both high LET radiation sources. Osteosarcoma, however, was more of a problem than leukaemia. Living and working in a high concentration of radon, another high LET -emitter, has also been suggested as a potential hazard (see Chapter 3). A study of 64000 miners found over one thousand radon-related lung cancers, but no overall excess of mortality from leukaemia. There was, however, a two-fold leukaemia excess in the first ten years after starting work. The cause of this excess is not clear.

Eighty per cent of annual exposure is environmental and this is estimated to cause about 11 per cent of all leukaemia deaths. Increased environmental exposures from fallout following accidents at nuclear installations have indicated small increases in leukaemia incidence in Norway and the USA. However, in the years since the Chernobyl accident in 1986—ample time to allow development of most leukaemias—there has been no evidence of increased incidence; there are reports, though, of an excess of thyroid cancers. The possibility that a small excess of childhood leukaemia in Seascale was due to the proximity of the Sellafield nuclear reprocessing plant in England received considerable publicity but it now seems to have been discounted in favour of some other agent, possibly infectious in origin.

1.12 Conclusion

Haemopoiesis occurs in a renewing tissue which is extremely vulnerable to cytotoxic damage. Radiation is a particularly toxic agent which can generate a wide range of damage to the haemopoietic stem cells; from chromosome damage which might or might not be repaired, to death.

Experimental leukaemia in animals can be induced by low and high LET radiation and demonstrable damage to the haemopoietic tissue and its regulatory microenvironment can be demonstrated. In humans, epidemiological studies are used to evaluate leukaemic radiation hazards. This summary chapter has extensively plagiarised the ideas expounded in the following chapters which give more detailed accounts of the varied aspects of radiation toxicology in bone marrow.

References

HALL, E.J. (1984) *Radiation and Life* (2nd edn). Oxford: Pergamon Press, pp. 254 ff. National Radiological Protection Board (NRPB, UK) (1989) *Living with Radiation,* pp. 62 ff.

2
Haemopoiesis

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2
Haemopoiesis

2.1
Introduction

Haemopoiesis (hematopoiesis if one is reading an American publication)—the word itself a combination of two Greek words ‘haima’ meaning blood and ‘poiesis’ meaning production—is the process by which the many types of blood cell are generated. In the adult mammal, this cell production takes place almost exclusively in the bone marrow although under some pathological conditions haemopoiesis may redevelop in the spleen and even in the liver. In some lower mammals, specifically the mouse, splenic haemopoiesis continues at a low rate throughout life. The products of this process fall into two main categories: the red blood cells (RBC) or erythrocytes whose purpose is to transport oxygen to all the tissues of the body; and the white blood cells (WBC) or leucocytes whose purpose is to protect the body against infection. The scale of production is massive. With a RBC count of 5×10^{12} per litre and a blood volume of 5 litres, an adult man has 2.5×10^{13} circulating erythrocytes. Their lifespan is about 115 days, so that the daily turnover—and therefore production—is about 2.2×10^{11}. Neutrophilic granulocytes which constitute 66 per cent of the leucocytes in the blood number about 4.5×10^{9} per litre. Their lifespan, however, is short (less than half a day) indicating an overall WBC production rate of about 10^{11} per day. This means that the haemopoietic tissue is generating some 3×10^{11} blood cells of all types per day. At about 10^{9} WBC or 10^{10} RBC per gramme this represents some 120 g of blood cells (equivalent to a quarter-pound steak, or enough for a black pudding) every day.

With these figures in mind, it is appropriate to tackle the question of how the production targets are met. Unlike most tissues of the body, bone marrow does not have any clearly recognisable tissue geometry. We see later that it does in fact have structure but, viewed under the microscope, a smear of marrow cells (Figure 2.1) reveals a diverse selection of cell types and stages of developmental maturation, all intermixed and without any apparent logical interrelationships. In spite of all this complexity, it is perhaps paradoxical that the mechanisms of regulation and functioning of haemopoietic tissue are probably the best understood of all tissues. We see below how a strict hierarchy of cells develops, first as undifferentiated multipotential cells which then respond to differentiation instructions to develop the specific blood cell lineages. We see how these various levels of differentiated cells do exist in a well organised tissue structure, such that they may always be in the right place to respond to the growth and differentiation factors generated by the microenvironment which encompasses and controls their dynamics. Finally, we see how the tissue can respond, or indeed be manipulated, to conditions which necessarily require changes in functional blood cell output.

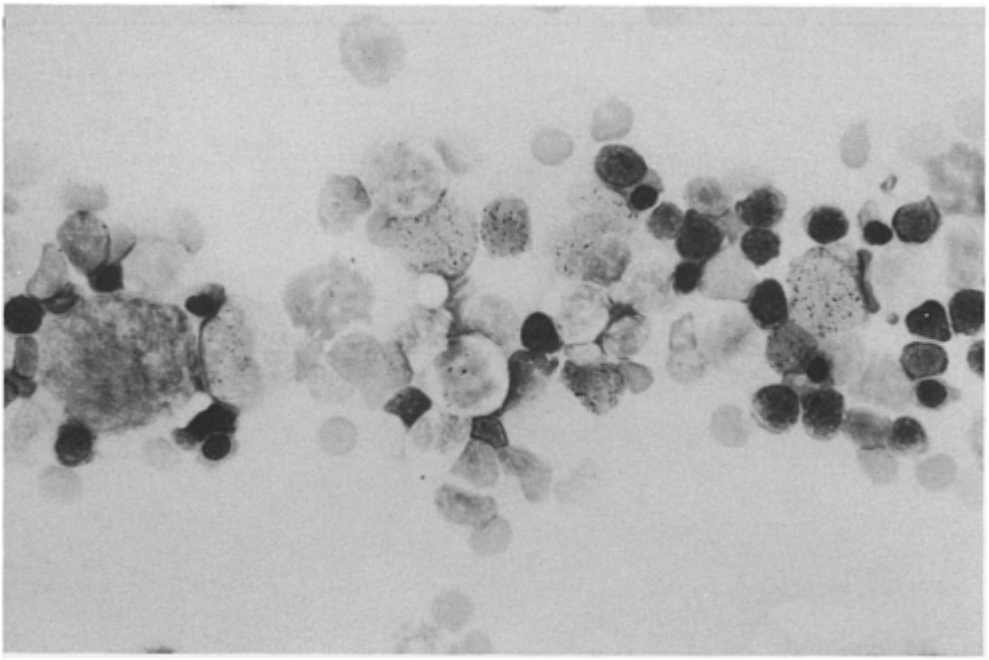

Figure 2.1 Smear of normal bone marrow cells stained with May Grünwald and Giesma. 865× (*see also* Colour Plate 1)

It should be recognised that while most of our knowledge of the regulation and function of haemopoietic tissue has been generated in animal (mainly mouse) studies both *in vivo* and *in vitro,* subsequent (though necessarily more limited) human studies have confirmed that human haemopoiesis has all the same physiological characteristics.

2.2
The Cellular Hierarchy

Haemopoiesis falls naturally into a four-tiered hierarchy of cell types (Figure 2.2) characterised by increasing differentiation and maturation, and coupled to a loss of the developing cells' capacities to maintain their own populations. The four categories (Figures 2.2 and 2.3) are known as:

1 (Multipotent) stem cells.
2 Committed progenitor cells—committed, that is, to generating a specific cell lineage.
3 Differentiated and maturing cell lineages.
4 Fully-functional blood cells, which comprise erythrocytes, granulocytes, monocytes, lymphocytes and platelets.

Interestingly, some other cells not directly associated with the blood, notably the epidermal Langerhans cells, are also products of the haemopoietic stem cell. The haemopoietic cell population is built up by a long series of sequential doubling cell proliferation cycles and the functional cells are generally released into the circulating blood at the end of this sequence, to complete their final maturation phase outside the marrow (Figure 2.2). This somewhat artificial division of what is essentially a continuum—a production line—is

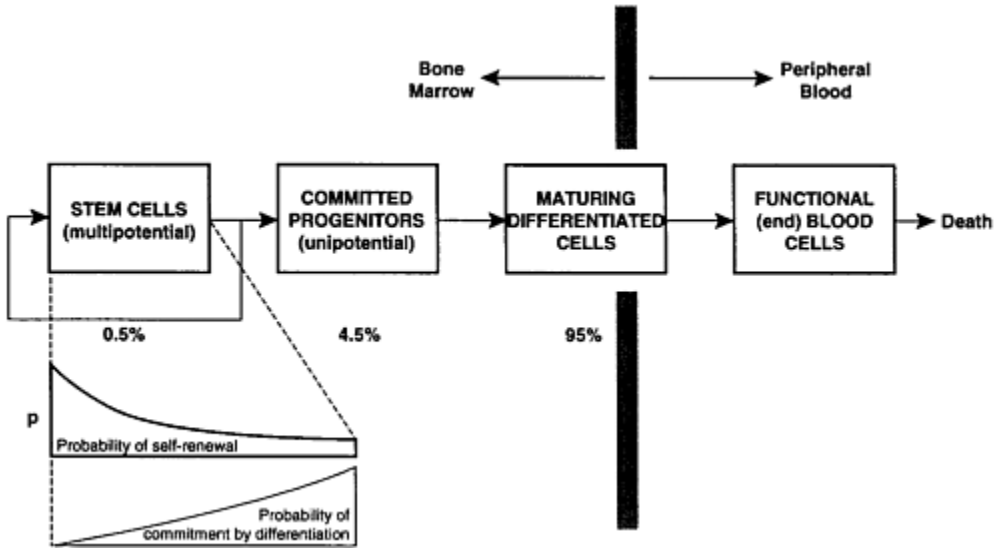

Figure 2.2 Haemopoietic cell hierarchy showing cell compartments in the bone marrow and peripheral blood. The numbers represent the approximate size—in cell number—of each compartment

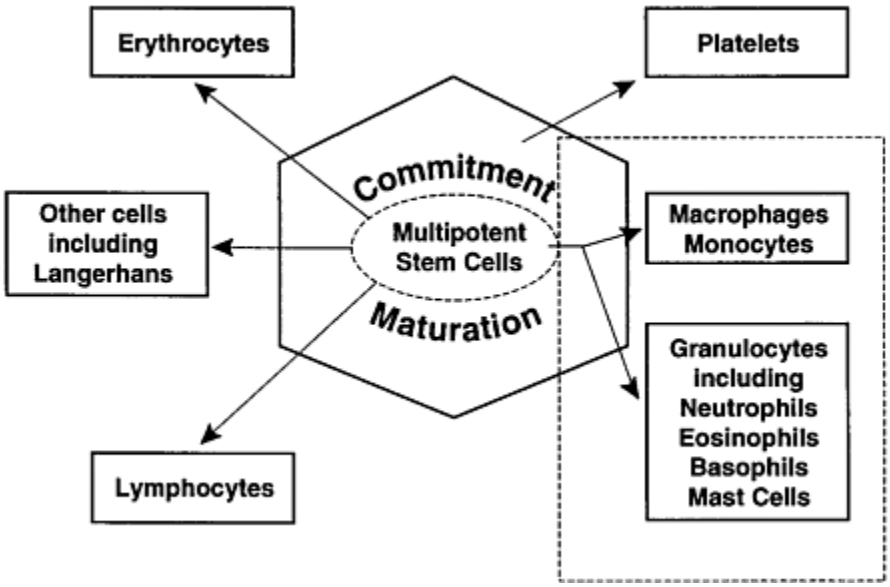

Figure 2.3 Differentiated cell lineages generated from the multipotent haemopoietic stem cells

crucial to understanding the mechanisms of haemopoiesis and its regulation. It is necessary therefore to look at each of these phases in turn.

2.2.1
Stem Cells

The stem cell population is clearly fundamental to the long-term maintenance of haemopoietic tissue. Without it, blood production would rapidly dry up as cells progressed down the maturational pipeline. The only necessary definition of a stem cell is that it shall be self-maintaining. That is, in the *normal* steady-state, a stem cell division will produce another stem cell. Clearly, there is no value in producing just stem cells. The other product of that stem cell division will be a cell that progresses into that maturational and developmental pipeline. A qualification to this statement is that if there has been damage to the stem cell population during regeneration, the balance of stem cell to developing cell production will be tipped towards stem cell growth. That is, the result of cell division will be more than one stem cell and less than one developmental cell. This means that one should not think in terms of *the* stem cell so much as the stem cell *population* which under normal circumstances maintains itself in spite of removal of cells by differentiation with an average probability of self-renewal *(p)* of 0.5. For $p>0.5$ the stem cell population will grow; for $p<0.5$ it will decline and haemopoiesis will eventually fail. We consider below how the stem cell population is controlled.

The requirement for a haemopoietic stem cell has been recognised for many years and a most elegant and readable history of its quest was presented by Lajtha (1980). He described a late nineteenth century duel between the dualistic and monophyletic concepts of blood cell formation. Although the debate ran for nearly forty years without any firm conclusion, the ideas of Pappenheim (1909) and Maximow (1910), pronouncing a lymphoid cell as the cell in question, began to hold sway. Nearly a century later, the most likely candidate is still a small to medium round cell which looks something like a lymphocyte (Visser *et al.,* 1984; Lord and Spooncer, 1986; Spangrude *et al.,* 1988)—but a lymphocyte it is not. For a start, there are far too many lymphocytes—about 20 per cent—in the marrow when the maximum estimate for multipotent cells is of the order of 0.5 per cent (Figure 2.2). Since those early attempts to recognise and define the stem cell, many cells have been candidates, and, over the years, progressively more and more primitive cells have been named.

This uncertainty in the exact identity of the stem cell, coupled with its very low frequency in a marrow population, i.e. it cannot be recognised morphologically (under the microscope), meant that classical approaches to its understanding were not appropriate. The modern concept of stem cells originates from 1961 when Till and McCulloch, exploring the radiosensitivity of haemopoietic cells, found that bone marrow cells injected into lethally irradiated mice generated clearly visible colonies of developing haemopoiesis 9 days (range 7–14 days) later in the spleen. The cells initiating the colonies were clearly 'stem cells' because each colony was derived from a single cell in the inoculated marrow (Becker *et al.,* 1963; Wu *et al.,* 1962) and in addition to recognisable erythroid and granulocytic cells, the colonies contained more cells which could themselves initiate further spleen colony development (Siminovitch *et al.,* 1963). Thus was born the *spleen colony assay*—a functional assay—for *haemopoietic stem cells*. The number of colonies on the surface of the spleen was shown to be directly proportional to the number of bone marrow cells injected. Thus a count of the colonies became a direct measure of the stem cell (defined as spleen colony forming units or CFU-S) component of haemopoietic tissue (and incidentally probably the origin of human bone marrow transplant therapy).

The CFU-S assay remains, nearly thirty-five years later, the basic stem cell assay. However, it is now recognised that the multipotent progenitor cell compartment or population has considerable fine structure and many investigators now require a much tighter definition of the stem cell. Different degrees of CFU-S production in growing colonies were recognised (Worton *et al.,* 1969), suggesting that some CFU-S express more self-renewal than others: CFU-S responses to alkylating agents required that different components of

the CFU-S population be postulated (Schofield and Lajtha, 1973): Sequential transplantation experiments revealed a declining capacity to give long-term regeneration (Siminovitch *et al.,* 1964) and later demonstrated pre-CFU-S (Hodgson and Bradley, 1979): Direct observation of the colonies revealed that those seen after eight days disappeared, to be replaced by different colonies appearing after twelve days (Magli *et al.,* 1982). Thus, the principle of age structure was developed (Schofield, 1978a; Rosendaal *et al.,* 1979) (Figure 2.4) in which a primitive stem cell develops, with cell division, through a pre-CFU-S subcompartment to CFU-S taking twelve days (CFU-S_{12}) and eight days (CFU-S_8) to develop as spleen colonies.

The evolution of this generation/age principle whereby a cell becomes maturationally older with each cell division now raises the question of whether all the cells in this somewhat artificially defined compartment are capable of self-renewal. The only real test of this is whether a cell can generate long-term marrow repopulation. It is usually assessed by detecting the presence of the Y chromosome in the marrow (and lymphoid organs) of female mice some 6–12 months after transplantation of male marrow. Cells with marrow repopulating ability (MRA), early pre-CFU-S, measured initially by their ability to generate CFU-S_{12} (Figure 2.4) can do this. By extrapolation, the equivalent human cells which can initiate haemopoiesis in long-term cultures (LTCIC) almost certainly have the same capacity. By contrast, many workers fail to re-establish marrow with the more mature progenitors (CFU-S_8 and even CFU-S_{12}) and consider that only the primitive and MRA cells should be recognised as stem cells. The present author, however, believes that failure to re-establish haemopoiesis may be due to a technical factor because, under appropriate transplantation conditions, CFU-S also can generate long-term haemopoiesis (unpublished results). This is not to belie age structure and it is unlikely that a cell can go backwards, i.e. de-differentiate and become more primitive. It seems probable, however, that the self-renewal capacity is falling as the cells progress through this multipotent compartment and is minimal at its end. With this picture in mind I shall continue to refer to the whole of this compartment as the 'stem cell compartment' and, unless specifically countered, it will represent stem cells throughout this book, particularly as discussion arises in Chapters 4, 5 and 8. However, we return to the question of self-renewal later.

Kinetically, the stem cells are quiescent. Although the population develops and maintains itself through cell proliferation, less than 10 per cent of the CFU-S and considerably fewer of the earlier cells are in DNA-synthesis at any one time. Most of the cells are in an out-of-cycle, stationary G_0 phase. Cells with marrow repopulating ability, for example, take four days to enter cell cycle even under conditions of severe demand for regeneration (Lord and Woolford, 1993). Turnover, therefore, is very slow and this appears to be a characteristic of most, if not all, stem cell populations.

2.2.2 Committed Progenitors

As the stem cells become more mature, the probability of their succumbing to a signal or instruction to differentiate increases. Such a signal induces specific changes (in gene expression) which commit the cell to a single line of development—erythroid, lymphoid, megakaryocytic (platelet) (Figure 2.3). At most, commitment may be bipotent. Granulocyte/macrophage progenitors which diverge after further growth also emerge. As with the stem cells, their frequency in the marrow is low (maximum of about 4.5 per cent), but unlike the stem cell population, they are proliferating rapidly (30–50 per cent in DNA synthesis). However, they have still not developed any morphologically recognisable characteristics.

The discovery of committed progenitor cells came from a desire to reduce animal experimentation by growing stem cells *in vitro*. The breakthrough came in 1965/6 when Pluznik and Sachs, and Bradley and

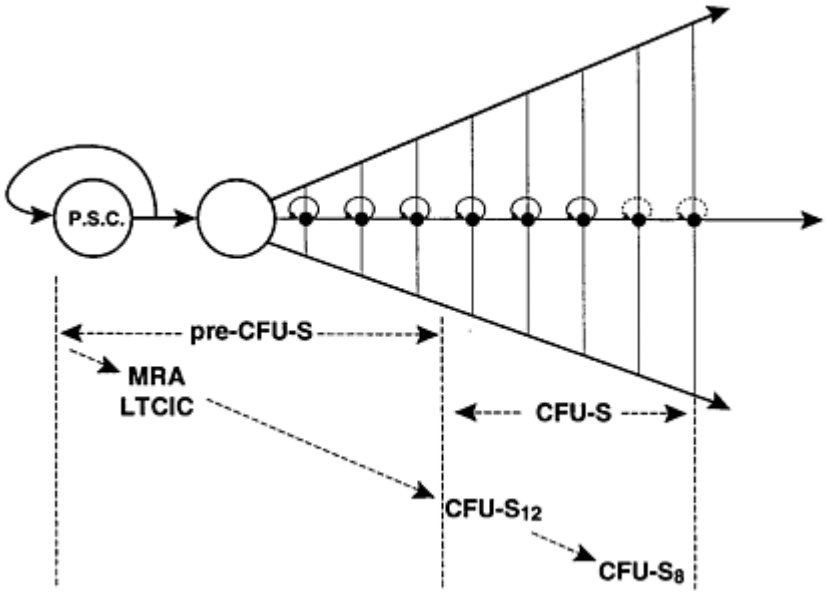

Figure 2.4 Fine structure of the multipotent haemopoietic stem cell compartment. P.S.C.=primitive stem cell. The small reverse circular arrows denote self-renewal and the reducing definition of this line conveys a continuously falling self-renewal probability. MRA=murine cells with marrow repopulating ability and LTCIC is the equivalent human cell which is capable of initiating haemopoiesis in long-term bone marrow cultures

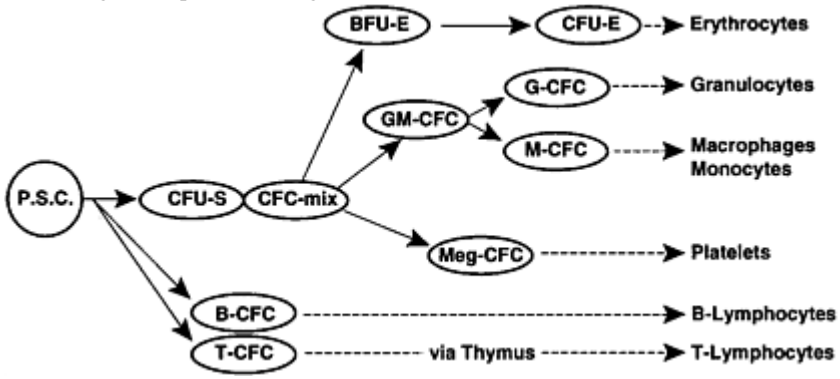

Figure 2.5 *In vitro* colony-forming cells generated by commitment from the multipotent stem cell compartment. P.S.C. —primitive stem cell

Metcalf independently grew colonies from bone marrow in a soft agar gel in a petri dish. Nourished initially by an underlayer of feeder cells, it was soon found that a medium conditioned by feeder cells was sufficient. For many years, however, only granulocyte and/or macrophage colonies could be grown, and the cells initiating these colonies became recognised as subordinate to, and derived from, the multipotential stem cells. Time and technical refinement enabled erythroid, megakaryotic or lymphoid colonies to be grown and, eventually, colonies of mixed cell type were possible. Although a mixed colony is technically the product of a multipotential cell, the colony initiating cell is distinguishable from the multipotent stem cells by its

continuous (no G_0 phase) and higher proliferation rate, as indeed is the entire committed colony-forming cell population.

The second stage of the haemopoietic developmental sequence was, therefore, established (Figure 2.5) with mixed colony-forming cells (CFC-mix)—perhaps overlapping slightly with the late stage CFU-S_8—giving rise to progenitors of the different individual blood cell lineages; the erythroid burst-forming units (BFU-E), the granulocyte/macrophage (GM) CFC, the megakaryocytic (Meg) CFC and a late-stage progenitor, the erythroid CFU-E. Although lymphocyte progenitors can be grown in a similar manner, it appears that these lineages peel off at an early stage (before CFU-S) of the multipotent stem cell population (Figure 2.5).

Although the committed populations amplify themselves by proliferation during colony growth, it is not clear that this is any real aspect of self-renewal. Rather it should be considered simply as maturational growth and development through the compartment.

A specific advantage of this *in vitro* technique is that the equivalent human progenitor cells can all be grown and assayed in a similar manner, underlining the comparable physiology of human and murine haemopoiesis. The only minor difference lies in a somewhat slower cell turnover in human tissue.

2.2.3
Maturing Cells

Developing from the committed, lineage restricted progenitor, perhaps by way of a further differentiation step, is the maturation phase. These cells are the morphologically recognisable and defined cell lineages which constitute 95 per cent of the total population. They are the cells that the clinicians have classically used to define the 'condition' of the bone marrow and, therefore, of haemopoiesis. The cells progress through straightforward maturation sequences which are defined by characteristic changes in appearance:

- From the earliest signs of haemoglobin synthesis in the proerythroblast, through increasing haemoglobinisation and nuclear condensation, to the acidophilic normoblast which then loses its nucleus leaving a reticulocyte to conclude its maturation to an erythrocyte in the peripheral blood.
- From a myeloblast through increasing cytoplasmic granulation and changing nuclear shape to release of polymorphonuclear granulocytes into the peripheral blood.
- From a megakaryoblast through endonuclear division which increases its size as a megakaryocyte out of all proportion with the rest of the marrow cells, to the release of tiny platelets into the peripheral blood.

Through the earlier stages of these maturation sequences, the numbers of cells are amplified by an accompanying series of cell proliferation cycles—about five in murine erythropoiesis, rather fewer (about three) in human erythropoiesis—and it is because of these final stages of cell proliferation that the maturing cells dominate the haemopoietic picture (Figure 2.1).

2.3
Developmental Haemopoiesis

So far I have described steady-state haemopoiesis in the adult mammal. This occurs almost exclusively in the bone marrow. Figure 2.6 illustrates, for the mouse, its course of development from the embryonic stage, through the foetal and neonatal stages to adulthood. In essence, the principles of blood production are the same throughout development, but there is a strict sequence of locational changes. Shortly after conception

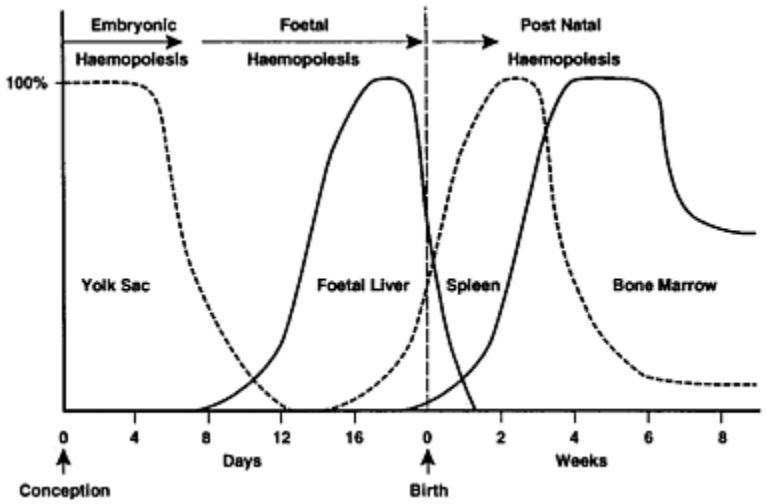

Figure 2.6 Diagrammatic evolution of haemopoietic tissue from conception to adulthood. This is not to scale and each phase of haemopoiesis is illustrated as a percentage of the peak activity for that organ

all haemopoiesis is in the blood islands of the embryonic yolk sac where CFU-S, albeit in extremely small numbers, can be detected. After implantation of the embryo in the epithelial lining of the uterus at about day 5, yolk sac haemopoiesis declines but the foetal liver develops from about day 7, primarily as a haemopoietic organ. Shortly before birth, haemopoiesis transfers via the spleen to the bone marrow. In humans, marrow haemopoiesis starts at around the fifth month of gestation. As the bone marrow expands after birth, first liver haemopoiesis disappears, followed by a decline to a very low level of splenic haemopoiesis. Up to about 8 weeks of age, the growth of the mouse demands expanding haemopoietic tissue, but beyond that age, as an adult animal, haemopoiesis settles into its steady-state adult production level of about half its maximum capacity. Again, a minor difference in human haemopoiesis is noticeable. In rodents, active haemopoiesis is retained throughout the skeleton, throughout life. In humans, probably because of lower cell turnover—the lifespan of red cells is three times longer than in the mouse, for example—and lower metabolic and physical activity, the shutdown in adult haemopoiesis is more complete. It disappears completely from the spleen, and fatty yellow marrow replaces active red marrow in the long bones, restricting haemopoiesis mainly to the ribs and bones with restricted cavities such as the iliac crest of the pelvic bones.

2.4
Microanatomy of the Haemopoietic Bone Marrow

An eminent experimental haematologist claimed that ‘hemopoietic populations exist together almost as free living cells, not constrained by organ-defining structures such as basement membranes, required where specific anatomical relationships underline functions’ (McCulloch, 1978). This, in effect, describes the classical understanding of haemopoiesis. By this time, however, it was becoming recognised that outside influences had some bearing on the performance of the haemopoietic cells. The existence of a microenvironment with a distinct contribution to long-term maintenance was becoming undeniable. Was this the equivalent of a basement membrane after all? More of this microenvironment later, but its existence

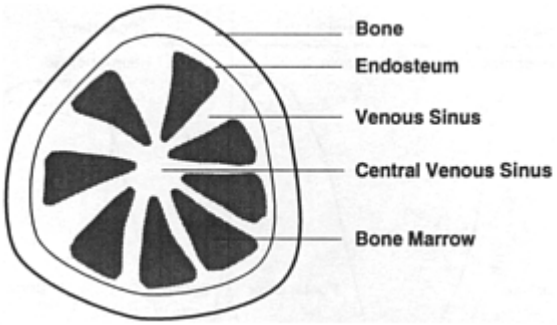

Figure 2.7 Diagrammatic illustration of a transverse section of a mouse femur as described by Weiss (1970)

and a definitive role in haemopoiesis suggest that the haemopoietic tissue itself must conform and be appropriately located. Indeed, it would be remarkable if a tissue with such complex developmental patterns and control processes did not conform, as do all other tissues, to a specific geometrical structure.

Although Weinbeck had noted in 1938 that the central part of a marrow cavity contained a greater proportion of mature cells than was found close to the bone surface, Weiss (1970, 1976) was probably the first to attempt to define microanatomical structure. He showed (Figure 2.7) that murine marrow is dissected by various sinuses draining into a large central venous sinus through which mature blood cells are released for transport into the circulation.

More recently, using a technique which fractionates bone marrow longitudinally, it has proved possible to map out the spatial distributions of the major progenitor cell populations. This subject has recently been reviewed extensively (Lord, 1992) and Figures 2.8 and 2.9 illustrate the main features.

Stem cells, measured as CFU-S, are not randomly distributed throughout the marrow tissue, but are two to three times more concentrated near to the bone surfaces than close to the central venous sinus. Their progeny, the committed progenitors, have a peak concentration approximately two-thirds of the distance from the central longitudinal axis of the femur, while the most mature cells are, as Weinbeck observed, about twice as concentrated near to that central sinus (Figure 2.8).

Further analysis of the fine structure of the stem cell compartment (Figure 2.9) revealed that, although the net proliferative activity is low, some cell proliferation is clearly necessary and this is confined mainly to where the CFU-S concentration is highest i.e. near the bone. The capacity for self-renewal (p) and the MRA, however, are highest close to the centre of the bone (Lord, 1992), revealing that the most primitive and highest quality cells of the stem cell compartment reside in this location.

These data allow one to paint a dynamic picture of the progress of haemopoiesis. The primitive stem cell resides close to the centre of the bone. As it matures, it becomes more peripheral, amplifying its population by additional proliferation. Near to the bone, the cells come under the influence of those differentiative signals (products of the microenvironment—see below) that convert them to committed progenitor cells. These are further amplified by proliferation, the pressure wave of cell numbers propelling the newly formed cells back towards the centre. Their concentration then reaches a peak after which they progress into the maturing compartment. This contains the bulk of the cell numbers and, as they undergo further proliferation, their number increases towards the central venous sinus whence they are released into the peripheral blood.

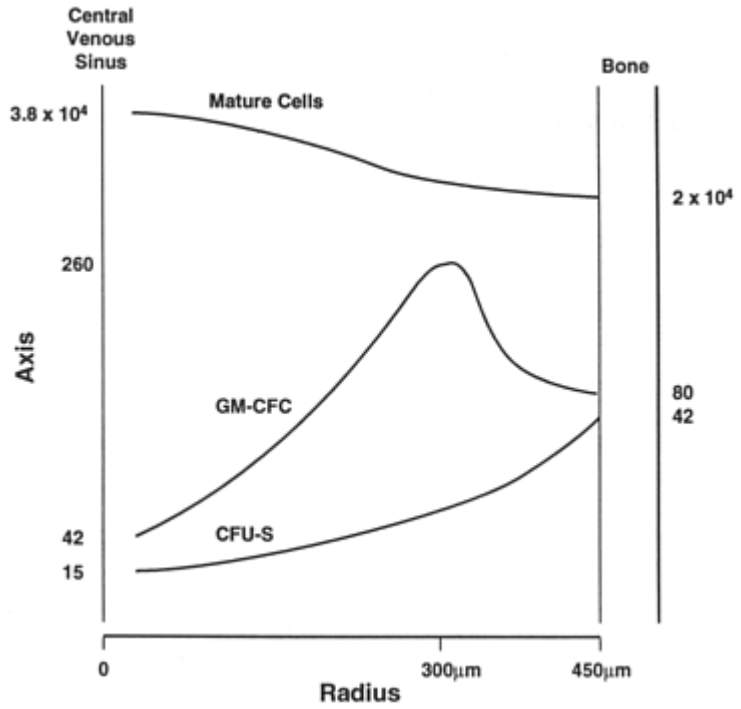

Figure 2.8 Spatial or radial distribution of CFU-S, GM-CFC and mature (granulocytic) cells in the mouse femur. The diagram (not to scale) depicts a longitudinal section of the bone with the ordinate being the central longitudinal axis along the line of the central venous sinus. Erythroid elements of the marrow have corresponding distributions (see Lord, 1992). Numbers are given as per 10^5 bone marrow cells

2.5
From Mouse to Man

Although human bones are considerably larger than mouse bones, it is interesting that structural dimensions are fairly similar. The trabecular structure of the human rib, the major site of adult haemopoiesis, is tubular and somewhat similar in cross-section to a bundle of mouse femora (see Lord, 1992). Furthermore, although in practice CFU-S cannot be measured for human marrow and the same type of fractionations cannot be made, there is some evidence that the committed progenitors are similarly distributed in the rib marrow cavities (Testa *et al.,* 1985).

The importance of this well defined spatial organisation for the cells will be evident when we consider the effects of bone and bone-seeking radionuclides in Chapters 4 and 5. For now, it is important that such an organisation could not be held together without a microenvironment which controls and directs it.

2.6
The Haemopoietic Microenvironment

As long ago as 1936, Perla showed that haemopoietic stromal tissue transplanted into ectopic sites in the body becomes revascularised and ultimately supportive of haemopoiesis again. Such cells transplanted under the kidney capsule generate bone and develop a microenvironment which eventually becomes

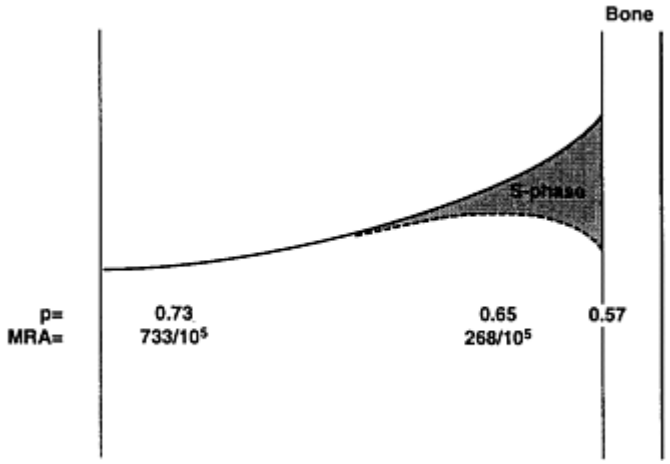

Figure 2.9 Longitudinal section (diagrammatic) of a mouse femur—see Figure 2.8 for full description—showing the kinetic properties of stem cells in different locations in the marrow. Self-renewal is measured under transplant conditions which stresses the cells to manifest their maximum self-renewal capacity (p). This does not correspond to performance under normal steady-state conditions but is taken to reflect the quality of the cells as stem cells, i.e. how primitive they are. The more primitive, the higher their capacity for self-renewal will be. MRA— marrow repopulating ability per 10^5 cells

colonised by haemopoietic stem cells and developing haemopoiesis (Friedenstein *et al.,* 1968). Friedenstein and colleagues (1967) had already described a fibroblastoid colony-forming cell (CFU-F) which they suggested was responsible for transferring the microenvironment of haemopoietic tissue. The first successful long-term culture of haemopoiesis (Dexter *et al.,* 1973) depended on the initial development of an adherent layer of reticular cells, fat cells, endothelial cells and macrophages which showed a striking resemblance to the cells described by Weiss (1976) lining the marrow sinusoids.

The importance of this stromal microenvironment was underlined by studies with genetically anaemic mutant mice; the W/W^v which has defective stem cells but can host normal stem cells and the Sl/Sl^d which has normal stem cells but is unable to support their development (see Harrison, 1978). Thus Sl/Sl^d bone marrow is able to cure the anaemia of the W/W^v mice. Similarly, in the long-term culture, the stromal adherent layer of the W/W^v mouse will support the growth of Sl/Sl^d marrow. An Sl/Sl^d adherent layer, however, will not support the growth of either (Dexter and Moore, 1977).

The requirement for a microenvironment was thus established and although excellent video films, taken by time lapse microcinematography in long-term cultures and demonstrating the movement of and interactions between haemopoietic tissue and its stromal microenvironment, are available, its role is still to be fully evaluated. Its main functional requirements are for stem cell lodgement, commitment and proliferation.

2.6.1 Lodgement

The necessity to provide a stable matrix in which haemopoiesis can develop is implicit in the well disciplined spatial organisation of the haemopoietic cells. In this respect, it is interesting that the one quantifiable stromal cell—CFU-F—is most concentrated in the centre of the bone cavity, in the same

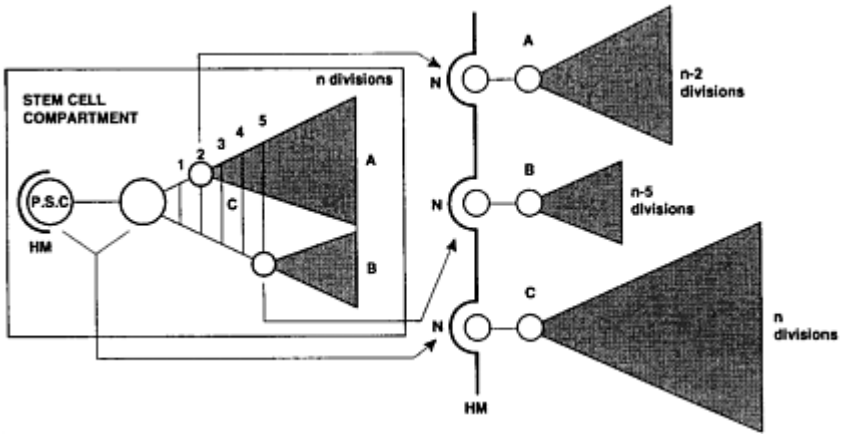

Figure 2.10 Performance of transplanted cells from different parts of the stem cell population. N— stem cell niche (Schofield, 1978b). HM—haemopoietic microenvironment. P.S.C.—primitive stem cell. This diagram represents bone marrow transplantation into a microenvironment that has been made available (emptied) by pretransplant conditioning

location as the more primitive stem cells (Xu and Hendry, 1981). Similarly macrophages, another element of the microenvironment, which generate an inhibitor of stem cell proliferation, are also most concentrated in this region. Other macrophages which generate a stimulator of stem cell proliferation are most concentrated at the bone surface where, not surprisingly, most proliferation is evident (Lord and Wright, 1984). Stromal cells with such regulatory functions could well represent important cellular elements of a hypothetical stem cell 'niche' proposed by Schofield (1978).

The practicalities of such a niche are that it can provide a home for the maintenance of stem cell integrity; that implanted cells can find the appropriate lodgement and thereby maintain their self-renewal capacity. Figure 2.10 illustrates this hypothesis. A primitive cell (or first daughter) would be fully preserved. A stem cell with only, say, two divisions left as a multipotent cell would be maintained by the niche as a self-renewing stem cell too, but with its output limited to its two remaining amplification divisions. In this way, the developing stem cell compartment is expressing the reducing self-renewal capacity described above and in Figure 2.4 but is retaining the definition of a stem cell throughout the compartment. Reduced stem cell production, as this would seem to indicate, might suggest a low ultimate blood output. Haemopoietic tissue, however, has ways of dealing with this, as we see later.

2.6.2
Commitment and Proliferation

Commitment is brought about by haemopoietic growth factors which were the logical outcome of purification and characterisation of those conditioned media necessary for growing the committed progenitor cell colonies *in vitro*. There is a wide variety of these factors, the principal ones being granulocyte colony stimulating factor (G-CSF), GM-CSF, M-CSF, Interleukin-3 (IL-3), stem cell factor (SCF), erythropoietin (EPO) and a recently discovered megakaryocyte CSF (Meg-CSF). Some, such as SCF, may not support colony growth on their own but may have important functions in promoting sensitivity to other factors or regulating self-renewal functions. These physiological regulators—some now in clinical use— are

principally the products of microenvironmental cells (e.g. macrophages). As such, their points of production and action are likely to depend on the distribution of the appropriate producer cells.

Since these same growth factors are required also to maintain the proliferative development of the colony-forming cells and their progeny, the maturing cells, the microenvironment is also instrumental in determining the rates of proliferation and cellular production. A treatise on these factors, however, is beyond the scope of this chapter. Their effects cover wide ranges of the developmental processes and often overlap. In addition, combinations of factors often produce wonderful synergies which compound to provide the appropriate levels of cell production. The interested reader is advised to look into the extensive specialist literature on growth factors (e.g. Lord and Dexter, 1992).

2.7
Dynamics of Blood Cell Production

The level of blood cell production is always required to meet the demands imposed on it. Under normal steady-state conditions this is merely to replace loss due to the natural lifespan and utilisation of the functional cells. Production is regulated at all levels by negative feedback, control signals which might be stimulators, such as the growth factors referred to above, or direct inhibitors. One classical feedback control loop is demonstrated with erythropoiesis where a stimulator (EPO) is involved (Figure 2.11). Red blood cells carry oxygen to the tissues which register their oxygen supply by the partial oxygen tension (pO_2) exerted. The kidney produces erythrogenin (which together with a plasma factor makes erythropoietin, EPO) in response to a falling pO_2. Thus, if the RBC level falls (haemorrhage possibly) then the pO_2 falls and EPO production increases to stimulate excess production from the erythrocyte precursor compartments. At high altitude, the same thing happens because of the low oxygen content of the atmosphere and we produce more RBC to compensate—a physiological phenomenon known to all athletes who compete in endurance events.

This increased production is the result of extra cell divisions taking place during the maturation process. In the same way, a reduced supply from the stem cell compartment would lead to a lower ultimate output. This, however, would be recognised by some ‘output monitor’ (the kidney in this case of erythropoiesis) which would then, via its feedback link, establish additional compensatory proliferation. This, for example, would be the outcome of low dose-rate chronic irradiation which damages the stem cell compartment, only to be compensated by extra proliferation during maturation. An example of direct inhibitory feedback control whereby the intermediate monitor (such as the kidney in the example above) is not required is the role of macrophage inflammatory protein-1 (MIP-1) which is known to suppress the proliferation of stem cells. Other examples of inhibitory molecules in haemopoiesis are a tetrapeptide (AcSDKP) a pentapeptide (pEEDCK) and the ubiquitous transforming growth factor- (TGF).

2.8
What Can Go Wrong?

Almost anything can go wrong, as will become clear in subsequent chapters. Basically, radiation is a well recognised mutagen and one must accept that the various leukaemias are likely to demonstrate characteristic cytogenetic changes. Leukaemia is characterised as a problem of uncontrolled cell proliferation, but it would be wrong to consider that leukaemia is necessarily the result of direct damage solely to haemopoietic cells. Just as likely as damage to a haemopoietic cell is damage to the microenvironment, and such damage may perturb the appropriate production of growth controlling factors. Specific chromosomal changes may not in themselves necessarily lead to leukaemia, but in combination with damage to the microenvironment

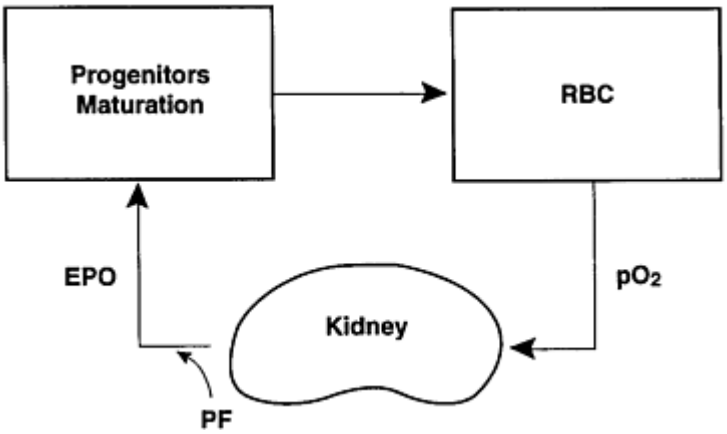

Figure 2.11 Negative feedback regulation of red blood cell (RBC) production by erythropoietin (EPO). N.B. The kidney produces erythrogenin (in response to a lowered pO_2) which combines with a plasma factor (PF) to make EPO

(radiation-induced or not; simultaneously or independently induced) the haemopoietic cells may be allowed or induced to develop in an uncontrolled manner.

In theory, any cell which is proliferating is potentially at risk from such events. In practical terms, the further a cell is along the development line, the more likely any induced lesion to that cell or its microenvironmental control is to produce only a small aberrant clone which cannot maintain itself. Stem cells which are naturally self-maintaining seem the more appropriate targets. Chronic myeloid leukaemia (CML) has well recognised damage which is characterised by the pH^1 chromosome. This marker appears not just in CML cells but also in erythroid and some lymphoid cells, clearly indicating that the leukaemia must have originated from a very early part of the stem cell compartment.

Not all leukaemias are considered as of stem cell origin, however. Lymphocytes are usually thought of as 'end' functional cells and lymphoid leukaemias as mature cell leukaemias. A word of warning about lymphocytes, however. Erythrocytes and granulocytes, as end cells, have matured beyond the possibility of further proliferative activity. However, lymphocytes have not. Antigenic stimulation can, and does, induce proliferation. Thus they are end cells which retain proliferative potential. Furthermore, some of their control properties are more akin to those of the stem cells than those of other developing lineages. Mechanistically, therefore, induction of a lymphoid leukaemia may not be entirely different from that of the granulocytic leukaemias. On the basis of this knowledge, the following chapters take a close look at radiation toxicology in bone marrow and its relationship to leukaemia.

Acknowledgement

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References

BECKER, A.J., MCCULLOCH, E.A. & TILL, J.E. (1963) Cytological demonstration of the clonal nature of spleen colonies derived from mouse marrow cells, *Nature,* **197,** 452–54.

BRADLEY, T.R. & METCALF, D. (1966) The growth of mouse bone marrow cells *in vitro, Austral J. Experimental and Medical Sciences,* **44,** 287–300.

DEXTER, T.M. & MOORE, M.A.S. (1977) *In vitro* duplication and 'cure' of haemopoietic defects in genetically anaemic mice, *Nature,* **269,** 412–14.

DEXTER, T.M., ALLEN, T.D., LAJTHA, L.G., SCHOFIELD, R. & LORD, B.I. (1973) Stimulation of differentiation and proliferation of haemopoietic cells *in vitro, J. Cellular Physiology,* **82,** 461–74.

FRIEDENSTEIN, A.J., CHAILAKHJAN, R.K., LATSINIK, N.V., PANASYNK, A.F. & KEILISS-BOROK, I.V. (1967) Stromal cell responsible for transferring the microenvironment of the haemopoietic tissue, *Transplantation,* **5,** 74–80.

FRIEDENSTEIN, A.J., PETRAKOVA, K.V., KURALESOVA, A.I. & FROLOVA, G.P. (1968) Heterotopic transplants of bone marrow. Analysis of precursor cells for osteogenic and haemopoietic tissues, *Transplantation,* **6**, 230–47.

HARRISON, D.E. (1978) Genetically defined animals valuable in testing aging of erythroid and lymphoid stem cells and microenvironments. In D.Bergsma & D.E.Harrison (eds), *Genetic Effects on Aging,* National Foundation March of Dimes Original Article Series. New York: Alan R.Liss, pp. 187–96.

HODGSON, G.S. & BRADLEY, T.R. (1979) Properties of haemopoietic stem cells surviving 5-fluorouracil treatment: evidence for a pre-CFU-S cell, *Nature,* **281**, 381–2.

LAJTHA, L.G. (1980) The common ancestral cell. In M.M.Wintrobe (ed.), *Blood Pure and Elegant*. New York: McGraw Hill, pp. 80–95.

LORD, B.I. (1992) The architecture of bone marrow cell populations. In M.J.Murphy, Jr, (ed.), *Concise Reviews in Clinical and Experimental Hematology*. Dayton, OH: Alpha Med Press, pp. 225–34.

LORD, B.I. & DEXTER, T.M. (eds) (1992) Growth factors in haemopoiesis. *Clinical Haematology,* vol. 5, no. 3. London: Ballière Tindall, pp. 499–789.

LORD, B.I. & SPOONCER, E. (1986) Isolation of haemopoietic spleen colony forming cells. *Lymphokine Research,* **5**, 59–72.

LORD, B.I. & WOOLFORD, L.B. (1993) Proliferation of spleen colony-forming units (CFU-$S_{8,}$ CFU-$S1_{2)}$ and cells with marrow repopulating ability, *Stem Cells,* **11,** 212–17.

LORD, B.I. & WRIGHT, E.G. (1984) Spatial organisation of CFU-S proliferation regulators in the mouse femur, *Leukaemia Research,* **8**, 1073–83.

MCCULLOCH, E.A. (1978) Stem cell functions and the clonal haemopathies of man. In B.I. Lord, C.S.Potten & R.J.Cole (eds), *Stem Cells and Tissue Homeostasis*. Cambridge: Cambridge University Press, pp. 139–56.

MAGLI, M.C., ISCOVE, N.N. & ODARTCHENKO, N. (1982) Transient nature of early haemopoetic spleen colonies, *Nature,* **295**, 527–9.

MAXIMOW, A. (1910) Untersuchungen über die Blut und Buidegewebe. III: Die embryonale Histogenese des Knockenmarks der Sangetiere, *Achiven Mikroskopie Anatomie,* **76**, 1–113.

PAPPENHEIM, A. (1909) A meeting of the German Haematologic Society, *Folia Haematologica (Leipzig),* **8**, 390–409.

PERLA, D. (1936) The regeneration of autoplastic splenic transplants, *Am. J. Pathology,* **12**, 665–75.

PLUZNIK, D.H. & SACHS, L. (1965) The cloning of normal mast cells in tissue culture, *J. Cellular Physiology,* **6**, 319–24.

ROSENDAAL, M., HODGSON, G.S. & BRADLEY, T.R. (1979) Organisation of haemopoietic stem cells: the generation age hypothesis, *Cell and Tissue Kinetics,* **12**, 17–29.

SCHOFIELD, R. (1978) The relationship between the spleen colony-forming cell and the hemopoietic stem cells: a hypothesis, *Blood Cells,* **4**, 7–25.

SCHOFIELD, R. & LAJTHA, L.G. (1973) Effects of isopropyl methane sulphonate (IMS) on haemopoietic colony-forming cells, *Br. J. Haematology,* **25**, 195–202.

SIMINOVITCH, L., MCCULLOCH, E.A. & TILL, J.E. (1963) The distribution of colony forming cells among spleen colonies, *J. Cellular and Comparative Physiology,* **62,** 327–36.

SIMINOVITCH, L., TILL, J.E. & MCCULLOCH, E.A. (1964) Decline in colony-forming ability of marrow cells subjected to serial transplantation into irradiated mice, *J. Cellular and Comparative Physiology,* **64**, 23–31.

SPANGRUDE, G.J., HEIMFELD, S. & WEISSMAN, I.L. (1988) Purification and characterisation of mouse hematopoietic stem cells, *Science,* **241**, 58–62.

TESTA, N.G., HENDRY, J.H. & MOLINEUX, G. (1985) Long-term bone marrow damage to experimental systems and in patients after radiation or chemotherapy, *Anticancer Research ,* **5**, 101–10.

TILL, J.E. & MCCULLOCH, E.A. (1961) A direct measurement of the radiation sensitivity of normal mouse bone marrow cells, *Radiation Research,* **14**, 213–22.

VISSER, J.W.M., BAUMAN, J.G.J., MÜLDER, A.H., ELIASON, J.F. & DE LEEUW, A.M. (1984) Isolation of murine pluripotent haemopoietic stem cells, *J. Experimental Medicine,* **59,** 1576–90.

WEINBECK, J. (1938) Die Granulopese des kindlichen Knockenmarker und ihre Reaktion auf Infectionen, *Beit Patologie Allgem Patologie,* **101,** 268–83.

WEISS, L. (1970) The histology of the bone marrow. In A.J.Gordon (ed.), *Regulation of Hematopoiesis,* vol. 1. New York: Appleton-Century-Croft, pp. 79–92.

WEISS, L. (1976) The hematopoietic microenvironment of the bone marrow: an ultrastructural study of the stroma in rats, *Anatomical Record,* **186,** 161–84.

WORTON, R.G., MCCULLOCH, E.A. & TILL, J.E. (1969) Physical separation of hemopoietic stem cells differing in their capacity for self-renewal, *J. Experimental Medicine,* **130,** 91–101.

WU, A.M., TIU, J.E., SIMINOVITCH, L. & MCCULLOCH, E.A. (1967) A cytological study of the capacity for differentiation of renal hemopoietic colony-forming cells, *J. Cellular Physiology,* **69,** 177–84.

XU, C.X. & HENDRY, J.H. (1981) The radial distribution of fibroblastic colony forming cells in mouse femoral marrow, *Biomedicine Express,* **35**, 119–22.

3
Human Radiation Exposure
Sources and levels

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3
Human Radiation Exposure
Sources and levels

3.1
General Introduction

Human radiation exposures to bone marrow span a wide range of dose from around 1 mSv y^{-1} from natural background radiation to several Sv in the case of medical irradiation for diagnostic or treatment purposes. Equally, these exposures cover the complete range of radiation type and quality, from medical X-rays and natural background -radiation to internally deposited natural -radionuclides and Auger emitters used in nuclear medicine.

This chapter discusses radiation exposure to bone marrow in approximate order of the size of population exposed. This means first considering exposure from natural background sources.

Radiation exposure to the UK population is summarised in the pie chart shown in Figure 3.1, published by the National Radiological Protection Board (NRPB). This is expressed in terms of the average effective dose received. In radiation protection, terms such as *dose, equivalent dose* and *effective dose* are frequently used. These have precise meanings and their definition is given in section 3.1.1. A full list of definitions may be found in ICRP 60 (1991).

A number of points need emphasising in relation to Figure 3.1. The chart is made up of the equivalent doses to particular body organs from a variety of radiation types, which in turn are averaged for the whole population to give the overall average effective dose. Consider, for example, the contribution from domestic radon and thoron exposure. This accounts for 50 per cent of the radiation exposure to the UK population as a whole. When inhaled, the lung receives a far higher equivalent dose from radon and thoron compared with other internal organs. The average indoor exposure of 20 Bq m^{-3} thus results in an annual lung equivalent dose of approximately 8 mSv. The contribution to the effective dose is obtained by multiplying this value by a lung organ weighting factor of 0.12, the perceived relative risk of death from cancer in that organ, yielding a rounded value of 1 mSv y^{-1}. In fact, this quantity refers to the effective dose, averaged over the whole population because the mean UK indoor radon exposure of 20 Bq m^{-3} is itself a population-weighted average, i.e. it is made up of the mean radon concentration in the various counties of the UK, inferred from representative indoor radon measurements, which is then weighted by the number of people resident in those counties.

A similar situation arises in the case of medical diagnostic exposure. In Figure 3.1 the highest exposure from artificial sources is that from medical exposures, principally X-rays, accounting for 12 per cent of the effective dose. In practice, however, this is made up of people who actually receive radiation diagnosis or treatment in hospital, averaged alongside the majority of the population who receive no exposure at all.

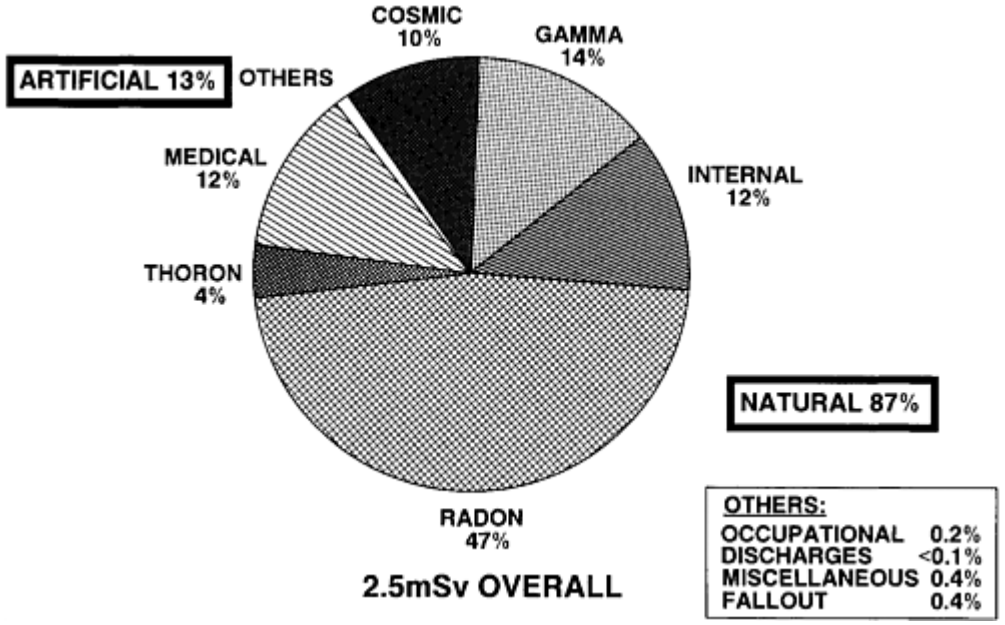

Figure 3.1 Pie chart showing the average effective radiation dose exposure to the UK population.

(*Source:* NRPB)

In considering human radiation exposure to bone marrow we also need to appreciate the clear difference between low LET and high LET radiation. In simple terms these refer to the rate of energy loss by ionisation of the radiation on a scale of biological interest, such as the diameter of double stranded DNA. In low LET radiation the rate of ionisation loss on this scale is very low, such that an individual low LET event in the cell nucleus, while capable of inducing a DNA single strand break, is unlikely simultaneously to break both strands of DNA. X-rays, -rays, -particles and sea-level cosmic ray electrons and muons fall into this category. On the other hand, high LET radiation is so-called because of the high ionisation rate that occurs on a nanometer scale, along the path of a single event such as the passage of an -particle. As a result, high LET radiation is considered highly efficient at inducing double strand breaks in DNA. In addition to -particles, fission neutrons and Auger emitters such as ^{99m}Tc and ^{201}Tl used in nuclear medicine also fall into the category of high LET radiation.

This quantitative difference between low and high LET radiation has been extensively investigated by workers in the field of microdosimetry (Paretzke, 1980; Wilson and Paretzke, 1980; Goodhead and Charlton, 1985). The relative importance of the two radiation types has been described by Goodhead (1988) and for natural exposures this is summarised in Figure 3.2. The annual effective dose from natural background low LET radiation is approximately 1 mSv. At this dose rate each cell nucleus of the body is traversed by approximately 1 low LET particle per year. In contrast, for the similar effective dose from natural background -radiation, only one in approximately five thousand cell nuclei are on average traversed by an -particle per year. Thus, in the case of -particles, most cells receive no hits at all, but of those which do, the radiation dose deposited is very large, ~0.5 Gy.

Another point to emphasise is that the radioactivity of a given radionuclide does little to indicate the dose received. This may be illustrated by comparing an average -particle emitter of typical energy around 0.5 MeV with an average -emitter of typical energy around 5.0 MeV. For the same activity the equivalent dose from

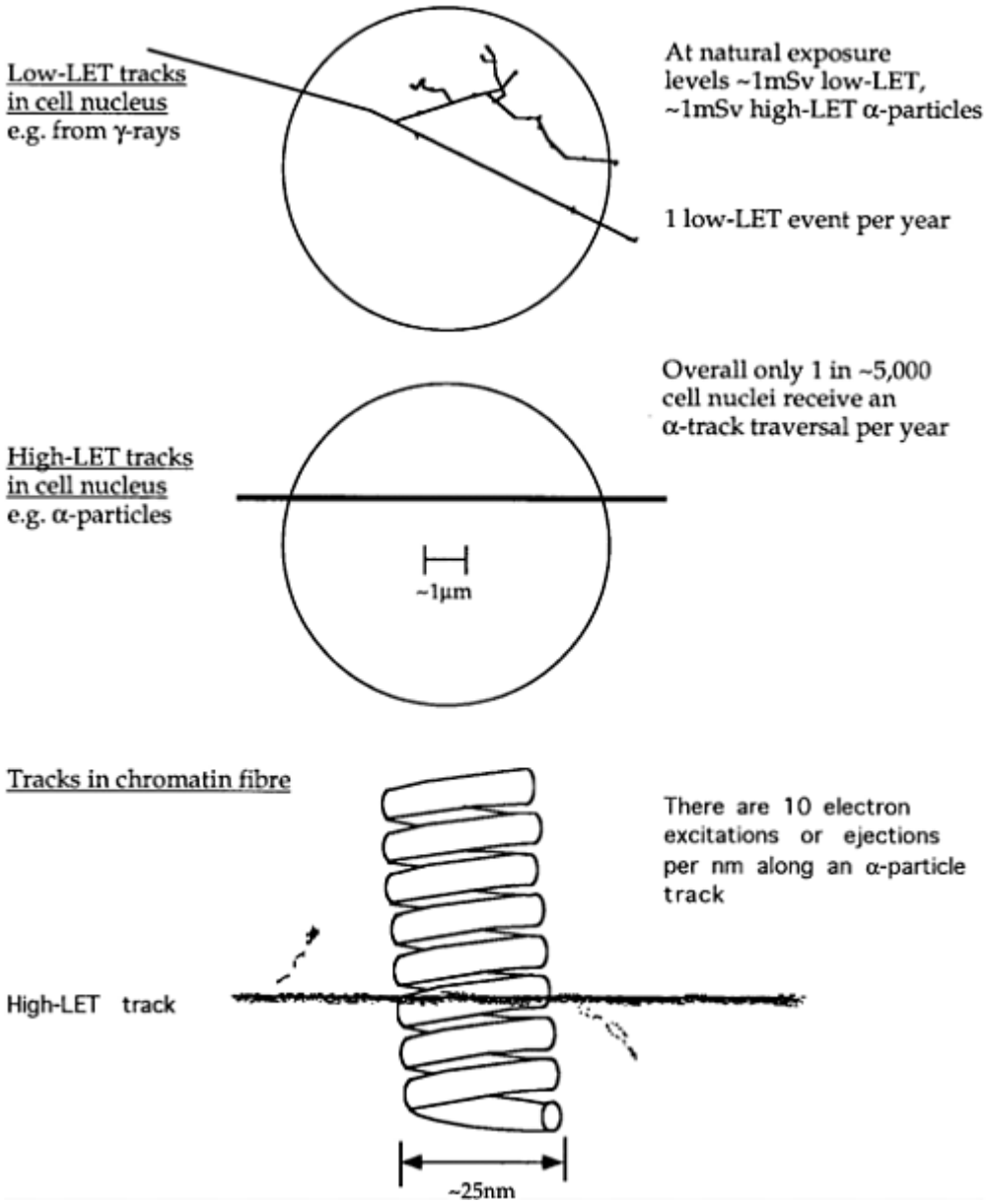

Figure 3.2 Schematic representation of low LET electron tracks from -rays passing through a cell nucleus in comparison with the passage of a high LET -particle through an elementary chromatin fibre of about 25 nm diameter. A true scale diagram would require that the clusters of ionisations near the track ends of the low-energy secondary electrons be very much more compact (Goodhead, 1988)

the -emitter is two hundred times greater than from the -emitter: firstly because the energy is ten times higher and secondly because the radiation weighting factor is twenty times greater. Thus an -emitter of

activity 1 Bq may deliver an equivalent dose rate equal to that of a 200 Bq -emitter. There are mitigating circumstances to this notion in the case of the marrow dose from radionuclides in bone. Alpha-radionuclides in bone irradiate the portion of marrow within -particle range of the bone surface. Since -particles have a greater range than -particles, at bone surfaces they irradiate a higher proportion of marrow. The subtleties of marrow dose from - and -emitters are discussed in section 3.3.2.

In view of the important differences between low LET and high LET radiation, the two types of radiation are considered separately in this chapter. In considering exposure to bone marrow, we concern ourselves solely with dose in units of gray, or equivalent dose in units of sievert. The equivalent dose for -particles is determined by multiplying the gray dose by its radiation weighting factor. In radiation protection this is currently set at 20, but it should be realised that for low doses the RBE of -particles for cancer induction as an end-point is not known.

There is increasing interest in early exposure to radionuclides, especially in the foetus. While for all ages the penetrating component of natural background radiation will give the same dose as the effective dose, far more detailed measurements are required to determine the marrow dose for ingested or inhaled radionuclides. For foetal dosimetry this requires knowledge both of transplacental transfer and distribution of radionuclides with respect to cells at risk in the foetus itself. Ongoing work in this area is also described.

3.1.1 Definitions

The following terms are used in this chapter reflecting those used in radiation research and radiation protection. A full set of definitions currently recommended for use in radiation protection may be found in ICRP 60 (1991).

LET, Linear energy transfer or LET is defined as L=dE/dl, where dE is the energy loss by a charged particle in traversing a distance dl. Its working units are keV μm^{-1}. More usually we define a restricted energy transfer L which is calculated for a given -ray cut-off energy, and which provides a measure of energy deposit close to the axis of the particle track. LET is used in situations where one seeks to determine the energy deposited on a scale of the order of DNA size.

High and low LET radiation. Gamma-radiation and -particle electrons are termed low LET radiations because of the sparse ionisation density produced on a nanometre scale. In contrast, heavy ions such as -particles and the recoil nuclei produced from fission neutrons are termed high LET radiations because of the high ionisation density they produce on a nanometre scale.

Organ dose. For radiation protection purposes, ICRP defines a tissue- or organ-averaged absorbed dose, D_T, given by:

$$D_T = \frac{\varepsilon_T}{m_T}$$

where $_T$ is the total energy imparted in a tissue or organ and m$_T$ is the mass of that tissue or organ. The unit for organ dose is the gray (Gy) whose SI units are J kg^{-1}.

Relative biological efficiency (RBE) and *radiation weighting factor*. In experimental studies, different types of radiation such as -particles and fission neutrons are shown to have a greater biological effect, or relative biological efficiency, compared with the same dose of - or X-rays. In practice, measured RBE values vary considerably depending on their end-point. Therefore, for radiation protection purposes a judgement of the RBE for -particles has been made, defined as the radiation weighting factor, w_R, whose value is currently set at 20. For fission neutrons w_R varies with energy but is set at 20 in the energy range > 100 keV to 2 MeV; w_R for -rays, X-rays and -particles is defined as 1.

Equivalent dose. The equivalent dose, $H_{T,R}$, in tissue or organ T *d*ue to radiation *R,* is given by:

$$H_{T,R} = w_R \cdot D_{T,R}$$

where $D_{T,R}$ is the average dose from radiation R in tissue or organ T, and w_R is the radiation weighting factor. Since w_R is dimensionless, the SI units of equivalent dose are again J kg^{-1}, but called sieverts, written Sv.

Tissue weighting factors and effective dose. In order to take account of the different radiation sensitivities of body organs, ICRP defines an effective dose, *E,* obtained by multiplying the equivalent dose to a particular organ by a weighting factor, w_T, for that organ. The latter represents the assumed relative risk of death from cancer. Mathematically this is written :

$$E = \sum_T w_T \cdot H_T$$

The unit of effective dose is again the sievert.

3.2
Naturally Occurring Low LET Sources

3.2.1
Cosmic Rays

The earth is bombarded with cosmic rays from outer space. These rays consist of protons and other nuclei, and electrons. All of these, however, are absorbed by the earth's atmosphere and the cosmic rays seen at sea-level represent the secondary components of nuclear interactions of the primary cosmic rays. The sea-level cosmic rays consist principally of muons, electrons, -ray photons and a small number of neutrons. The intensity of this secondary component varies with altitude above sea-level and, as a result, the dose rate at high altitude locations such as Mexico City is higher than at sea-level. There is some variation in the intensity of the sea-level cosmic rays across the earth owing to the effect of the earth's magnetic field, although variations across most individual countries are small. The world average annual effective dose at sea-level is around 270 μSv. The high-LET neutron component contributes only 30 μSv to this figure.

3.2.2
Terrestrial Gamma-rays

Many of the naturally occurring radionuclides in the uranium and thorium radioactive decay series emit -radiation and the presence of these radionuclides in the

Table 3.1 Natural background -radiation absorbed dose rate in air for the five highest and five lowest countries (in nGy h^{-1} and Sv y^{-1})

	Outdoor		Indoor	
	nGy h^{-1}	μSv y^{-1}	nGy h^{-1}	μSv y^{-1}
Five highest				
Hong Kong	161	1410	182	1590
Namibia	120	1050	140	1230
Australia	93	810	103	900
Portugal	83	730	105	920

	Outdoor		Indoor	
	nGy h^{-1}	μSv y^{-1}	nGy h^{-1}	μSv y^{-1}
Romania	80	700	–	–
Five lowest				
Canada	25	220	–	–
Iceland	28	250	23	200
Egypt	32	280	–	–
Netherlands	33	290	65	570
UK	35	310	61	530

Source: UNSCEAR, 1993, p. 76.

earth and in building materials provides a source of natural background radiation to the population (Table 3.1). The presence of these radionuclides in building materials invariably means that the dose received indoors is higher than that received outdoors. However, the picture is not simple and there are significant variations in different countries throughout the world. This is illustrated in Figure 3.3 which shows the ratio of indoor to outdoor absorbed dose rates in air from terrestrial radiations for various countries (UNSCEAR, 1993). The ratio is less than one for only a few countries: France, Bulgaria, Japan, Iceland and the USA.

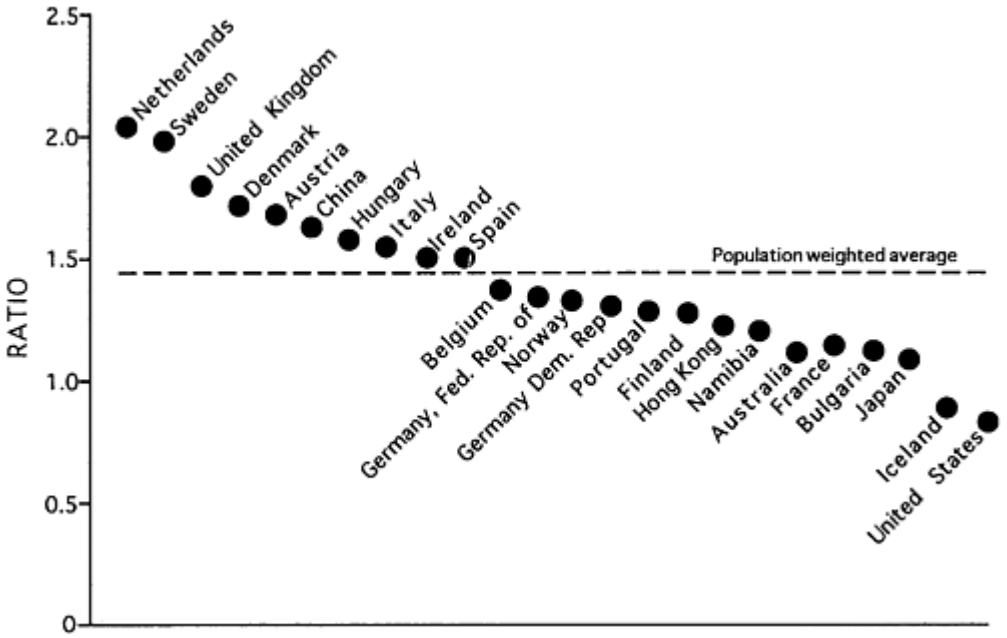

Figure 3.3 Ratio of indoor to outdoor absorbed dose rates in air from terrestrial radiation.
(*Source:* UNSCEAR, 1993, p. 76)

3.2.3
Potassium-40

The body content of potassium is about 0.18 per cent. The element has a naturally occurring radioactive isotope ^{40}K whose uptake by the body results in a source of background radiation exposure (UNSCEAR, 1993, p. 43). Potassium-40 decays mainly by -emission. The isotopic abundance of ^{40}K of 1.18×10^{-4} results in an average body activity of 55 Bq kg^{-1} of body weight. This results in an annual effective dose of 165 μSv for adults and 185 μSv for children, most of the dose being delivered by -particles.

3.3
Naturally Occurring Long-lived Alpha-radionuclides

3.3.1
Introduction

Historically, studies of long-lived -radionuclides in the skeleton concentrated on those affecting certain high exposure groups such as the radium dial painters, the thorotrast patients and nuclear workers potentially exposed to actinides.

Radionuclides, including the -emitters, were crudely classified into so-called bone-volume or bone-surface seekers, depending on their mode of uptake in bone. Elements which were chemically, and therefore metabolically, similar to calcium were classed as volume-seekers, under the assumption that for non-acute exposure they would distribute uniformly throughout the bone volume. Radium and uranium fell into this category, along with lead. The importance of the latter arises from the skeletal burden of ^{210}Pb which decays to -emitting ^{210}Po. Elements such as plutonium and americium, which have no physiological analogue, were assumed to deposit on bone surfaces, without subsequent distribution within bone volume.

The classification of radionuclides into volume and surface seekers was adopted by the International Commission on Radiological Protection in their ICRP Publication 30 (1979) and this appears to hold in the ICRP Publication 67 (1993) (Table 3.2). Existing measurements in human bone show this classification to be too simplistic as most radionuclides display a long-term surface retention component in bone. This phenomenon is discussed in section 3.3.6.

Traditionally therefore, -emitters are usually considered to irradiate haemopoietic cells in bone marrow by virtue of their presence in bone. The short range of the -particle, ~40 μm in tissue, means that cells residing close to bone surfaces should be thought of as those mostly at risk. Ironically, the -radionuclides dominating natural exposures, ^{210}Po, ^{222}Rn and its short-lived -emitting daughter nuclei ^{218}Po and ^{214}Po have no affinity for bone. In practice, ^{210}Po is associated with bone because, as indicated above, it usually arises from the decay of ^{210}Pb in bone.

Table 3.2 Model calculations of mean residence times on bone surfaces (1/rate of loss) of bone-seeking radionuclides as described in ICRP (1993)

Bone-seeking -radionuclides[a]	days, trabecular bone surfaces	days, cortical bone surfaces
Radium	1.44	1.44
Lead	0.997	0.997
Uranium	7.22	7.22
Plutonium	1350	8120

Bone-seeking -radionuclides[a]	days, trabecular bone surfaces	days, cortical bone surfaces
Neptunium	1350	8120
Americium	1350	8120

[a] According to these values, radium and lead behave as classical volume-seekers, and the actinides as surface-seekers, although direct measurements in human bone show a long-term surface retention component. Uranium bone surface deposits would be removed by 1–2 months after deposition, and the distribution pattern would then be a 'volume' type.

Research into the radiobiology of -emitters in experimental animals has traditionally been dominated by those associated with high exposure groups, notably radium and plutonium; rather less attention was paid to polonium and, until recently, radon was all but ignored.

Worldwide, extensive measurements of ^{210}Po and ^{210}Pb have been made in human tissues, especially the skeleton. Much of this was carried out in the 1950s and 1960s. The levels found were mostly of academic interest and the consequent dose to bone marrow was only occasionally considered.

In the UK, serious interest in natural emitters can be traced to the Black Report (1984) into the excess cases of childhood cancer near the British Nuclear Fuels' reprocessing plant at Sellafield. This was the first time that public attention had been focused on radionuclides in bone, but the concern remained with man-made discharges and their possible carcinogenic effect. Although existing research clearly showed that the levels of discharge emitters in bone were considerably lower than those for natural background radionuclides (Table 3.3), intense efforts continued to be put into research into the radiobiology of man-made -radionuclides and in establishing their levels in tissues of the general population.

Table 3.3 Summary of average levels of long-lived -radionuclides in the human skeleton from autopsy measurements

-radionuclide	Typical levels (Bq kg^{-1})	Key references
^{210}Po/^{210}Pb	0.7–3.0	Czeglédi (1987), Hill (1965), Parfenov (1974), Henshaw *et al.* (1988)
^{226}Ra	0.2–0.6	Fisenne and Keller (1981), Walton *et al.* (1959)
^{238}U	~0.05	Welford and Baird (1967), Hamilton (1972), Singh *et al.* (1985), Nozaki *et al.* (1970)
^{232}Th	~0.005	Singh *et al.* (1985)
^{239}Pu	<0.001–0.005	Burkinshaw *et al.* (1987), Popplewell *et al.* (1985, 1988)

Today it is generally appreciated that, for all but special exposure groups, the two most important -emitters contributing to marrow dose in man are polonium and radon. Most recently, interest has begun to focus on these natural -radionuclides *vis-à-vis* their possible role in the aetiology of some cancers in man, especially leukaemia.

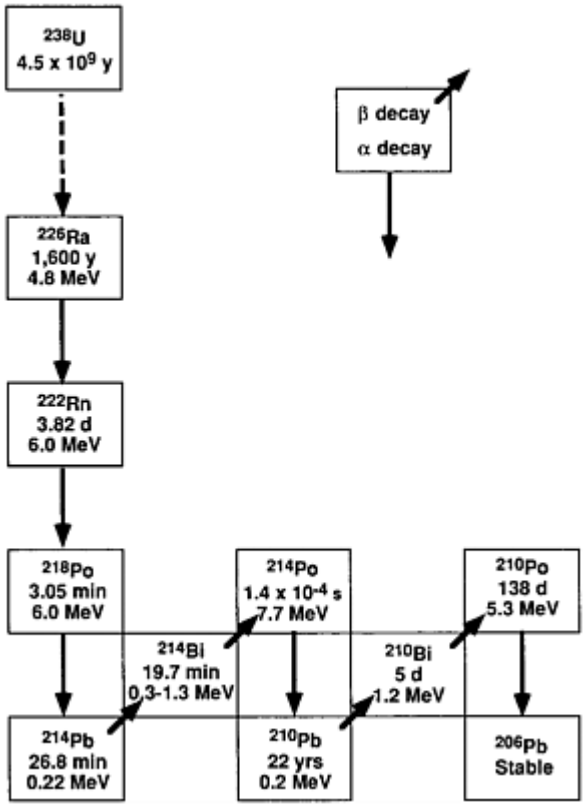

Figure 3.4(a) Relevant parts of the natural radioactive decay chains: ^{238}U

3.3.2
Polonium-210 in Bone

Polonium-210 ranks alongside ^{222}Rn as being the most important source of radiation dose to bone marrow in the general population. This -radionuclide occurs at the end of the ^{238}U natural radioactive decay chain, as shown in Figure 3.4 . As such, most ^{210}Po occurs in association with ^{210}Pb, the latter having a half-life of twenty-two years, decaying via five-day half-life ^{210}Bi to ^{210}Po. The ^{210}Po which is found in association with ^{210}Pb is said to be *supported* by ^{210}Pb, otherwise the term *unsupported* ^{210}Po is often used.

Polonium-210 itself is not a bone-seeker. Ingested or inhaled in unsupported form, it distributes fairly uniformly in the body, although higher levels are taken up by the liver and kidneys. In the bloodstream, 90 per cent of ^{210}Po is linked to the erythrocytes, more specifically with the protein fraction of the haemoglobin (Campbell and Talley, 1954). Polonium-210 in citrate form binds preferentially to DNA, and this in part may explain the observed anomalously high RBE values found in some *in vitro* experiments (Rao *et al.* 1991). The level of ^{210}Po in marrow has been measured by radiochemical analysis with values in the range 0.10–0.90 Bq kg^{-1} (UNSCEAR, 1988; Ladinskaya *et al.,* 1973; Bradley, 1990; Baratta and Ferri, 1966). This translates to a marrow dose in the range 55–500 μSv y^{-1}.

Most ^{210}Po in bone, however, arises from the presence of ^{210}Pb in bone (Osborne, 1963; Hill, 1965; Parfenov, 1974). The review by Parfenov (1974) showed values ranging from 0.7 to 1.9 Bq kg^{-1} with an average of 1.4 Bq kg^{-1}. Most, but not all, of this is supported by ^{210}Pb with an average $^{210}Po/^{210}Pb$ ratio of 0.

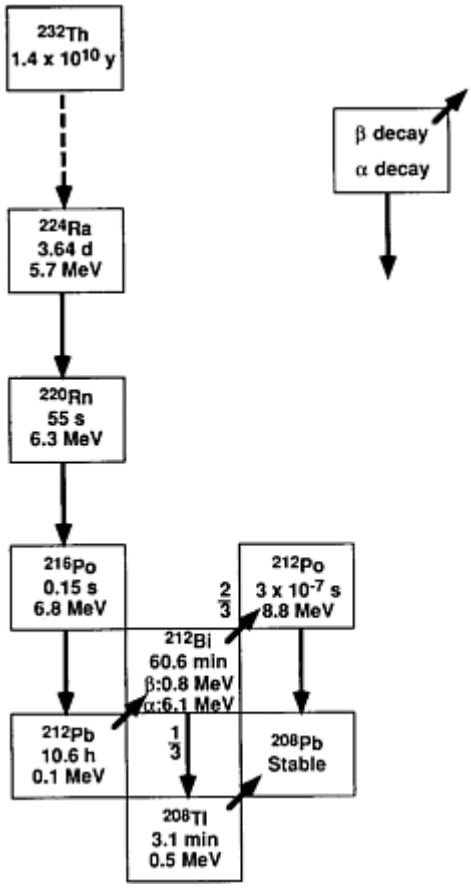

Figure 3.4(b) Relevant parts of the natural radioactive decay chains: ^{232}Th

7. A study by Czeglédi (1987) found values for ^{210}Po in the range 1.0–3.0 Bq kg^{-1}. Henshaw *et al.* (1988) analysed -particle autoradiographs of bone samples from adults and children in the UK, obtaining average values of 1.5 and 0.3 Bq kg^{-1} respectively.

Under the assumption that ^{210}Pb is a volume-seeker, it is possible to estimate the dose contribution to marrow in trabecular bone from the subsequent decay of ^{210}Po within the bone volume. Extensive measurements have been made of the size and shape of trabecular spaces in human bone, expressed as the frequency distribution of path lengths across the spaces (Beddoe, 1977; Beddoe *et al.* 1976, Spiers, 1974; Spiers and Beddoe, 1977; Whitehouse, 1974). A typical example of the path length distribution for the third lumbar vertebra of males aged 1.7, 9 and 44 years is shown in Figure 3.5 (Beddoe *et al.,* 1976). Using such distributions, Spiers *et al.* (1978) derived factors for converting -activity concentration in bone to the equivalent in trabecular marrow space. For volume-seekers the factors vary with location in the skeleton and are in the range 0.019–0.083.

These data may be used to estimate the marrow dose derived from ^{210}Pb in bone. In the absence of precise human data, estimated values are shown in Table 3.4 as a function of age. These are somewhat lower than those indicated by some of the autopsy measurements quoted above. The contribution from ^{210}Bi

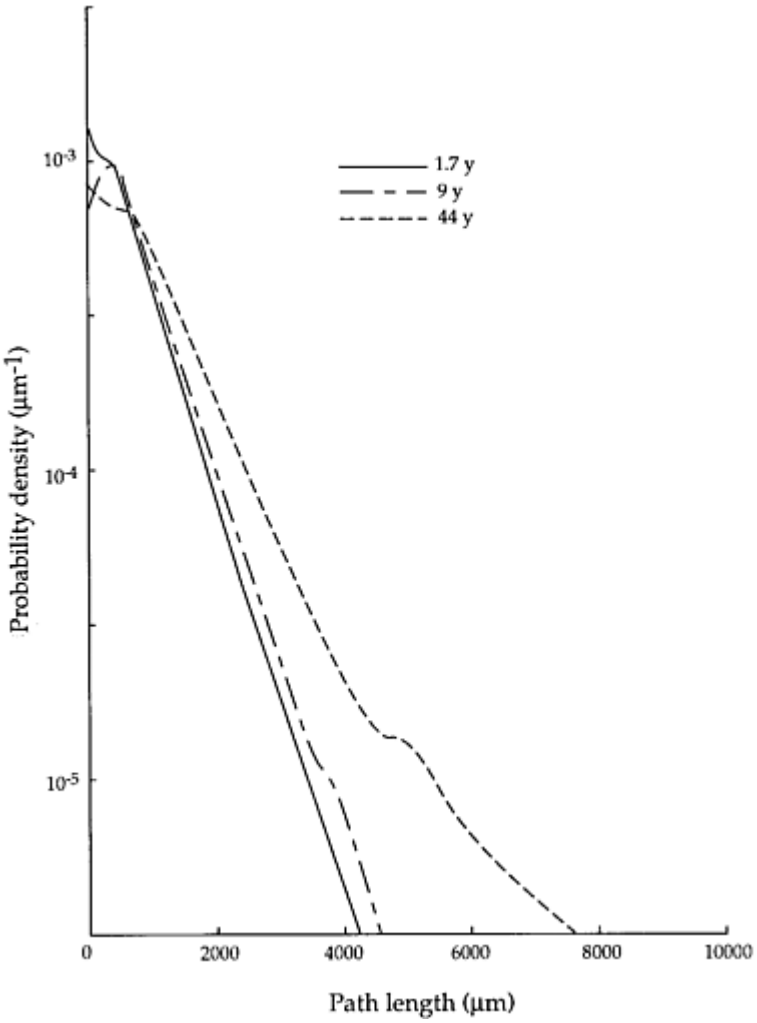

Figure 3.5 Path length distributions through trabecular spaces of the third lumbar vertebra of males aged 1.7 years, 9 years and 44 years. (Source: Beddoe *et al.,* 1976)

should be noted: this arises since -particles emitted at bone surfaces have a greater range than -particles and therefore irradiate a higher proportion of marrow.

3.3.3
Radium-226 in Bone

In a worldwide study, Fisenne and Keller (1981) measured a median population weighted concentration of ^{226}Ra of 0.85 Bq kg^{-1} of calcium corresponding to

Table 3.4 Activity in bone and absorbed doses in red bone marrow from ^{210}Pb, ^{210}Bi and ^{210}Po

Age (years):	0.25	1	5	10	15	25
Activity in bone, Bq kg^{-1} wet weight						
^{210}Pb	0.5	0.75	1.0	1.25	1.5	1.5
^{210}Bi	0.5	0.75	1.0	1.25	1.5	1.5
^{210}Po	0.180	0.465	0.710	0.963	1.23	1.32
^{210}Po/^{210}Pb	0.36	0.62	0.71	0.77	0.82	0.88
Absorbed dose in red marrow from trabecular bone, μGy y^{-1}						
^{210}Pb	0.0107	0.0160	0.0214	0.0253	0.0303	0.0303
^{210}Bi	0.159	0.239	0.318	0.376	0.451	0.451
^{210}Po	0.192	0.496	0.757	0.969	1.16	1.16
^{210}Po μSv y^{-1}	3.84	9.92	15.1	19.4	23.2	23.2
Total equivalent dose, μSv y^{-1}						
	4.2	10.7	16.2	20.8	24.8	24.8

Note: ^{210}Bi and ^{210}Po are assumed to be supported by ^{210}Pb in mineral bone, and the ^{210}Po/^{210}Pb ratio is calculated from age-dependent Pb retention times in bone (ICRP, 1993), in the absence of precise human data on age-dependent ^{210}Po/^{210}Pb ratio in bone. Doses to red bone marrow are based on the ^{210}Pb–^{210}Po dosimetry in Thomas (1994). The activity of ^{210}Pb in bone at different ages is given arbitrary values based on several published measurements of ^{210}Pb and stable Pb in bone which show a low degree of consistency.

~0.23 Bq kg^{-1} of wet bone. A study of Walton *et al.* (1959) of samples from the UK gave values for ^{226}Ra of 1.6 Bq kg^{-1} of calcium or 0.6 Bq kg^{-1} of wet bone. These values are approximately an order of magnitude lower than for ^{210}Po in bone with correspondingly lower contribution to marrow dose.

3.3.4
Other Natural Alpha-radionuclides in Bone

A number of workers have measured natural uranium in bone (Welford and Baird, 1967; Hamilton, 1972; Singh *et al.,* 1985; Nozaki *et al.,* 1970) and reported values in the range 0.01–0.09 Bq kg^{-1}. Singh also made measurements of thorium isotopes in bone and found maximum values of 0.1 Bq kg^{-1}.

3.3.5
Discharge and Fallout Sources

Detailed measurements have been carried out of the plutonium content of bone and bone marrow in tissues of the general public in the UK. Much of this appears to be from fallout rather than from discharges from nuclear reprocessing. Popplewell *et al.* (1985, 1988) compared Pu activity of concentrations in rib, vertebra and femur of former residents of West Cumbria, Central Scotland, North-east England and Oxfordshire. The concentrations were somewhat similar in all regions with little evidence of higher levels in tissues from West Cumbria, where the British Nuclear Fuels' Sellafield reprocessing plant is situated. In all cases, levels were generally below 0.01 Bq kg^{-1}. Burkinshaw *et al.* (1987) obtained similar results for tissues from Yorkshire. Mean values in bone were <0.02 Bq kg^{-1}, and in bone marrow ~0.001 Bq kg^{-1}.

3.3.6
Bone Surface Retention of Alpha-radionuclides

There is considerable evidence that the classification of -radionuclides into surfaceand volume-seekers is too simplistic. The detailed behaviour in the skeleton of animals of several of the -radionuclides in Table 3.2 has been described by Priest and Van de Vyver (1992), showing evidence of surface retention behaviour of the so-called volume-seekers. Evidence has emerged in several studies in man to suggest that most radionuclides exhibit a long-term surface retention component such that the dose to neighbouring marrow is rather greater than would be implied from the results of whole bone activity concentration determined by standard radiochemical analysis.

Bone surface -spectroscopy following protracted exposure to each of the -radionuclides ^{226}Ra, ^{241}Am and ^{239}Pu has revealed enhanced levels at bone surfaces, extending to a depth of a few microns (Schlenker, 1984; Schlenker and Oltman, 1986; see Table 3.5). In the case of ^{226}Ra and ^{241}Am, surface concentrations were observed in human bone where the radionuclide burdens had been acquired several decades previous to death. This finding is particularly interesting in the case of ^{226}Ra, which is considered to be the classic volume-seeking radionuclide, because of its strong ionic behaviour and similarity to calcium.

Recently, Salmon *et al.* (1994) used the spectroscopic capabilities of -sensitive TASTRAK plastic autoradiography to study the depth distribution of ^{210}Pb-

Table 3.5 Measured thicknesses of concentrated deposits of bone-seeking elements at bone surfaces, by various experimental techniques

Species	Bone-seeking element	Experimental method	Derived thickness of the bone surface concentrated deposit (microns)
Dog[a]	^{226}Ra	-spectroscopy by surface barrier detector	1.2
Human[a]	^{239}Pu	-spectroscopy by surface barrier detector	0.87
Human[a]	^{241}Am	-spectroscopy by surface barrier detector	1.1
Human[b]	^{241}Am	-spectroscopy by surface barrier detector	0.6
Human[b]	^{226}Ra	-spectroscopy by surface barrier detector	2–4.3
Human[c]	^{210}Pb/^{210}Po	TASTRAK spectroscopy	<3 μm

[a] Schlenker (1984).
[b] Schlenker and Oltman (1986).
[c] Salmon *et al.* (1994). Note that the ^{210}Pb/^{210}Po was received through chronic natural exposure.

supported ^{210}Po at bone surfaces of human bone from natural exposures. Such data are particularly difficult to obtain since autoradiographs must be stored for several years to accumulate enough -particle tracks for analysis. The -particle range distribution recorded on autoradiographs of bone surface and cut sections within the bone volume were compared, yielding evidence of enhanced ^{210}Pb-supported ^{210}Po activity at the bone surface. These results are illustrated in Figures 3.6–3.8. Figure 3.6 shows calibration spectra for thin and thick -particle sources. Figure 3.7 shows the range spectra obtained from cortical and trabecular bone surfaces which may be compared with those from cut bone surfaces shown in Figure 3.8. In Figure 3.7 the

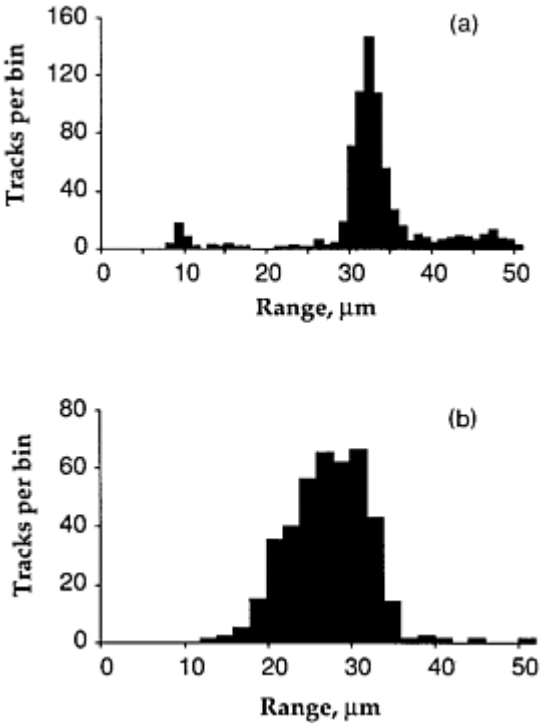

Figure 3.6 (a) The range spectrum recorded on a TASTRAK plastic detector exposed to a thin (Mylar covered) ^{252}Cf source, emitting -particles of 5.23 MeV with a residual range of 32.4 μm in the plastic; (b) the spectrum obtained from a thick -particle source of ^{241}Am uniformly embedded in a block of resin. (*Source:* Salmon *et al.,* 1994)

spectra display features of enhanced surface activity similar to the thin source calibration spectrum. Analysis of these spectra suggests that ^{210}Po activity is enhanced by a factor of ~4 at the bone surface and extends to a depth ~3 μm.

The above findings of persistent thin bone surface deposits of bone-seeking metals, either decades after intake or after continuous lifetime exposure needs to be reconciled with the process of bone remodelling. The rate of remodelling in adult cortical bone is about 2.5–3% by volume and 15–20% y^{-1} by cortical bone surface area (Schlenker and Oltman, 1986). Thus it might be expected that a superficial bone surface deposit of a radionuclide would be removed by bone resorption within five to seven years. The persistence of bone surface radionuclide deposits, despite remodelling activity, points to the importance of secondary deposition, the process by which nuclide ions removed from bone by osteoclast resorption are returned to the bone surface during the apposition of new bone.

Such remodelling has implications for marrow dose because the process involves removing the radionuclide from the bone surface into marrow. An example of the distribution of ^{210}Po activity across an -autoradiograph of human bone at natural exposure is illustrated in Figure 3.9. The sample consisted of a triangular section of the cranium. Each dot represents the decay of ^{210}Po and each cross the decay of ^{226}Ra. Whereas the ^{226}Ra activity is well confined to the bone section, the ^{210}Po activity is spread across the whole autoradiograph. This activity is well above the background count on control plastics and has been interpreted as being indicative of the potential activity in trabecular marrow spaces (Henshaw *et al.,* 1988). The activity is associated with the fluids in the wet unfixed bone sample. It could therefore represent the

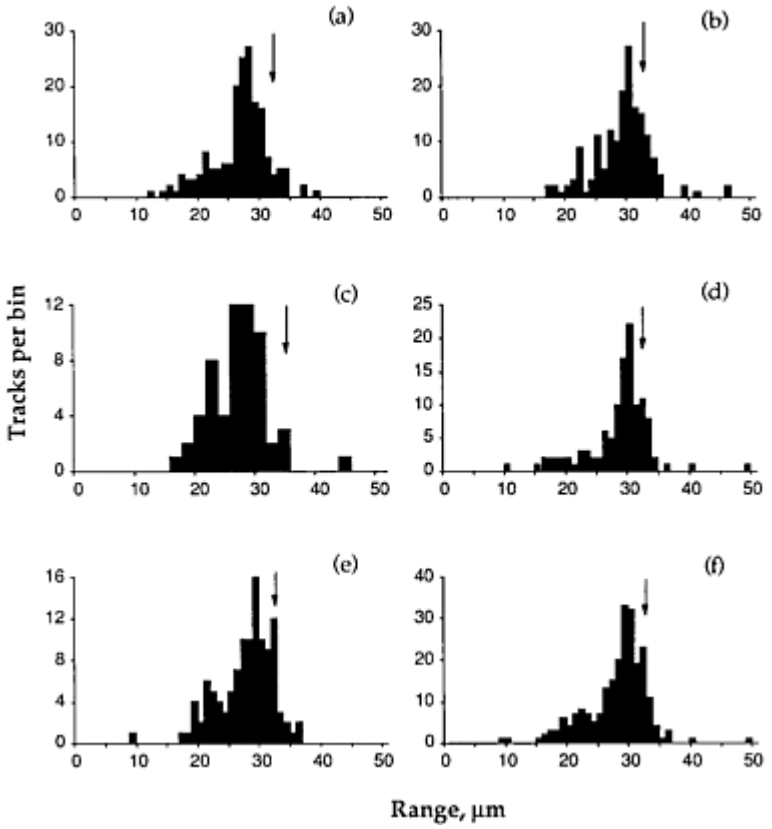

Figure 3.7 Spectra of -particle ranges measured at cortical and trabecular bone surfaces by TASTRAK spectroscopy. Arrows show the ^{210}Po -particle range in TASTRAK, 32.7 μm. The spectra (a), (b) and (c) are from cranial periosteal surfaces. Spectra from trabecular surfaces of the cranium are shown in (d) and (e). The combined spectra from (d) and (e) are shown as spectrum (f). The bin width is 2 μm in (c) and 1 μm in the other five spectra. (*Source:* Salmon *et al.,* 1994)

activity in marrow *per se* or it could be indicative of the release of ^{210}Po following the decay of ^{210}Pb at the bone surface. Whatever the explanation, the ^{210}Po shows a quite different behaviour from ^{226}Ra which remains confined to the bone section itself.

3.3.7
Alpha-radionuclides in Teeth

Measurements of -radionuclides in teeth are of particular interest because they represent a readily obtainable source of human material whose assay may be used as an indicator of levels in bone proper.

There have been a number of radiochemical measurements of ^{210}Po and ^{210}Pb in teeth and some of these have shown a correlation between ^{210}Po in teeth and cumulative radon exposure in the general population (Clemente *et al.,* 1982, 1984; Toth *et al.,* 1982). Activity concentration values have ranged up to 9.0 Bq kg $^{-1}$. Lovaas and Hursh (1968) found values of ^{226}Ra in teeth in the range 0.2–0.9 Bq kg^{-1} wet weight. The authors also showed that measurements of both ^{210}Pb and ^{226}Ra in teeth may be used for the assessment of

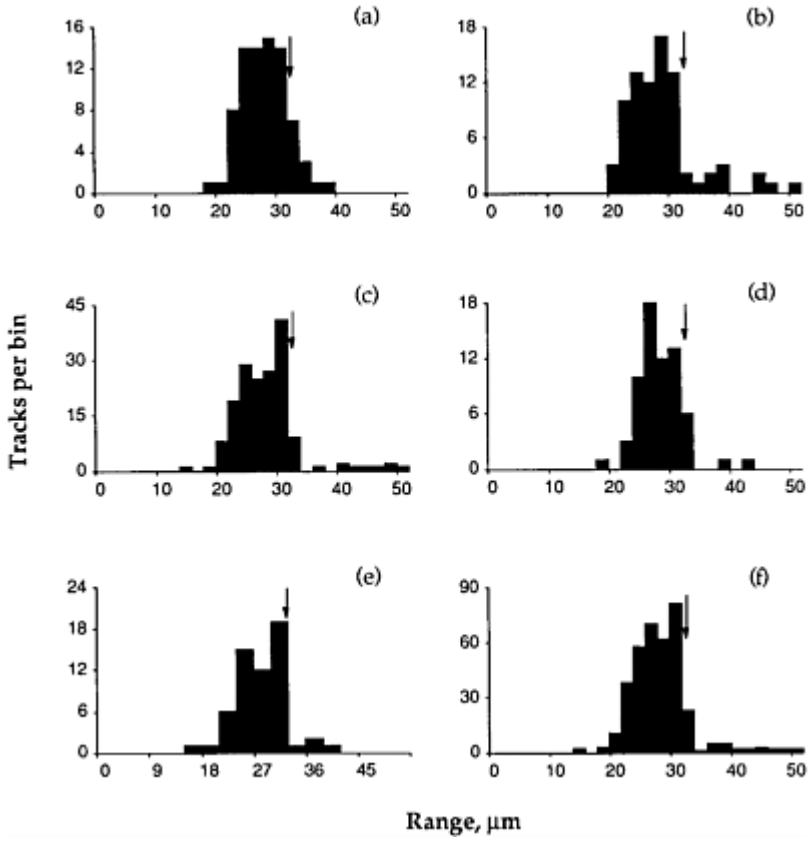

Figure 3.8 Spectra of -particle ranges measured at cut surfaces of human cortical bone by TASTRAK spectroscopy. Arrows show the ^{210}Po -particle range in TASTRAK, 32.7 μm; the spectrum (a) is from the cranial longitudinal section, spectra (b)–(e) are transverse sections of femur, case 181, and spectrum (f) is the combined spectrum of exposures (b)–(e). The bin width is 3 μm in (e) and 2 μm in the other five spectra. (*Source:* Salmon *et al.,* 1994)

the level of these nuclides in compact bone. Uranium-238 and ^{232}Th levels have been measured by Carpenter (1992) in UK teeth from orthodontic extractions yielding mean values of 0.91 Bq kg^{-1} (range 0. 07–5.40 Bq kg^{-1}) and 0.17 Bq kg^{-1} (range 0.02–0.44 Bq kg^{-1}) respectively.

At Bristol University a national survey has been carried out of -activity in children's teeth (Henshaw *et al.,* 1994a). As in bone, most activity arises from natural background sources. The survey has used the -autoradiographic techniques discussed above in relation to studies in bone (Fews and Henshaw, 1983; Hatzialekou *et al.,* 1988; Henshaw *et al.,* 1988). The advantage of this approach is that the total activity concentration is measured in a single tooth sample and spatial distribution information is obtained. Limited spectral information is also obtained enabling the absolute activity concentration and spatial distribution of both ^{210}Pb-supported ^{210}Po and ^{226}Ra to be determined.

Deciduous teeth and permanent teeth extracted from juveniles for orthodontic purposes were obtained from throughout the UK by arrangement with district dental officers. Each tooth was sliced into two longitudinal sections and sandwiched between two TASTRAK -sensitive plastic track detectors. In this way activity was measured on the outer enamel surface of the tooth and on a central section of dentine, pulp cavity and outer annular enamel.

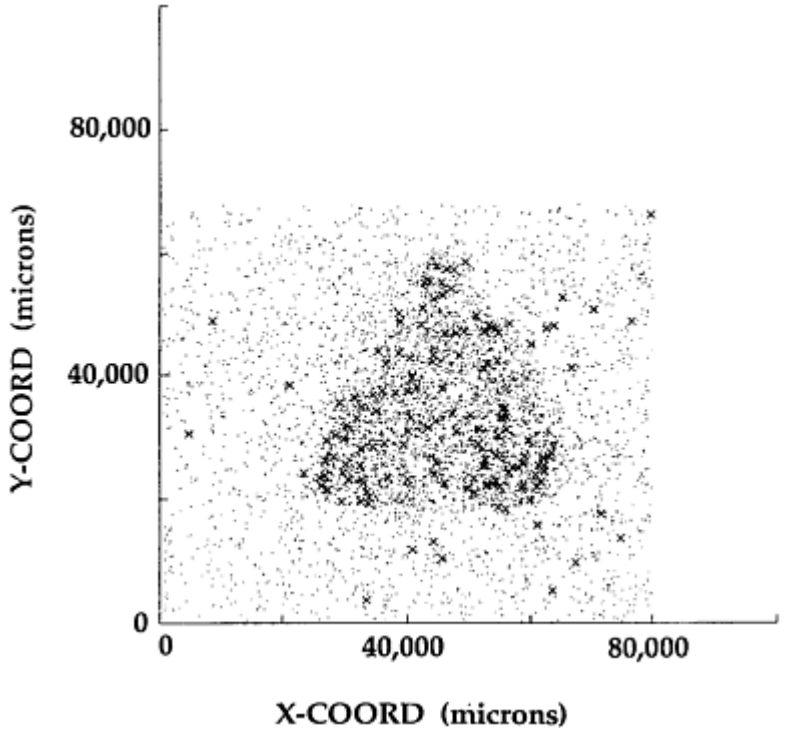

Figure 3.9 The x–y distribution of -particle tracks on a TASTRAK autoradiograph from a cranium fragment which was mounted wet and unfixed against the plastic. Dots and crosses represent -decays from ^{210}Po and ^{226}Ra respectively. Whereas the ^{226}Ra activity is confined to the bone itself, activity from ^{210}Po covers the whole of the autoradiograph, indicating that this component is also associated with the fluids leaching out of the sample

An immediate feature of the spatial distribution of -activity is that it is grossly non-uniform, illustrated by the example in Figure 3.10b. This shows the spatial distribution of activity on the dentine surface of the upper right 4 tooth taken from a 13 year old Bristol boy. The activity is preferentially associated with the annular enamel and with the pulp. Closer examination of the actual -tracks shows that the activity from the annular enamel is predominantly composed of ^{210}Po, but around the pulp there is a visible excess of ^{226}Ra. The excess activity concentration in the annular enamel of the tooth is also seen by comparing the activities recorded on separate autoradiographs of the outer enamel and dentine surfaces. These activities are displayed in the form of scatter diagrams in Figure 3.11 for deciduous and permanent teeth respectively. In both cases there is a clear excess of activity concentration on the outer enamel surface, but this is more pronounced in the case of permanent teeth. In these examples, the mean activity concentration on the outer and inner surfaces are 9.4 Bq kg^{-1} and 5.2 Bq kg^{-1} respectively for deciduous teeth. For the permanent teeth the respective values are 8.5 and 3.1 Bq kg^{-1}.

Of particular interest to the absolute -activity levels is the fact that throughout the UK the mean level of Pu in teeth obtained by Priest and Van de Vyver (1992) was only 4.2 mBq kg^{-1}. This is a factor of > 1000 less than that for natural background sources. Even in West Cumbria, the highest single measurement was only 20 mBq kg^{-1}. These observations reinforce the conclusion that discharge -emitters constitute a negligible contribution to the burden of -radioactivity in teeth and bone.

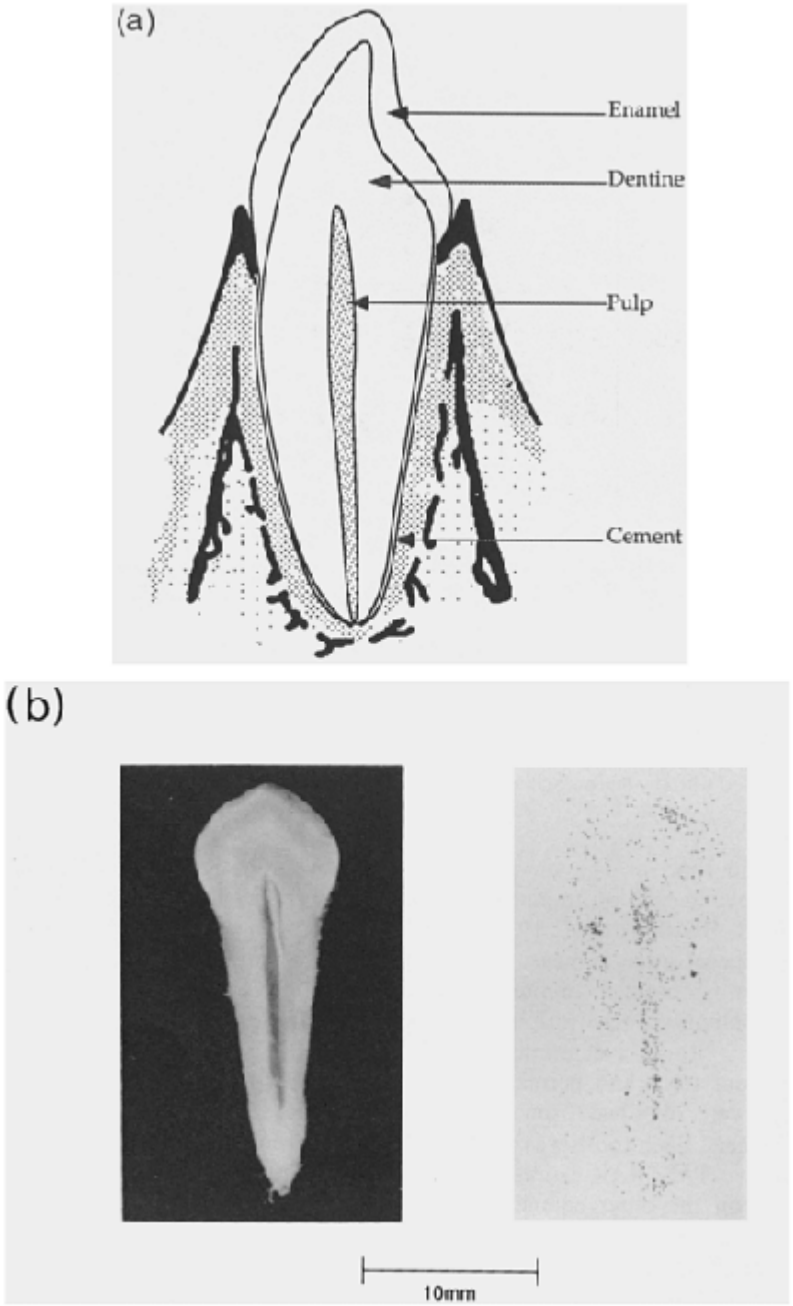

Figure 3.10 (a) Morphology of the inner section of a permanent tooth; (b) the spatial distribution of -particle activity on the inner section of the upper right 4 tooth taken from a 13 year old Bristol boy. The activity is preferentially associated with the outer tooth surface and with the pulp. Closer examination of the actual -tracks shows that the activity on the outer tooth surface arises predominantly from ^{210}Po, but around the pulp the activity arises predominantly from ^{226}Ra

The non-uniform activity distribution in teeth gives clues to the behaviour of -radionuclides in bone and consequently our understanding of marrow dose. The observations in teeth suggest that ^{210}Pb/^{210}Po concentrates at the interface of enamel with saliva or blood, by means of unidirectional ionic exchange with calcium. In contrast, ^{226}Ra concentrates in the predentine band at the interface with pulp and with systemic blood circulation, where its uptake is permitted by the incomplete calcification in this band. The observations not only clearly illustrate that lead and radium are in no sense bone volume-seekers, but also that they behave quite differently from each other.

The measurements in teeth by Henshaw *et al.* (1994a) form the basis of more detailed ongoing studies of age-dependent uptake of -radionuclides in teeth and their microdistribution, and of the geographical variation in activity concentration with respect to environmental factors such as domestic radon exposure and urban pollution.

3.3.8
Alpha-radionuclides in the Foetus

Data on the transfer of radionuclides from the mother to the developing foetus in humans are sparse. Rajewsky *et al.* (1965) measured ^{226}Ra levels in foetal tissues from Frankfurt, obtaining mean values of 0.05 Bq kg^{-1} in wet bone and ~4 mBq kg^{-1} in soft tissues. The authors found no evidence of variations with foetal age. Bradley and Prosser (1993) and Bradley and Ewers (1994) made radiochemical measurements of $^{239–240}$Pu, ^{210}Pb and ^{210}Po in termination tissues from Oxfordshire and West Cumbria, and used mass spectrometry to assay ^{239}Pu, ^{238}U and ^{232}Th. Concentrations of ^{210}Po in the foetus were in the range 4–60 mBq kg^{-1}, levels for ^{210}Pb were similar, but generally somewhat lower. This implies that ^{210}Po transfers to the foetus in both supported and unsupported form. Levels of other natural radionuclides were below 10 mBq kg^{-1}, but ^{239}Pu was present at a level <50 μBq kg^{-1}. The estimated radiation dose during gestation from these -radionuclides was 30 μSv, mainly from ^{210}Po.

Henshaw *et al.* (1994b) have used TASTRAK plastic autoradiographs to map the spatial distribution of -activity in autopsy foetal tissues. This technique has the ability to detect total -activity in a small (<5 g) unconcentrated sample, but at the expense of detection threshold, which is in the range 20–50 mBq kg^{-1}. The authors found highest activity concentrations in the foetal skeleton, most of this being consistent with the presence of ^{210}Po. The evidence suggested that the activity concentration increased with gestational age (Figure 3.12) commencing with the early stages of skeletal development at about week 12 of gestation and reaching ~0.6 Bq kg^{-1} at birth. This apparent trend was in proportion to the rate of skeletal calcification as shown in Figure 3.13. The estimated dose to the haemopoietic cells in the foetus is 70 μSv.

Activity concentrations were also measured in thirty-two foetal teeth. The average concentration found was 2.05 ±0.31 Bq kg^{-1}. This figure is particularly interesting because it is about one-quarter of the level seen in deciduous teeth at around age 10 years, implying that a significant fraction of -activity in these teeth is acquired prenatally.

3.3.9
Distribution of Cells at Risk in the Foetus

There is scope for considerably more detailed studies of the dose to haemopoietic cells in the human foetus. The majority of bones in the foetus are formed by endochondral ossification, in which a cartilage model is first laid down. This occurs around the sixth week of gestation. Proliferation of the cartilage cells facilitates

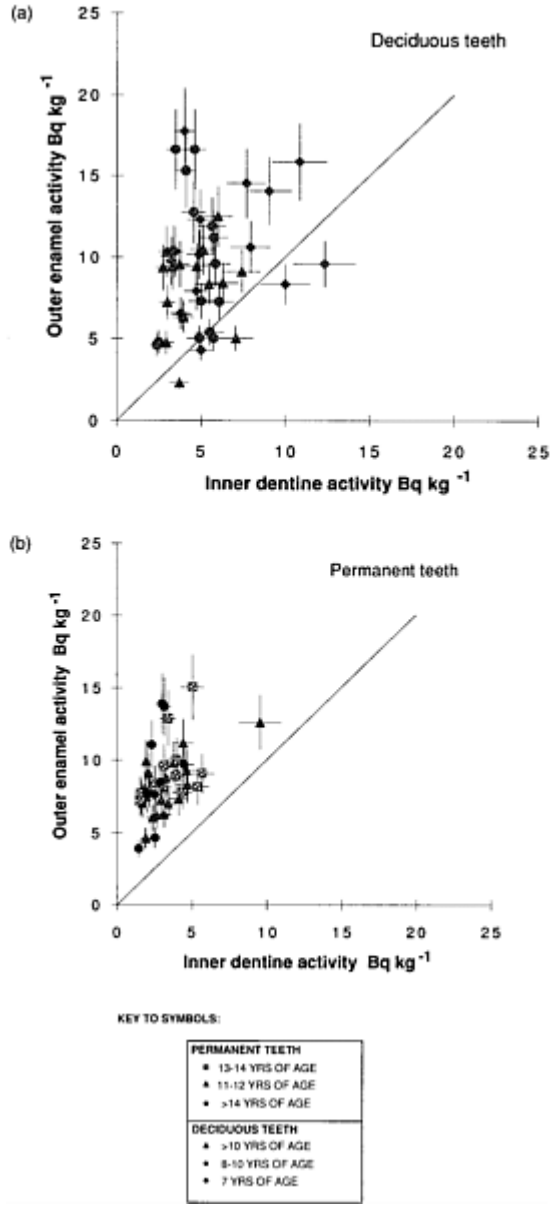

Figure 3.11 Scatter diagrams comparing total -activity on the outer enamel tooth surface with that on the face of a central longitudinal section comprising pulp, dentine and annular enamel: (a) for deciduous teeth; (b) for permanent teeth

growth of the model. By about the twelfth week of gestation, the chondrocytes show distinct hypertrophy and this signals the onset of calcification of the cartilage.

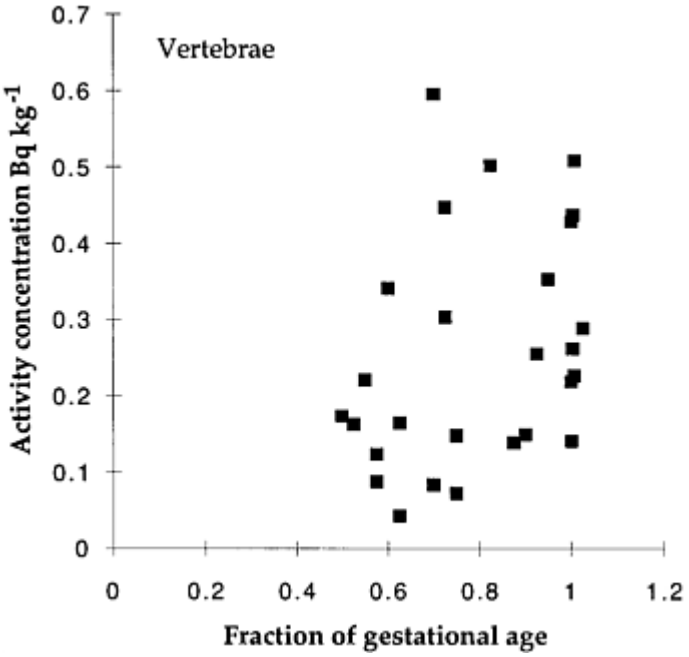

Figure 3.12 Activity concentration measured in human foetal vertebra at natural exposure levels using TASTRAK plastic detectors, plotted as a function of gestational age

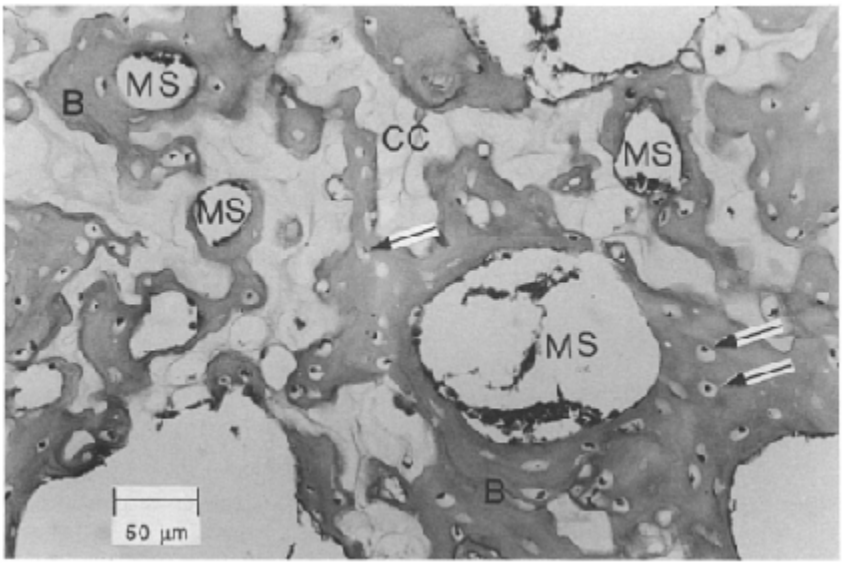

Figure 3.14 The marrow spaces in trabecular bone of a foetal lumbar vertebra at 40 weeks gestation, from the central portion of a transverse section. B—bone, dark pink; CC—calcified cartilage, pale pink; MS—marrow space; arrows show osteocytes

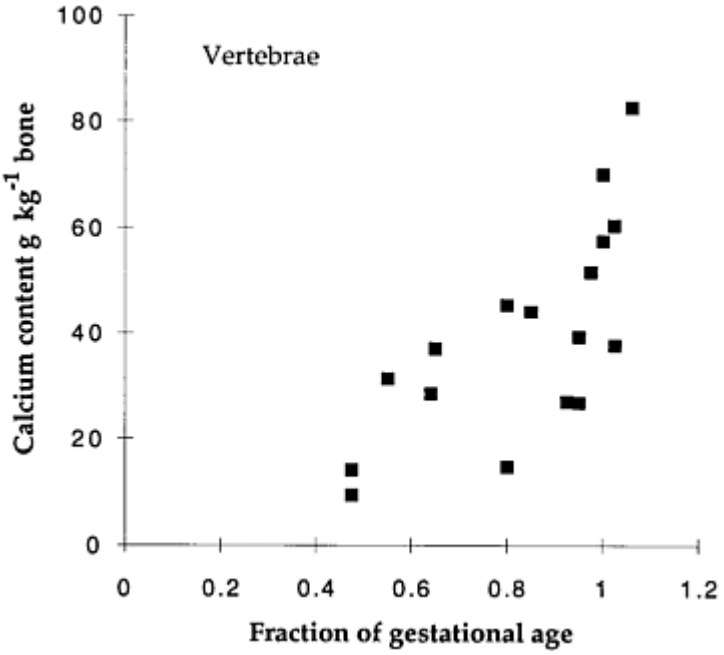

Figure 3.13 Calcium concentration in human foetal vertebra as a function of gestational age

A collar of intramembranous periosteal bone forms around the shaft of long bones and on the inner surface of vertebrae, and it is from the periosteum that perivascular cells invade the rows of hypertrophied chondrocytes. This invasion and consequent erosion of the cartilage leaves spicules of calcified cartilage along which osteoblasts align themselves, particularly at sites where bone formation is active. The osteoblast cells are responsible for the production and deposition of bone matrix, and thereafter are involved in the process of calcium deposition. In this way, the spicules of calcified cartilage grow to create anastomosing bone trabeculae.

As the osteoblasts continue to affect the deposition of calcium, they become enclosed in the bone matrix as mature bone cells, osteocytes, responsible for maintaining the bone matrix. Figure 3.14 illustrates the marrow spaces in trabecular bone of a foetal lumbar vertebra at forty weeks gestation, from the central portion of a transverse section. Initial trabeculae still contain remnants of calcified cartilage. The immigration of blood-borne haemopoietic cells into the cavities of the ossifying skeleton takes place at the end of the first trimester of foetal development, that is, at about twelve weeks gestation.

As discussed above ^{210}Pb crosses the placenta along with calcium and is taken up by the foetal skeleton. Unlike the adult, trabecular spaces in the foetal skeleton are comparatively small, such that haemopoietic cells in marrow are generally within -particle range of a bone surface. This is illustrated in Figure 3.15 which shows the distribution of path lengths across trabecular spaces in a foetal lumbar vertebra at forty weeks gestation.

The target cells for acute lymphoblastic leukaemia, ALL, the major leukaemia subtype in young children, are the lymphoid stem cells and progenitor cells, and there is little information on their spatial distribution in the foetal skeleton. Various antibodies are now available for histological staining of primitive haemopoietic cells. At Bristol, the antibodies CD34 and CD38 are being used to map the number and spatial distribution of myeloid stem cells and primitive lymphoid cells respectively in foetal bone marrow from different

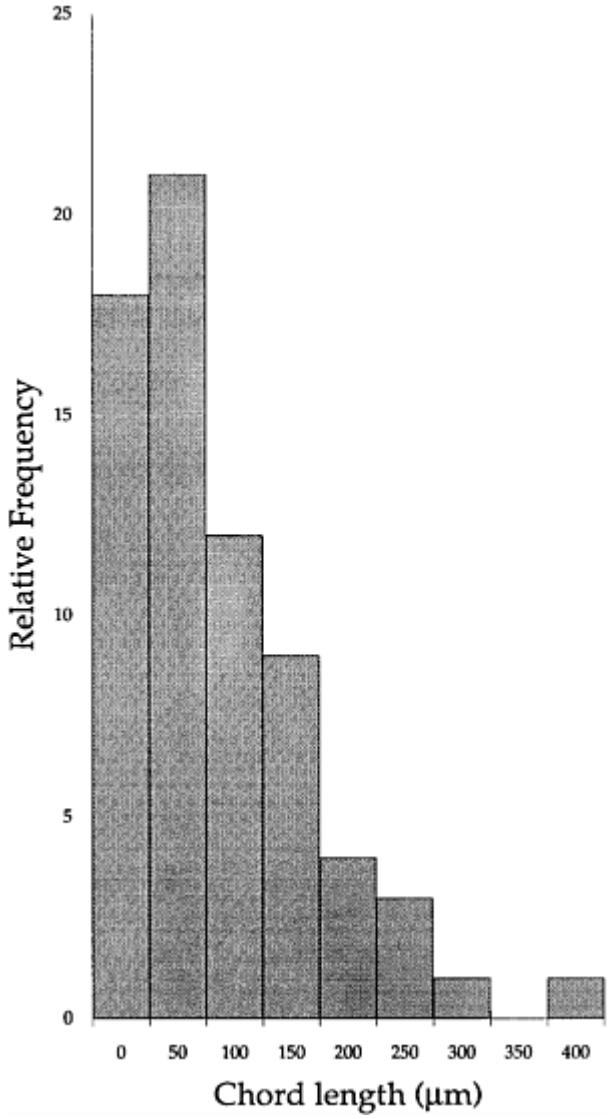

Figure 3.15 The distribution of path lengths across trabecular spaces in the central portion of a foetal lumbar vertebra at 40 weeks gestation

haemopoietic sites. This information will be used to calculate the -radiation dose to $CD38^+$ cells close to bone surfaces from ^{210}Pb-supported ^{210}Po.

Cuevas *et al.* (1994) have demonstrated the first use of CD38 on paraffin sections using a microwave technique on a range of normal tissues. At Bristol, foetal bone marrow in the vertebra has been stained successfully using CD38 with the technique described by Cuevas *et al.* Figure 3.16 shows $CD38^+$ cells (brown) within -particle range of a trabecular bone surface.

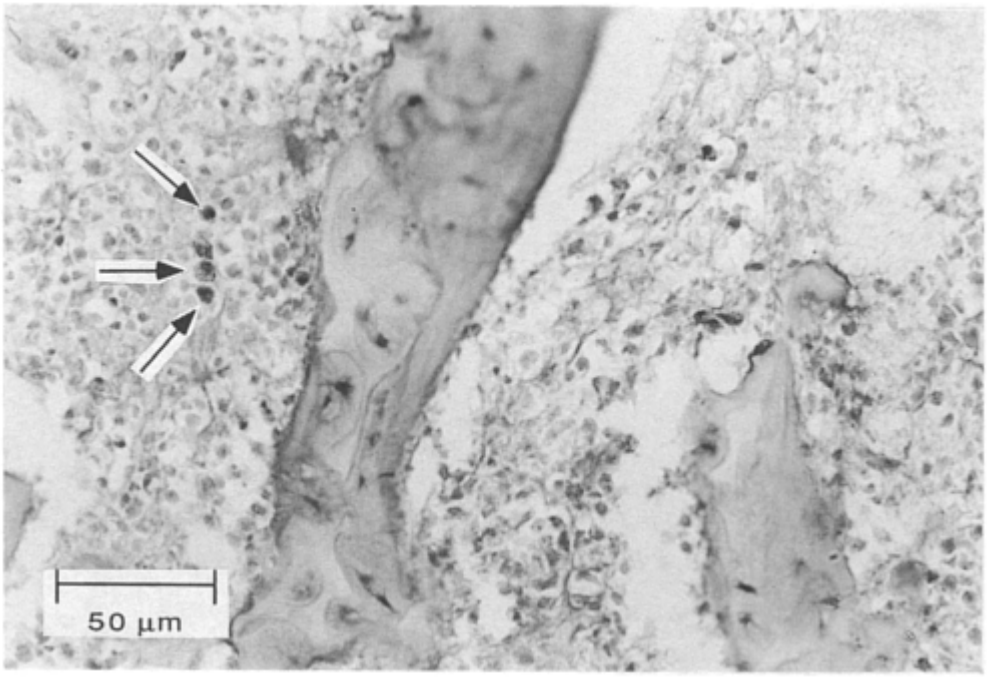

Figure 3.16 CD38^{+} cells (brown) within -particle range of a trabecular bone surface of a lumbar vertebra at 40 weeks gestation (*see also* Colour Plate 2)

There are essential differences in the histology of foetal bone marrow compared with adult marrow. It is important to note that foetal marrow contains a higher proportion of developing lymphocytes, and therefore more target cells for leukaemogenesis, and that there are no fat cells present.

3.3.10 Environmental and Geographical Features of Long-lived Alpha-radionuclides in Bones and Teeth

Of interest here are the global, regional and local variations of ^{210}Po in the human skeleton. Many examples of these were reviewed by Parfenov (1974). In northern latitudes the food chain from lichen to reindeer to man in the Arctic and Sub-Arctic regions of Alaska, in Canada and Greenland, Scandinavia and the USSR can lead to significant elevations of ^{210}Po intake. Lichens, the staple diet of reindeer during the winter, grow on rocks and have a surprising capacity to concentrate ^{210}Po. Typical levels are 400 Bq kg^{-1} dry weight. Levels up to several hundred Bq kg^{-1} have been measured in soft tissues of reindeer and the related Alaskan species of caribou, up to three orders of magnitude higher than in normal human tissues. Analyses of human tissues from Arctic regions show values up to 25 Bq kg^{-1} in bone and up to 0.6 Bq kg^{-1} in placenta (Parfenov, 1974, p. 126), about an order of magnitude higher than normal world levels.

Polonium-210 in children's teeth is proving a valuable assay for small-scale local exposure. The UK national survey of -activity in children's teeth discussed in section 3.3.7 has revealed unexpected evidence

of higher levels of ^{210}Po in both deciduous and permanent teeth of children living near the major motorways in the UK (Henshaw *et al.,* 1995).

Linear regression analysis showed that for distances up to 10 km from the motorway, significant higher activity concentrations were found near the major motorways in the UK, for which data were available (Table 3.6 and Figure 3.17). For distances greater than 10 km the associations broke down. The results are surprising in view of the number of potentially confounding factors such as the closeness of major urban areas, where -activity concentration in teeth was also found to be raised, and the fact that there was no information on the length of time that children

Table 3.6 Correlations of ^{210}PO -activity concentration in children's teeth with distance of residence from a UK motorway

Motorway/tooth type		Maximum distance (km)	Number of teeth	Average activity concentration (Bq kg^{-1})	Slope with respect to motorway (Bq kg^{-1} km^{-1})	p^a
ALL DATA		10	220	8.93±0.26	0.40	<0.001
M5/6	All	10	58	9.32±0.55	0.86	<0.05
M1/A1	All	10	105	7.80±0.41	0.33	<0.05
M25	All	10	15	12.9±1.5	–	ns[b]
M5/6	Deciduous	10	26	10.1±0.9	1.2	=0.05
M1/A1	Deciduous	10	37	8.74±0.40	1.1	<0.001
M5/6	Permanent	10	31	8.51±0.73	–	ns
M1/A1	Permanent	10	65	6.98±0.57	–	ns
M1/5/6/A1	Permanent	2	13	7.53±1.57	8.6	<0.002
M5/6	Permanent	2	8	8.63+2.32	12	<0.001

[a] Probability that slope is zero.
[b] ns=not significant.
Source: Henshaw *et al.,* 1995.

had lived at their given address. Measurements of ^{210}Po in air with -sensitive plastic detectors confirmed the presence of high levels near motorways, much of which was in gravitationally settled form, indicating that this component was attached to particulates. For detectors exposed for up to six days, typical values were 1.2 (range 0.6–1.7) Bq m^{-2} for detectors hung on the bumpers of cars using non-motorway routes in Bristol, 0.7 (range 0.5–1.5) Bq m^{-2} at fixed locations in central Bristol, but 13 (4.6–26) Bq m^{-2} on the M4 and M5 motorways.

Polonium-210 in exhaust emissions arises from several sources: from leaded petrol (Marsden, 1964) or from its presence in oil as part of trace ^{238}U radioactive decay chain products. A study of Savitz and Feingold (1989) showed elevated levels of childhood cancer in general and childhood leukaemia in particular, in relation to living near to heavy traffic. There are many pollutants in motor vehicle exhausts but the presence of ^{210}Po may be unique in terms of what is known of its carcinogenic potential at environmental exposure levels. It is relevant that ^{210}Po levels, adhered to the outer tooth enamel, can be presumed to be an indicator of deposition and retention on bone surfaces in general and of the level of ^{210}Po transferred to the foetus. In other words, the measured levels of ^{210}Po in teeth should translate *pro-rata* into -radiation dose to bone marrow.

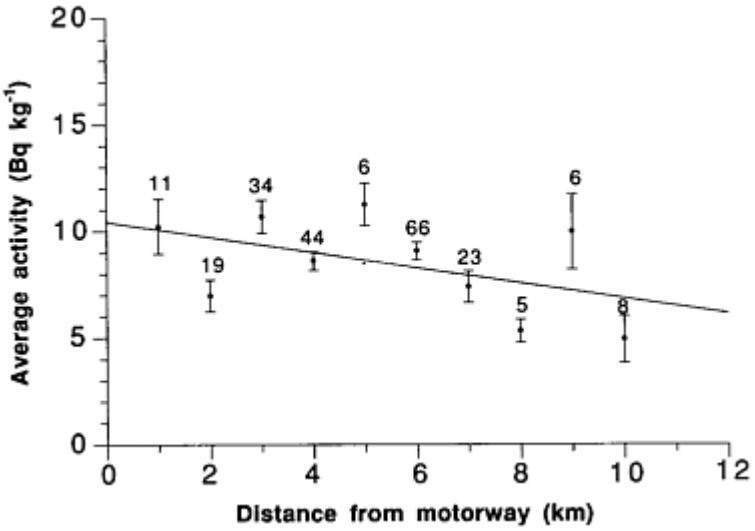

Figure 3.17 Alpha-activity concentration in children's teeth as a function of distance of place of residence from a motorway. Each point shows the mean and error in mean activity concentration in teeth per kilometre interval

3.4
Natural Exposure to Radon, Thoron and their Short-lived Daughter Nuclei

3.4.1
Introduction

Radon, more precisely ^{222}Rn, is the only natural -radionuclide, and indeed the only example of natural ionising radiation, to be firmly linked to cancer in man. The link has been established for lung cancer in uranium and certain other underground miners exposed at high radon levels in mining conditions. It is on this basis that radon exposure in the home is calculated to be responsible for about 6 per cent of the lung cancer incidence in the UK. On radiation protection grounds, radon is undoubtedly a public health issue, given that in the UK alone up to 100000 people may be exposed in their homes at dose rates in excess of the ICRP limit for nuclear workers of 20 mSv y^{-1}, that is, above 400 Bq m^{-3}.

This state of affairs has resulted in the introduction of maximum recommended airborne radon concentrations or action limits above which remedial measures should be taken to reduce exposure. In the UK the recommended action limit for domestic exposure is 200 Bq m^{-3}. In the workplace the limit is 400 Bq m^{-3} and this is a legal requirement under the United Kingdom Ionising Regulations (HMSO, 1985). This is the first time that radiation protection action has taken place in relation to public exposure to radiation from natural sources.

To date, little attention has been paid to the radon-derived dose to body organs and tissues other than the lung. In most situations, including the lung, it is the short-lived -emitting daughter nuclei, ^{218}Po and ^{214}Po, rather than ^{222}Rn itself that deliver the dose to cells at risk. Radon daughter plateout on the surface of the skin results in a substantial dose to the sensitive cells residing in the basal layer about 50 μm below, estimated at 2.5 (range 1.7–17) mSv y^{-1} for the UK average indoor radon exposure of 200 Bq m^{-3} (Eatough

and Henshaw, 1992). The wide range reflects the large variations in published data on plateout onto surfaces of radon daughters.

Apart from the pioneering work of Austrian researchers (Pohl and Pohl-Rüling, 1967; Hofmann *et al.,* 1979), and recent work at Bristol, little interest has been shown in the radon-derived dose to internal organs other than the lung, and the dose to bone marrow has been previously considered to be small. However, it turns out that, at the average UK radon exposure, the radon- and thoron-derived equivalent dose to red bone marrow exceeds that from all other high LET sources and, at the action limit of 200 Bq m^{-3}, the equivalent dose exceeds that from all natural sources, including those from low LET radiation.

3.4.2
Radon- and Thoron-derived Dose to Bone Marrow

Detailed calculations of the radon (^{222}Rn) and thoron (^{220}Rn) derived equivalent dose to red bone marrow were performed at Bristol by Richardson *et al.* (1991). The authors took into account previously unconsidered factors such as: (1) the presence of fat cells in haemopoietically active marrow in which radon is sixteen times more soluble than in blood; (2) the separate inhalation of radon and thoron short-lived daughter nuclei in air which distribute around the body reaching active marrow, and (3) the contribution from the decay of ^{210}Pb-supported ^{210}Po in bone by the additional component in bone accumulated from protracted radon exposure.

The starting point for the determination of the level of radon itself in marrow is its solubility. Nussbaum and Hursh (1957, 1958) measured radon solubility in various tissues, including human abdominal fat at 37° C, obtaining a value of 6.3, compared with 0.4 in blood. Conventionally, bone marrow is categorised as either red, where haemopoiesis takes place, or yellow, consisting entirely of marrow fat. This classification is simplistic to the point of being erroneous. In the newborn, nearly all the bone marrow contains active haemopoietic tissue with negligible fat content. In the growing child, the red bone marrow is gradually replaced by yellow marrow. The percentage of active haemopoietic tissue gradually decreases with age in the axial skeleton of the child and the adult. As any medical textbook shows, red marrow in adults contains fat in the form of fat cells whose contents are composed predominantly in triglyceride. These cells are on average 60 μm in diameter. An example is shown in Figure 3.18, taken from the rib of a 87 year old female.

Richardson *et al.* (1991) first calculated the dose to red marrow as a function of the fraction of fat present and for various assumed sizes of fat cells. The results are shown in Figure 3.19. For fat fractions of zero and 100 per cent, the respective annual equivalent doses are 0.78 and 12.2 μSv y^{-1}, for 1 Bq m^{-3} radon exposure.

Pohl and Pohl-Rüling (1967) studied the distribution of radon and thoron daughters in various organs of guinea pigs following inhalation of ^{222}Rn, ^{220}Rn, ^{212}Pb and their decay products. After the lung, highest levels were found in the kidneys and blood. The results indicate that, in man, 30 per cent and 50 per cent of radon and thoron short-lived daughter nuclei, when inhaled, cross the alveolar membrane of the lung. Richardson *et al.* (1991) used the kinetics of the organ distribution in guinea pigs, carried over to man, to determine the marrow dose following daughter inhalation in normal domestic air.

Finally, the dose contribution from ^{210}Po following protracted radon exposure was calculated using the data of Clemente *et al.* (1984) and others for uranium miners and radon spa area inhabitants. The results of these calculations are summarised in Figure 3.20, which shows mean values of the total equivalent dose to haemopoietic marrow derived from the average radon and thoron exposure in UK homes of 20 Bq m^{-3}, and assuming a mean fat cell diameter of 100 μm. Following birth, the total equivalent dose rises sharply, and this is associated with the higher breathing rate and lung development in young children. The steady rise after age 20 results from the progressive ingrowth of fat in marrow. Interestingly, the equivalent dose in children

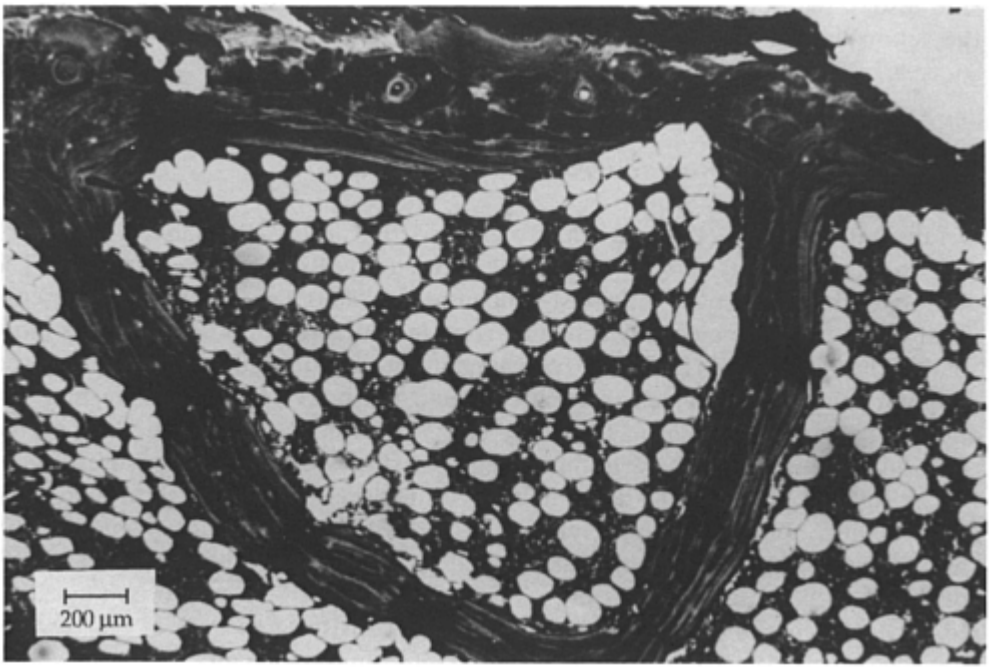

Figure 3.18 Fat cells in a trabecular space of a rib from an 87 year old female

under 4 years is higher from thoron than from radon. This results from the higher breathing rate coupled with the comparatively long half-life of the thoron daughter ^{212}Pb (10.6 hours) decaying to -emitting ^{212}Po.

Although extensive indoor ^{222}Rn monitoring is being carried out worldwide (UNSCEAR, 1993, p. 72), the short half-life of thoron, (55 s) is such that it is technically difficult to assay. Only a few measurements in houses have been carried out (UNSCEAR, 1993, p. 52, Cliff *et al.,* 1992). The importance of thoron with respect to marrow dose in children may provide an impetus for more extensive measurements.

3.4.3
Radon and Thoron Dose to the Foetus

Estimates have been made of the radon- and thoron-derived dose to the human foetus (Richardson, 1991, 1992). Radon-222 will undergo transplacental transfer according to its solubility, and the data of Bradley and Prosser (1993) imply that both lead and polonium isotopes will also transfer. In an experiment in guinea pigs, Richardson (1992) found no transfer of radiobismuth, and on this basis it may be assumed that the radon daughter ^{214}Bi does not undergo transplacental transfer in humans. The position of ^{214}Bi in the radon daughter decay chain (Figure 3.4a) together with the kinetics of transport of radon daughters around the mother's body is considered to result in a negligible dose from radon short-lived daughters to foetal organs. The thoron daughter ^{212}Pb will, however, transfer to the foetus.

The above considerations are summarised in Table 3.7 which shows the estimated foetal dose from radon and thoron for various radon exposures. The ^{210}Po value at 20 Bq m^{-3} was taken from Figure 3.12 and scaled for higher exposures using the data of Clemente *et al.* (1984).

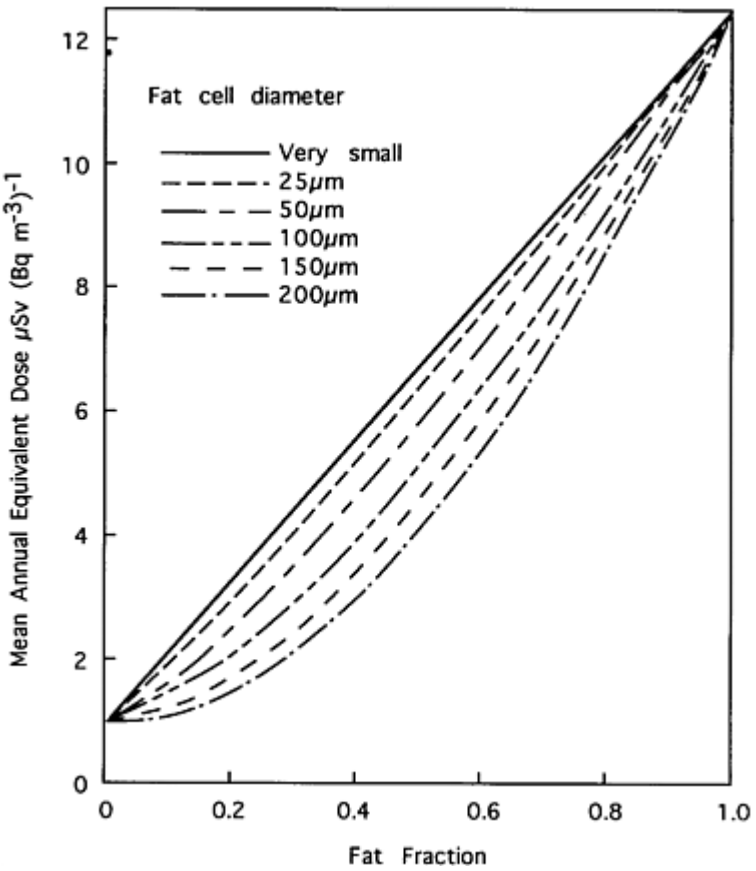

Figure 3.19 The mean annual equivalent dose to haemopoietic tissue for the point decay of ^{222}Rn, ^{218}Po and ^{214}Po, estimated for variable fraction and fat cell diameters of minimal size to 200 μm, for 1 Bq m^{-3} radon concentration in inhaled air

3.4.4
Spatial and Number Distribution of Fat Cells in Bone Marrow

In the absence of experimental data on the size and spatial distribution of fat cells in human bone marrow, Richardson *et al.* (1991) assumed an average diameter of 100

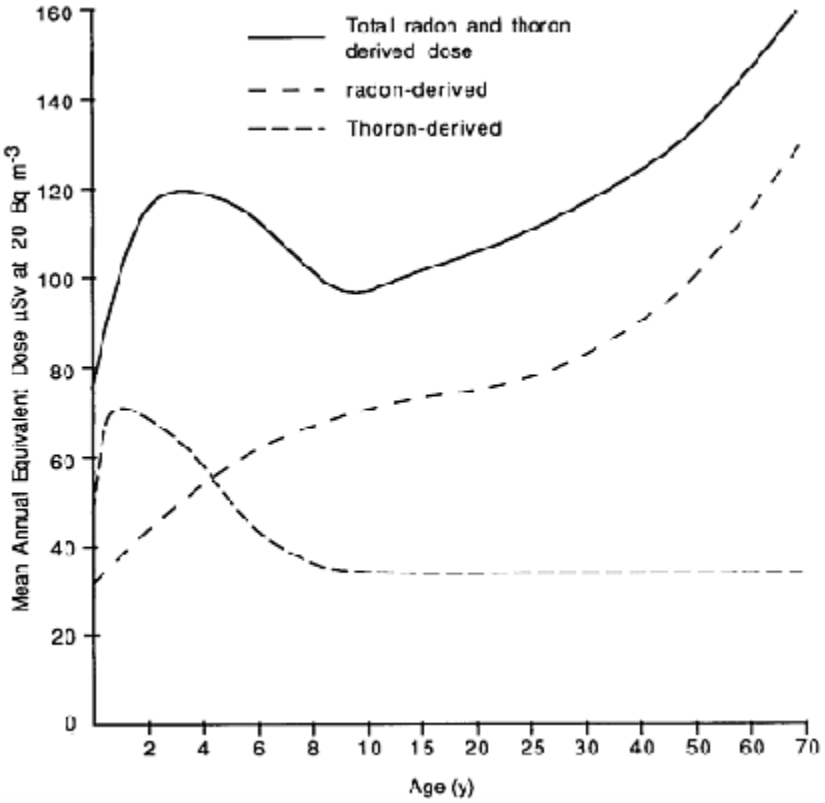

Figure 3.20 The mean values of the total equivalent dose to haemopoietic marrow derived from radon and thoron at the average UK exposure, estimated from birth to 70 years of age (*Source:* Richardson *et al.,* 1991)

Table 3.7 Radon- and thoron-derived equivalent dose to the human foetus at three representative indoor levels: 20 Bq m $^{-3}$ (UK average); 42 Bq m^{-3} (world average); 1000 Bq m^{-3} (Swiss action limit for existing homes)

Radionuclide	Dose μSv at 20 Bq m^{-3}	42 Bq m^{-3}	1000 Bq m^{-3}	Comment
Pure ^{222}Rn	12	25	600	Fat-free marrow
^{222}Rn daughters	1	2	50	No transfer of bismuth
^{220}Rn daughters	23	48	1150	Transfer of ^{212}Pb
^{210}Po in foetal skeleton[a] (Henshaw *et al.,* 1994b)	70	72	156	Measured at 20 Bq m $^{-3}$, scaled for higher exposures
Total	106	147	1956	

[a] The ^{210}Po value is measured at 20 Bq m^{-3} and is scaled for higher radon exposures using the data of Clemente *et al.* (1984). The large contribution from ^{210}Po arises from the small size of trabecular spaces in the foetal skeleton.

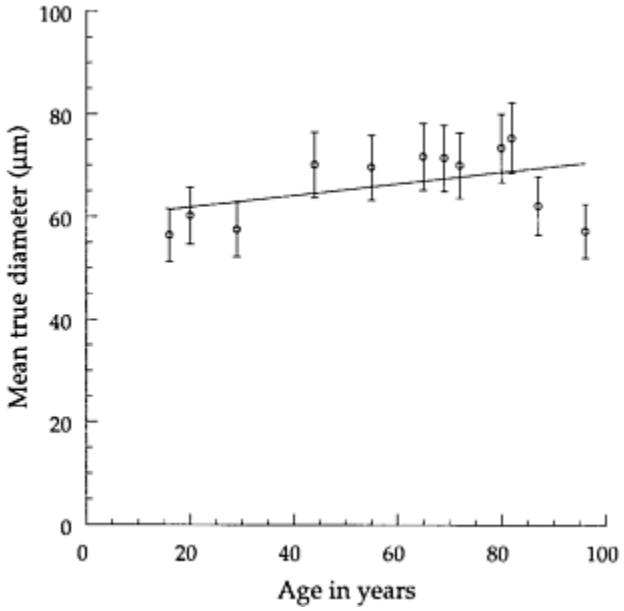

Figure 3.21 Mean diameter of fat cells in human rib as a function of age

μm and a random distribution in deriving the mean equivalent dose from domestic radon exposure. Since that publication, work has been initiated at Bristol to determine the actual size and spatial distribution of fat cells, by the analysis of autopsy and biopsy samples.

Allen *et al.* (1995) have examined the size distribution of fat cells in human rib as a function of age. The results are illustrated in Figure 3.21. The mean diameter of fat cells rises from ~55 μm at age 16–19 years to ~65 μm at age 82–96 years. The spatial distribution was shown to be random, with evidence that saturation of trabecular spaces with fat occurs first near the bone surface. These data were used to calculate the radon-derived doses to haemopoietic tissue for exposure to a unit radon concentration of 1 Bq m^{-3}, obtaining values of 3.5 and 5.2 μSv y^{-1} at ages 16 and 80 years respectively. These experimental values are slightly higher than that obtained by Richardson *et al.* (1991). For a given fat fraction in marrow, lower fat cell size results in a higher dose to haemopoietic tissue because a larger fraction of -energy will escape the fat cell.

3.4.5
Distribution of Cells at Risk in Bone Marrow

Monoclonal antibody staining is a technique very much at the forefront of modern haematological methods. The first demonstration of the use of CD34 stain for primitive haemopoietic cells in paraffin sections of human bone marrow was made by Soligo *et al.* (1991). At the Department of Medical Physics, Royal Adelaide Hospital, and the Division of Tissue Pathology, Institute of Medical and Veterinary Science, Adelaide, research is being carried out into the spatial distribution of $CD34^{+}CD38^{-}$ stem cells in serial sections of normal bone marrow with respect to fat cells and bone surfaces in various regions of the marrow using microwave-mediated antigen retrieval techniques (Leong and Milios, 1993) and image analysis (Fews, 1992). The information obtained will be used to calculate the -radiation dose to individual stem cells and to the haemopoietic tissue as a whole. Figure 3.22 shows one $CD34^{+}$ cell (brown) in normal adult bone marrow, in the centre of the haemopoietic tissue and close to several fat cells. Following the calculations of

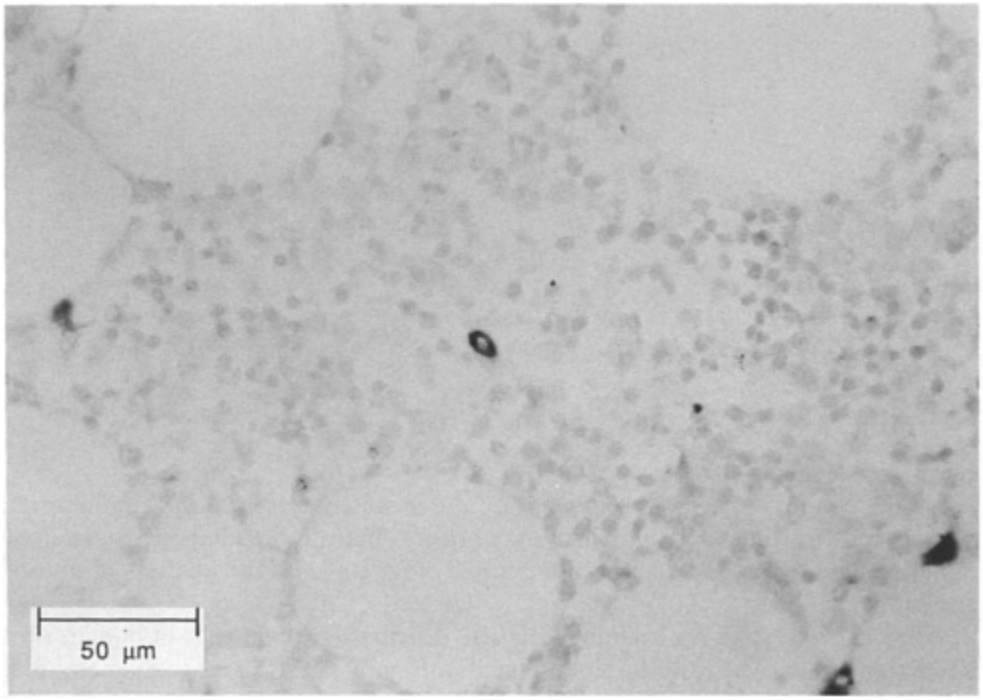

Figure 3.22 A $CD34^+$ cell (brown) in normal adult bone marrow, in the centre of the haemopoietic tissue and close to several fat cells (*see also* Colour Plate 3)

Allen *et al.* (1995), Monte Carlo modelling of the -radiation dose to stem cells is also being undertaken at Montreal and Adelaide (Charlton and Utteridge, 1995).

3.4.6
Radon and Leukaemia

The question of whether domestic radon exposure is implicated in the incidence of leukaemia in the general population is beyond the scope of this chapter. However, it should be mentioned here that several geographical associations have been observed suggesting a link between domestic radon exposure and the incidence of leukaemia in adults and children, and of other childhood cancers (Alexander *et al.,* 1990a,b; Henshaw *et al.,* 1990; Eatough and Henshaw, 1990, 1993, 1994; Viel, 1993). These links are being pursued in a number of case-control studies, such as the national study of childhood cancer in the UK. Results from these studies, however, are not expected for several years.

3.5
Special High Exposure Groups

3.5.1
Introduction

Here the various instances in which population groups have received high bone marrow doses from ionising radiation are reviewed. The Japanese atomic bomb survivors have formed the basis of much of the radiation risk factors employed in radiation protection, especially for leukaemia induction. Unfortunately, this group, as well as most of the others discussed below, received their exposures in such unusual circumstances that they provide us with only the crudest of risk factors. Furthermore, the exposures, generally in the order of sieverts, were such as to require huge extrapolation to be of interest to low-dose exposures, with the inherent difficulties that this introduces.

3.5.2
Japanese Atomic Bomb Survivors

Although the survivors of the atomic bombs dropped on the cities of Hiroshima and Nagasaki in 1945 form the basis of many of the risk factors employed in radiation protection, substantial uncertainties in exposure have been evident. The latest revision of exposure estimates were designated DS86 and were documented in two Radiation Effects Research Foundation reports (RERF87, RERF88), and summarised in the BEIR V report (1990, p. 190). The reassessment of A-bomb dosimetry consisted of a careful review of information on the number of fissions that occurred in the A-bomb explosions and detailed calculations of neutron and -ray transport. This was followed by Monte Carlo calculations of the radiation field within Japanese houses, and the organ dose received by survivors having various shielding circumstances. The dose reassessment is considered to be more accurate and more soundly based than previous estimates; nevertheless, efforts to reconcile differences between some measured and calculated values are continuing.

In the case of Hiroshima, the average equivalent dose to bone marrow estimated by DS86 was 1 Sv. This is almost exactly half of the previous estimate and was achieved mainly by a significant reduction in the estimated neutron dose. A similar situation pertains in Nagasaki. Here the average equivalent dose to bone marrow is again estimated to be 1 Sv; this compares with 1.5 Sv estimated previously. For the 75991 survivors studied in Life Span Study II (Shimizu *et al.,* 1987), these new average dose estimates span a range of exposure from 0.006 to 4.0 Gy. Such a significant change in dose estimate provides little confidence in cancer risk estimates derived from the A-bomb data (even without the difficulties of extrapolating to other exposure circumstances), although the difficulties in making precise estimates of the dose received are undoubted. One important conclusion from DS86 was that the neutron doses were so low in both cities such as to provide no information on the RBE for neutrons for human carcinogenesis.

3.5.3
Radium Dial Painters

During the early part of this century, radium was used as a luminous paint to coat watch dials. The practice involved workers, mainly women, who painted the dials by hand using a small brush. To carry out the intricate paintwork, they would often lick the brush to a sharp point. This led to the intake of huge doses of radium, up to 10000 μCi (3.7×10^8 Bq). The practice of using radium for luminising took place at a time

when the toxic effects of radioactivity were not widely appreciated and the consequences for the radium dial painters were truly disastrous. In 1924, Blum reported the first cases of 'radium jaw', this being just one of the examples of the bone cancers that were contracted by these workers.

Rundo *et al.* (1986) reviewed the data on US radium dial painters. Two interesting features were that no cancers occurred for intakes below 2.2×10^6 Bq and that although bone tumours were widely observed, essentially no cases of leukaemia occurred for the whole range of exposures from 3.7×10^3 to 3.7×10^8 Bq.

In the UK, radium dial painting was not a major industry until the Second World War, when it was used for luminous dials on aircraft. By this time the adverse effects of radium intake were known and, consequently, codes of practice ensured that the intake for UK workers was comparatively low. Baverstock *et al.* (1981) and Baverstock and Papworth (1986) reviewed the health of 1110 female workers. As a result of the comparatively low intakes, only one case of osteosarcoma was observed and, interestingly, no case of leukaemia. Thorne and Vennart (1975) estimated the marrow dose from radium, obtaining a value of 0.54 Sv per MBq systemic intake, suggesting that most UK workers received a marrow dose from -particles below 0.24 Sv. Workers also received an external -radiation dose from non-ingested radium in the workplace, estimated at below 0.33 Sv.

A further group exposed to radium was the patients injected with ^{224}Ra for the treatment of tuberculosis and ankylosing spondylitis, mainly between 1946 and 1950. Detailed studies of German patients were carried out and these were reviewed by Mays *et al.* (1986). Younger patients aged 1–20 years were typically injected with 1.04×10^6 Bq per kg body weight, while adults were given a lower activity of 6×10^5 Bq per kg body weight. The resulting average skeletal doses for the younger age group were around 10.6 Gy, and 2.1 Gy for the adults. The overwhelming malignant effects were the induction of bone sarcomas. As with the other radium exposed groups, few cases of leukaemia were seen and it is doubtful whether these could be attributed to the radium intake (see also Chapter 5).

In terms of radiation exposure to bone marrow, the experience of the radium dial painters and the patient groups treated with radium is remarkable because of the lack of induction of leukaemia. The US dial painters would have received marrow doses up to 200 Sv, which is well in excess of a cell-killing regime. On the other hand, the UK dial painters were exposed to much lower doses and it has also been argued that the lack of leukaemia implies that cells at risk reside within the body of marrow well away from bone surfaces. Whatever the explanation, the radium-exposed individuals have provided nothing by way of risk factors for leukaemia induction.

3.5.4 Thorotrast Patients

Starting in the 1920s and continuing until the 1950s, thorotrast, a stabilised 25 per cent colloidal solution of thorium dioxide was injected into patients as an X-ray contrast medium. Van Kaick *et al.* (1986) reviewed its use in Germany where it was introduced in 1929 and where 100000 injected patients were examined between 1930 and 1950. Thorotrast is not a bone-seeker: 60 per cent and 30 per cent of intake are taken up by the liver and kidneys, respectively, with 9 per cent taken up by bone marrow and only 2 per cent by calcified bone. The resulting doses to red bone marrow were typically 0.09 Gy y^{-1} (1.8 Sv y^{-1}) (Kaul and Noffz, 1978).

The use of thorotrast resulted in widespread malignancies amongst these patients, cancer of the liver and liver cirrhosis being the most common. Leukaemia did occur but there was also a large number of cases of bone marrow failure, suggestive of a high cell-killing dose to marrow.

The thorotrast patients are often used as a source of risk factors for leukaemia induction, but this must be seen against the background of the high doses as evidenced by the incidence of bone marrow failure and also the fact that most malignancies in the patients occurred in the liver.

3.5.5
Workers at Nuclear Installations

The cases of high exposure to man-made -radionuclides in the nuclear industry are few and provide no incontrovertible evidence of health effects (Bair *et al.,* 1989). Such cases as have been reported stem largely from the US Transuranium Registry which was formed in 1968 to catalogue cases of exposure in the US nuclear industry.

McInroy *et al.* (1989), in reviewing their own case histories, provided some examples of cases of high exposure to plutonium and americium. The authors quote six cases where body burdens were estimated either by urine analysis or by subsequent postmortem radiochemical analysis. Five cases involved plutonium exposure, and whole-body estimates of the levels present ranged from ~570 Bq to 4 kBq. In the one case of exposure to ^{241} Am, postmortem analysis yielded a whole body burden of 5.4 kBq. For the plutonium exposed cases, 54 per cent of the body burden was, on average, found in the skeleton. There was no separate estimate of the mean marrow dose.

Although these and similar cases provide examples of significant body burdens of transuranic radionuclides, they do not provide any information on cancer risk. It should also be emphasised that these cases are historic, and procedures today should preclude such high exposures in the nuclear industry.

3.5.6
Uranium and Related Underground Miners

Exposure of uranium and related underground miners to radon daughter nuclei has been extensively studied in relation to the incidence of lung cancer (BEIR IV, 1987, Lubin *et al.,* 1994). Typical exposures were in the range 5–120 working level months per year, corresponding in domestic terms to the range 700–18000 Bq m^{-3} y^{-1}. Using the results from section 3.4 these suggest marrow doses in the range 4.5–117 mSv y^{-1}. In addition to this high LET component, there will be a low LET component from external -radiation, although estimates of exposures are generally not available.

3.6
Occupational and Other Low Dose Exposure Groups

3.6.1
Various Groups Monitored for Exposure to Ionising Radiation

The 1993 UNSCEAR report (annex D) has considered many occupational groups which, although exposed to ionising radiation, fall well within the limit of 20 mSv y^{-1} recommended by the International Committee on Radiological Protection (ICRP Publication 60, 1991). In most cases the calculated or measured effective dose may be taken to apply to bone marrow. Thus, airline pilots receive 5–8 μSv h^{-1} at the average aircraft altitude of 10–12 km due to the increased flux of cosmic rays. In general, workers in the nuclear industry, either at nuclear power stations or those involved in fuel reprocessing, have monitored average doses below 3 mSv y^{-1}, with only 10 per cent exceeding 15 mSv y^{-1}. Military personnel exposed to radiation doses from

nuclear weapons receive on average only 0.7 mSv y^{-1}. Hospital personnel involved in all medical uses of radiation are particularly efficient at minimising their exposure and receive average doses ~0.5 mSv y^{-1}.

3.6.2
Chernobyl Residents

The accident at Chernobyl Reactor Number 4 on 26 April 1986 released around 2 tons of nuclear fuel comprising some 10^{19} becquerels of radioactivity into the atmosphere. A further 18 tons remain in the reactor, in dangerous condition.

A substantial literature exists on the Chernobyl accident and a detailed assessment of the radiation dose received by the local population was made by the International Atomic Energy Agency Chernobyl Project Technical Report (ICP, 1991). The most important contributor to dose was from ^{131}I which is rapidly taken up by the thyroid. The uptake by young children is much higher than in later years and doses to the thyroid of up to 30 Sv in children less than 1 year old were estimated (ICP, 1991, Table 4). This has resulted in a significant increase in incidence of thyroid cancer in children (CEC, 1993; Stsjazhko *et al.,* 1995).

In terms of marrow dose, the important contaminating radionuclides are ^{137}Cs and ^{90}Sr. Caesium is not itself a bone-seeker, rather it distributes homogeneously throughout the body. Levels in the body can be monitored externally from the 662 keV -ray emitted following the -decay to ^{137}Ba. In 1990 the ICP made measurements in about nine thousand people from a number of villages in the Chernobyl area. Mean doses to red bone marrow were estimated to be 0.1–0.4 mSv y^{-1}, but the range extended up to 6 mSv y^{-1}.

Strontium-90 is a bone-seeker which decays by -emission to ^{90}Y, but unfortunately there is no associated -ray emission. This means that the presence of ^{90}Sr in the human body cannot be measured externally. Instead, estimates of marrow dose have to be made by applying generalised transfer factors to ^{90}Sr deposition on the ground. Such data were used to estimate marrow doses to the population in the above villages, obtaining mean values in the range 2–23 mGy (ICP, 1991, Table 17).

Caesium-137 and ^{90}Sr have respective half-lives of 30 and 29 years and so will undergo long-term uptake by the local population.

It is generally assumed that transuranic radionuclides such as plutonium, although released from the reactor, were deposited mainly in the 30 km radius exclusion zone. Of concern here is the question of so-called hot particles, grains of plutonium and other radionuclides in the size range 0.1–10 μm which may be inhaled or ingested, giving high local doses to tissues. Current research aims to quantify such particles and their likely contribution to marrow dose.

3.7
Medical Exposures to Radiation

3.7.1
Diagnostic X-Rays

Of the medical uses of radiation, the examination of patients with X-rays for diagnostic purposes is by far the most frequent. Such examinations are performed in all kinds of health care establishments, including chiropractic and podiatric clinics in many countries. A detailed review of medical exposures to artificial radiation is given in UNSCEAR (1993, annex C).

Although the doses from diagnostic X-ray examinations are generally relatively low, the magnitude of the practice makes for a significant radiological impact. In the UK, for instance, 150 chest X-rays were carried

out per 1000 of the population, during the period 1980–84. Taking all X-rays, the average for developed countries is 890 per 1000 of the population with a rather wide range of 320–1290 per 1000. These figures do not imply that every member of the population receives on average around one X-ray per year; rather it indicates the wide use of X-rays for diagnostic purposes for those referred by their doctor.

The doses to patients from diagnostic X-ray examinations vary widely. In certain cardiac procedures, entrance surface doses of several gray occur. Computerised tomography is being used more frequently with typical doses of 5 mSv. Doses from chest X-rays on the other hand are small, typically 0.1 mSv. UNSCEAR (1993, p. 249) estimate a mean dose to patients from diagnostic X-rays of 1.2 mSv with a range of 0.3–2.2 mSv. By their nature, penetrating X-rays also irradiate bone marrow and these figures can be taken as indicative of marrow dose.

3.7.2
Diagnostic Use of Radiopharmaceuticals

Of the many different radionuclides used in nuclear medicine examinations, ^{99m}Tc and ^{131}I with respective half-lives of six hours and eight days, are the most important. In conjunction with external -counting, these radionuclides are used for diagnostic investigation of a number of organs and tissues such as bone, the cardiovascular system, liver, spleen and the thyroid. Technetium-99m is of special interest because as a result of its -emission to the ground state ^{99}Tc, electron vacancies are produced in the inner atomic shells. These vacancies trigger a cascade of very low energy electrons, known as Auger electrons, many of which have ranges less than the diameter of the nucleus of a cell. Such concentrations of electrons constitute a local deposition of high LET radiation, with the result that the local dose to cells is not adequately represented by the overall dose estimates.

As a rule, the dose per procedure is less for ^{99m}Tc due to its shorter half-life, but ^{131}I is widely used, especially in developing countries. Patient doses vary widely depending on the procedure. Thyroid examinations using ^{131}I can result in a dose approaching 100 mSv, but for ^{99m}Tc the effective dose is under 1 mSv. For bone examinations using ^{99m}Tc phosphate, the delivered dose is around 7 mSv.

3.7.3
Radiation Therapy

Treatments by radiotherapy are intended to deliver high doses to target organs to eliminate malignant or benign conditions. The circumstances of patients faced with a life-threatening disease is such that scattered dose to normal tissue is of secondary importance. Attempts to calculate doses to non-target organs are often difficult and secondary effects associated with such doses are difficult to estimate.

Patients undergoing whole body radiation for the treatment of leukaemia receive doses in the range 8–25 Gy, and for lymphoma the range is 35–45 Gy. Radiation treatment for solid tumours using ^{60}Co can deliver a scattered dose to red bone marrow and depending on the exact treatment regimen dose values can range up to 70 mSv per gray of dose to the tumour. The most common use of radiopharmaceuticals in treatment therapy is in thyroid cancer using ^{131}I. The scattered dose to red bone marrow in this case is typically 20 mSv.

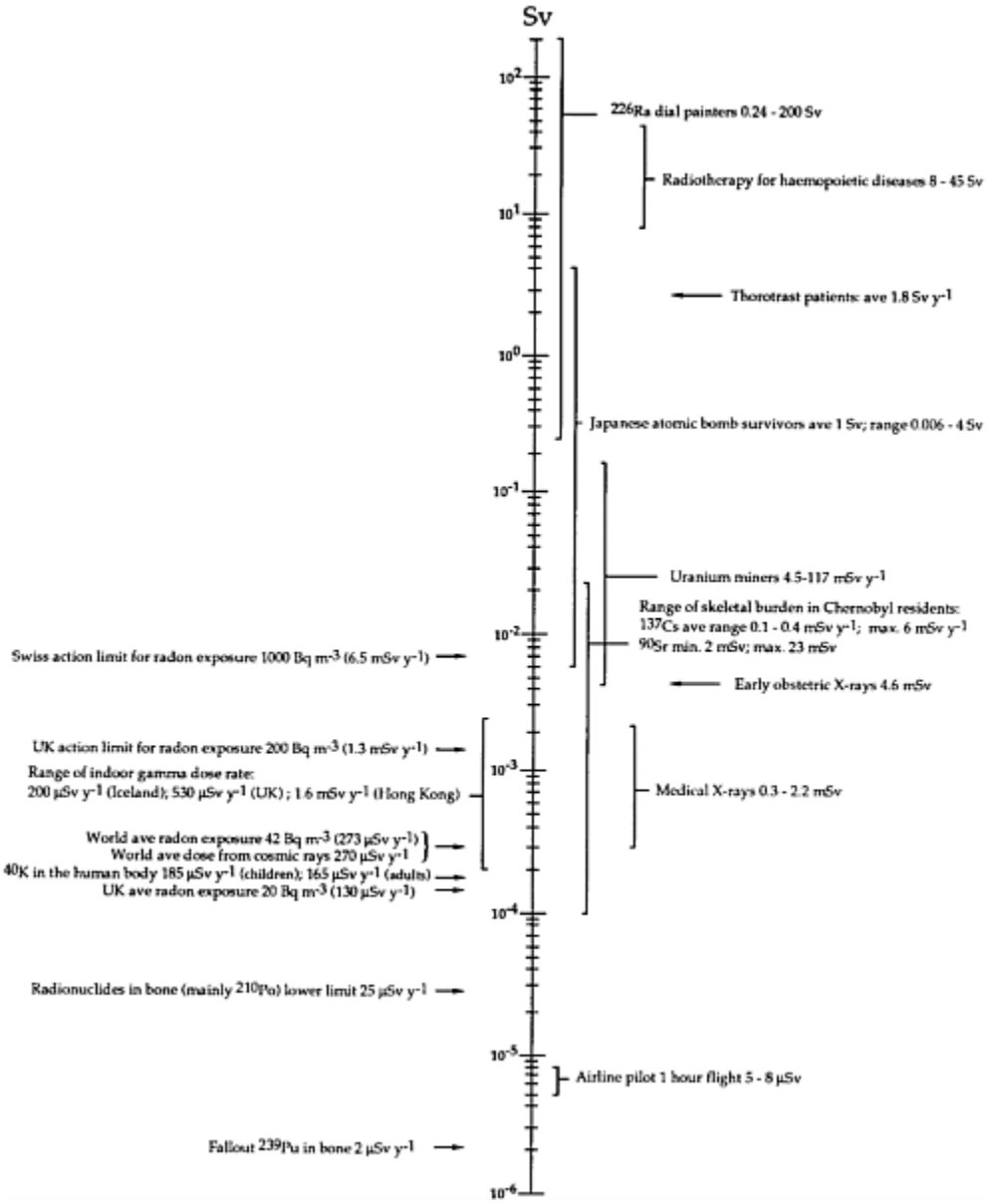

Figure 3.23 Summary human radiation exposure to bone marrow

3.7.4
Obstetric X-rays and Childhood Cancers

During the late 1930s and the 1940s, pregnant women received routine pelvic X-rays as part of antenatal care. Stewart *et al.* (1956, 1958) found early evidence that the incidence of childhood cancer was linked to

this radiographic practice. Stewart and Kneale (1970) examined childhood cancer data for the period 1943–65 which revealed an excess risk directly related to the foetal dose from these obstetric X-rays. This work received much criticism (Burch, 1970, 1978), the strongest coming from the Hiroshima and Nagasaki atomic bomb data (Ishimaru *et al.,* 1981; Jablon and Kato, 1970) which revealed no excess mortality from leukaemia or other cancers of children exposed *in utero,* and from Totter and MacPherson (1981) who suggested that women more at risk were more likely to be X-rayed during pregnancy.

Several other studies gave strong support to Stewart's findings (Harvey *et al.,* 1985; Mole, 1974; Monson and MacMahon, 1984; Diamond *et al.,* 1973). The practice of routine obstetric X-rays continued in some form until the early 1970s, but it is now accepted that there is a causal relationship between *in utero* irradiation and childhood cancer (UNSCEAR, 1986, 1988). The total risk of death from leukaemia is estimated to be in the region of $1.0–1.25\times10^{-2}$ Gy^{-1} of exposure to the foetus.

3.8 Summary

Figure 3.23 summarises human radiation exposure to bone marrow. For the natural exposures, the importance of radon is clear. For the occupational exposures, the uranium miners provide a group which is of potential interest for epidemiological studies, in relation to possible leukaemia risk. Instances of much higher exposure are generally historic and provide little valuable information of the potential health effects of exposure at lower doses.

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References

ALEXANDER, F.E., MCKINNEY, P.A. & CARTWRIGHT, R.A. (1990a) Letter. Radon and leukaemia, *Lancet,* **335**, 1336–7.

ALEXANDER, F.E., MCKINNEY, P.A., RICKETTS, T.J. & CARTWRIGHT, R.A. (1990b) Community lifestyle characteristics and risk of acute lymphoblastic leukaemia in children, *Lancet,* **336,** 1461–5.

ALLEN, J.E., HENSHAW, D.L., KEITCH, P.A., FEWS, A.P. & EATOUGH, J.P. (1995) Fat cells in red bone marrow of human rib: their size and spatial distribution with respect to the radon-derived dose to the haemopoietic tissue, *Int. J. Radiat. Biol.* (submitted for publication).

BAIR, W.J., PARK, J.F., DAGLE, G.E. & JAMES, A.C. (1989) Overview of biological consequences of exposure to plutonium and higher actinides, *Radiat. Prot. Dosim.,* **26**, 1–4, 125–35.

BARATTA, E.J. & FERRI, E.S. (1966) Radionuclides in selected human tissues, *Am. Indust. Hyg. Assoc. J.* **27**, 438–43.

BAVERSTOCK, K.F. & PAPWORTH, D.G. (1986) The UK radium luminiser survey: significance of a lack of excess leukaemia. In W.Gössner *et al.* (eds), *The Radiobiology of Radium and Thorotrast*. Munich: Urban & Schwarzenberg, pp. 22–6.

BAVERSTOCK, K.F., PAPWORTH, D.G. & VENNART, J. (1981) Risks of radiation at low dose rates , *Lancet,* i: 430–3.

BEIR IV (Committee on the Biological Effects of Ionising Radiation) (1987) *Health Risks of Radon and Other Internally Deposited -emitters*. Washington, DC: National Academy of Science/National Research Council.

BEIR V (Committee on the Biological Effects of Ionising Radiation) (1990) *Health Effects of Exposure to Low Levels of Ionizing Radiation*. Washington, DC: National Research Council/National Academy Press.

BEDDOE, A.H. (1977) Measurements of the microscopic structure of cortical bone, *Phys. Med. Biol.,* **22**(2), 298–308.

BEDDOE, A.H., DARLEY, P.J. & SPIERS, F.W. (1976) Measurements of trabecular bone structure in man, *Phys. Med. Biol.* **21**, 589–607.

BLACK, SIR D. (1984) *Investigation of the Possible Increased Incidence of Cancer in West Cumbria,* Report of the Independent Advisory Group.

BRADLEY, J. (1990) The distribution of ^{210}Po in human bone. Unpublished report, NRPB.

BRADLEY, E.J. & EWERS, L.W. (1994) The transfer and resulting radiation dose from polonium, thorium and other naturally-occurring radionuclides to the human fetus. Paper presented at Health Effects of Internally Deposited Radionuclides: Emphasis on Radium and Thorium, 18–21 April 1994, Heidelberg (proceedings in press).

BRADLEY, J. & PROSSER, L. (1993) Radionuclides in human fetal tissues, *Radiological Protection Bulletin* **148** (Dec), 28–31.

BURKINSHAW, L, BAYHREYNI-TOOSSI, M.T. & SPIERS, F.W. (1987) Plutonium content of tissues of members of the general public, *J. Soc. Radiol. Prot.,* **7** (1).

BURCH, P.R.J. (1970) Prenatal radiation exposure and childhood cancer, *Lancet,* ii, 1189.

(1978) Does fetal irradiation cause childhood malignancies? *Br. J. Radiol.* **51,** 146.

CAMPBELL, J.E. & TALLEY, L.H. (1954) Association of Polonium-210 with blood, *Proc. Soc. Exp. Biol. Med.,* **87**, 221.

CARPENTER, R.C. (1992) Environmental and Medical Sciences Division, AEA Technology, Harwell, UK. Personal communication.

CEC (1993) *Thyroid Cancer in Children Living near Chernobyl: Expert Panel Report on the Consequences of the Chernobyl Accident,* D.Williams, A.Pinchera, A.Karaoglou & K. H.Chadwick (eds), Report EUR 15248 EN.

CHARLTON, D.E. & UTTERIDGE, T.D. (1995) Personal communication.

CLEMENTE, G.F., RENZETTI, A., SANTORI, G., POHL-RÜLING, J. & STEINHÄUSLER, F. (1982) Pb-210-Po-210 tooth content and radon daughter exposure, *Proc. 2nd Spec. Symp. on Natural Radiation Environment*. Bombay: Wiley Eastern, pp. 269–74.

CLEMENTE, G.F., RENZETTI, A., SANTORI, G., STEINHÄUSLER, F. & POHL-RÜLING, J. (1984) Relationship between the ^{210}Pb content of teeth and exposure to Rn and Rn daughters, *Health Phys,* **47**, 253–62.

CLIFF, K.D., GREEN, B.M.R., MAWLE, A. & MILES, J.C.H. (1992) Thoron daughter concentrations in UK homes, *Radiat. Prot. Dosim.,* **45**, 1–4, 361–6.

CUEVAS, E.C., BATEMAN, A.C., WILKINS, B.S. *et al.* (1994) Microwave antigen retrieval in immunocytochemistry: a study of 80 antibodies, *J. Clin. Pathol.,* **47**, 448–52.

CZEGLÉDI, P. (1987) Natural contents of ^{226}Ra and ^{210}Po in young human bones and teeth, *Isotopenpraxis,* **23**, 272–7.

DIAMOND, E.L., HOCHMELER, H. & LILIENFELD, A.M. (1973) The relationship of intrauterine radiation to subsequent mortality and development in leukaemia in children, *Am. J. Epidemiol,* **97**, 283–313.

EATOUGH, J.P. & HENSHAW, D.L. (1990) Radon and cancer of the reproductive system, *Lancet,* **335**, 1292.

(1992) Radon and thoron associated dose to the basal layer of the skin, *Phys. Med. Biol.,* **37**, 955–67.

(1993) Radon and monocytic leukaemia in England, *J. Epid. and Comm. Health,* **47**, 506–7.

(1994) Radon exposure and myeloid leukaemia, *Int. J. Epidemiol,* **23**, 430–1.

(1995) The theoretical risk of non-melanoma skin cancer from domestic radon exposure, *J. Radiat. Prot.* **15**, 45–51.

FEWS, A.P. (1992) Fully automated image analysis of etched tracks, *Nucl. Instr. and Meth. in Phys. Res.,* **B71,** 465–78.

FEWS, A.P. & HENSHAW, D.L. (1983) Alpha-particle autoradiography in CR-39: a technique for quantitative assessment of -emitters in biological tissue, *Phys. Med. Biol.,* **28**, 459–74.

FISENNE, I. & KELLER, H.W. (1981) Worldwide measurements of ^{226}Ra in human bone: estimate of skeletal -dose, *Health Physics,* **40**, 163–71.

GOODHEAD, D.T. (1988) Spatial and temporal distribution of energy, *Health Phys.,* **55**, 231–40.

GOODHEAD, D.T. & CHARLTON, D.E. (1985) Analysis of high LET radiation effects in terms of local energy deposition, *Radiat. Prot. Dosim.,* **13**, 253–8.

HAMILTON, E.I. (1972) The concentration of uranium in man and his diet, *Health Physics* **22**, 149.

HARVEY, E.B., BOICE, J.D., HONEYMAN, M. *et al.* (1985) Prenatal x-ray exposure and childhood cancer in twins, *New Engl. J. Med.,* **312**, 541–5.

HATZIALEKOU, U., HENSHAW, D.L. & FEWS, A.P. (1988) Automated image analysis of -particle autoradiographs of human bone, *Nucl. Instr. Meths.,* **A263**, 504–14.

HENSHAW, D.L., HATZIALEKOU, U. & RANDLE, P.H. (1988) Analysis of alpha-particle autoradiographs of bone samples from adults and children in the UK at natural levels of exposure, *Radiat. Prot. Dosim.,* **22**, 231–42.

HENSHAW, D.L., EATOUGH, J.P. & RICHARDSON, R.B. (1990) Radon: a causative factor in the induction of myeloid leukaemia and other cancers in adults and children? *Lancet,* **335**, 1008–12.

HENSHAW, D.L., ALLEN, J.E., KEITCH, P.A. & RANDLE, P.H. (1994a) The spatial distribution of naturally occurring ^{210}Po and ^{226}Ra in children's teeth, *Int. J. Radiat. Biol,* **66**, 815–26.

HENSHAW, D.L., ALLEN, J.E., KEITCH, P.A., SALMON, P.L. & OYEDEPO, L. (1994b) The microdistribution of polonium-210 with respect to bone surfaces in adults, children and fetal tissues at natural exposure levels. Presentation at an international seminar, Health effects of internally deposited radionuclides: Emphasis on radium and thorium, 18–21 April 1994, Heidelberg, Germany. Proceedings: G.van Kaick, A.Karaojglou & A.M.Kellerer (eds), World Scientific: CEC publication: EUR 15877EN.

HENSHAW, D.L., KEITCH, P.A. & JAMES, P.R. (1995) Lead-210, polonium-210 and vehicle exhaust pollution, *Lancet,* **245**, 324–5.

HILL, C.R. (1965) Polonium-210 in man. *Nature,* **208**, 423–8.

HMSO (1985) *Exposure to Radon, Ionising Radiations Regulations*. London: HMSO.

HOFMANN, W., STEINHÄUSLER, F. & POHL, E. (1979) Dose calculations for the respiratory tract from inhaled natural radioactive nuclides as a function of age. I: Compartmental deposition, retention and resulting dose, *Health Phys.* **37**, 517–32

ICP (1991) *Assessment of Radiological Consequences and Evaluation of Protective Measures,* International Chernobyl Project, Technical Report. Vienna: IAEA.

ICRP (1979) Limits for intakes of radionuclides by workers, *Annals of the ICRP,* Publication 30, **2**(3/4). Oxford: Pergamon.

ICRP (1991) 1990 Recommendations of the International Commission on Radiological Protection, *Annals of the ICRP (UK)* Publication 60 **21** (1–3). Oxford: Pergamon.

ICRP (1993) Age-dependent doses to members of the public from intake of radionuclides. Part 2: Ingestion dose coefficients. *Annals of the ICRP (UK),* Publication 67, **23**, (3/4). Oxford: Pergamon.

ISHIMARU, T., ICHIMARU, M. & MIKAMI, M. (1981) *Leukaemia incidence among individuals exposed in-utero, children of atomic bomb survivors and their controls: Hiroshima and Nagasaki, 1945–79.* RERF TR/11–81.

JABLON, S. & KATO, H. (1970) Childhood cancer in relation to prenatal exposure to atomic bomb radiation, *Lancet,* ii, 1000–3.

KAICK, G.VAN., MUTH, H., KAUL, A. *et al.* (1986) Report on the German thorotrast study. In W.Gössner *et al.* (eds), *The Radiobiology of Radium and Thorotrast.* Munich: Urban & Schwarzenberg, pp. 114–18.

KAUL, A. & NOFFZ, W. (1978) Tissue dose in thorotrast patients, *Health Phys.* **35**, 113–21.

LADINSKAYA, L.A. *et al.* (1973) Pb^{210} and Po^{210} content in air, water, foodstuffs and the human body, *Arch. Environ. Health,* **27**, 254–8.

LEONG, A.S.-Y. & MILIOS, J. (1993) An assessment of the efficacy of the microwave antigen-retrieval procedure on a range of tissue antigens, *Appl. Immunohistochemistry,* **1**, 267–74.

LOVAAS, A.I. & HURSH, J.B. (1968) Radium-226 and lead-210 in human teeth and bones, *Health Physics,* **14**, 549–55.

LUBIN, J.H., BOICE, J.D., JR, HORNUNG, R.W. *et al.* (1994) *Lung Cancer and Radon: A Joint Analysis of Eleven Underground Miners Studies.* Publication no. 94–3644, US National Institutes of Health, Bethesda, MD.

MARSDEN, E. (1964) Incidence and possible significance of inhaled or ingested polonium, *Nature,* **203**, 230.

MAYS, C.W., SPIESS, H., CHMELEVSKY, D. & KELLERER, A. (1986) Bone sarcoma cumulative tumor rates in patients injected with ^{224}Ra. In W.Gössner *et al.* (eds), *The Radiobiology of Radium and Thorotrast.* Munich: Urban & Schwarzenberg, pp. 14–21.

MCINROY, J.F., KATHREN, R.L & SWINT, M.J. (1989) Distribution of plutonium and americium in whole bodies donated to the United States Transuranium Registry, *Radiat. Prot. Dosim.,* **26**, 1–4, 151–8.

MOLE, R.M. (1974) Ante-natal irradiation and childhood cancer: causation or coincidence? *Br. J. Cancer* **30,** 199–208.

MONSON, R.R. & MACMAHON, B. (1984) Prenatal x-ray exposure and cancer in children. In J.D.Boice & F.J.Fraumeni (eds), *Radiation Carcinogenesis: Epidemiology and Biological Significance.* New York: Raven Press, pp. 97–105.

NOZAKI, T., ICHIKAWA, M., SASUGA, T. & INARIDA, M. (1970) Neutron activation analysis of uranium in human bone, drinking water and diet, *J. Radioanal. Chem.,* **6**, 33–40.

NUSSBAUM, E. & HURSH, J.B. (1957) Radon solubility in rat tissues, *Science,* **125,** 552.

(1958) Radon solubility in fatty acids and triglycerides, *J. Phys. Chem.,* **62,** 81–4.

OSBORNE, R.V. (1963) Lead-210 and polonium-210 in human tissues, *Nature,* July 20, p. 295.

PARETZKE, H.G. (1980) Advances in energy deposition theory. In R.H.Thomas & V. Perez-Mendez (eds), *Advances in radiation protection and dosimetry in medicine.* New York: Plenum, pp. 51–73.

PARFENOV, Y.D. (1974) Polonium-210 in the environment and in the human organism, *Atomic Energy Review,* **12,** 75–143.

POHL, E. & POHL-RÜLING, J. (1967) The radiation dose received by inhalation of air containing Rn222, Rn220, Pb212 (ThB) and their decay products. *Anais da Academia Brasileira de Ciências ,* **39,** 393–404.

POPPLEWELL, D.S., HAM, G.J., JOHNSON, T.E. & BARRY, S.F. (1985) Plutonium in autopsy tissues in Great Britain, *Health Phys..* **49,** 304–9.

POPPLEWELL, D.S., HAM, G.T., DODD, N.J. & SHUTTLER, S.D. (1988) Plutonium and ^{137}Cs in autopsy tissues in Great Britain, *Sci. Total Environ.,* **70,** 321–34.

PRIEST, N.D. & VAN DE VYVER, F. (1992), *The Distribution and Behaviour of Heavy Metals in the Skeleton and Body: Studies with Bone-seeking Radionuclides.* Bora Raton: CRC Press.

PRIEST, N.D., MITCHELL, P.D., HENSHAW, D.L. & STRANGE, L. (1992b) A study of geographical variations in the concentration of plutonium-239/240 and strontium-90 in teeth in the British Isles, *Proceedings of the International Conference on Determination of Low Levels of Radionuclides in Biological Samples, Rio de Janeiro, October 1992.*

RAJEWSKY, B., BELLOCH-ZIMMERMANN, V., LÖHR, E. & STAHLHOFEN, W. (1965) ^{226}Ra in human embryonic tissue, relationship of activity to the stage of pregnancy, measurement of natural ^{226}Ra occurrence in the human placenta, *Health Phys.,* **11,** 161–9.

RAO, D.V., NARRA, V.R., HOWELL, R.W., LANKA, V.K. & SASTRY, K.S.R. (1991) Induction of sperm head abnormalities by incorporated radionuclides: dependence on subcellular distribution, type of radiation, dose rate, and presence of radioprotectors, *Radiat. Res.,* **125,** 89–97.

RERF87 (Radiation Effects Research Foundation) (1987) *US-Japan Joint Reassessment of Atomic Bomb Radiation Dosimetry in Hiroshima and Nagasaki.* Final Report, vol. 1, Hiroshima, Japan.

RERF88 (Radiation Effects Research Foundation) (1988) *US-Japan Joint Reassessment of Atomic Bomb Radiation Dosimetry in Hiroshima and Nagasaki.* Final Report vol. 2, Hiroshima, Japan.

RICHARDSON, R.B. (1991) Radon, other natural alpha-emitters, and their relevance to the induction of leukaemia. PhD thesis, University of Bristol.

(1992) Transfer of radiobismuth to the fetus in guinea-pigs, *Radiat. Prot. Dosim.,* **41,** 169– 72.

RICHARDSON, R.B., EATOUGH, J.P. & HENSHAW, D.L. (1991) Dose to red bone marrow from natural radon and thoron exposure, *Br. J. Radiol.,* **64**, 608–24.

RUNDO, J., KEANE, A.T., LUCAS, H.F., SCHLENKER, R.A., STEBBINGS, J.H. & STEHNEY, A.F. (1986) Current (1984) status of the study of ^{226}Ra and ^{228}Ra in humans at the center for human radiobiology. In W.GÖSSENER *et al.* (eds) *The Radiobiology of Radium and Thorotrast.* Munich: Urban & Schwarzenberg, pp. 14–21.

SALMON, P.L., HENSHAW, D.L., ALLEN, J.E. KEITCH, P.A. & FEWS, A.P. (1994) TASTRAK spectroscopy of natural ^{210}Po -activity at bone surfaces: evidence for a concentrated surface deposit less than 3 μm deep, *Radiat. Res.,* **140**, 63–71.

SAVITZ, D.A. & FEINGOLD, L. (1989) Association of childhood cancer with residential traffic density. *Scand. J. Work Environ. Health,* **15,** 360–3.

SCHLENKER, R.A. (1984) The distribution of radium and plutonium in human bone. In N. Priest (ed.) *Metals in Bone,* Lancaster: MTP Press, pp. 127–47.

SCHLENKER, R.A. & OLTMAN, B.G. (1986) High concentrations of ^{226}Ra and ^{241}Am at human bone surfaces: Implications for the ICRP 30 bone dosimetry model, *Radiat. Prot. Dosim.* **16,** 195–203.

SINGH, N.P., LEWIS, L.L. & WRENN, M.E. (1985) Uranium, thorium and plutonium in bones from the general population. In N.Priest (ed.) *Metals in Bone.* Lancaster: MTP Press, pp. 231–42.

SHIMIZU, Y., KATO, H., SCHULL, W., PRESTON, S., FUJITA, S. & PIERCE, D. (1987) *Comparison of Risk Coefficients for Site-specific Cancer Mortality Based on the DS86 and T 65R Shielded Kerma and Organ Doses TR 12–87.* Life Span Study Report II, part 1. Hiroshima: RERF.

SOLIGO, D. *et al.* (1991) Identification of CD34$^+$ cells in normal and pathological bone marrow biopsies by QBend10 monoclonal antibody, *Leukaemia,* **5**, 1026–30.

SPIERS, F.W. (1974) Radionuclides and bone from ^{226}Ra to ^{90}Sr, *Br. J. Radiol.,* **47**, 833–44.

SPIERS, F.W. & BEDDOE, A.H. (1977) 'Radial' scanning of trabecular bone: consideration of the probability distributions of path lengths through cavities and trabeculae, *Phys. Med. Biol.,* **22**, 670–80.

SPIERS, F.W., WHITWELL, J.R. & BEDDOE, A.H. (1978) Calculated dose factors for the radiosensitive tissues of bone irradiated by surface-deposited radionuclides, *Phys. Med. Biol.,* **23**, 481–94.

STSJAZHKO, V.A., TSYB, A.F., TRONKO, N.D., SOUCHKEVITCH, G. & BAVERSTOCK, K.F. (1995) Childhood thyroid cancer since accident at Chernobyl, *Br. Med. J.,* **310,** 801.

STEWART, A., WEBB, J., GILES, D. & HEWITT, D. (1956) Malignant disease in childhood and diagnostic irradiation *in utero, Lancet,* **447**.

STEWART, A., WEBB, J. & HEWITT, D. (1958) A survey of childhood malignancies, *Br. Med. J.,* 1495–508.

STEWART, A. & KNEALE, E.W. (1970) Radiation dose effects in relation to obstetric X-rays and childhood cancer, *Lancet,* i 1185–9.

THOMAS, P.A. (1994) Dosimetry of ^{210}Po in humans, caribou, and wolves in Northern Canada, *Health Phys.,* **66,** 678–90.

THORNE, M.C. & VENNART, J. (1975) The toxicology of ^{90}Sr, ^{226}Ra and ^{239}Pu, *Nature,* **263**, 555–8.

TOTH, A., PETER, J., SZABO, J., BETA, I. & LAKATOS-NOVOTNY, S. (1982) Analysis of human teeth for Pb-210 and Po-210, *Proc. 2nd Spec. Symp. on Natural Radiation Environment.* Bombay: Wiley Eastern, pp. 275–82.

TOTTER, J.R. & MACPHERSON, H.G. (1981) Do childhood cancers result from pre-natal X-rays? *Health Phys.* **40**, 511–24.

UNSCEAR (1986) *Genetic and Somatic Effects of Ionizing Radiation.* Report to the General Assembly, with annexes. United Nations Sales Publications no. E.86.IX.9. New York: United Nations.

(1988) *Sources, Effects and Risks of Ionizing Radiation.* Report to the General Assembly, with annexes. United Nations Sales Publications no. E.88.IX.7. New York: United Nations.

(1993) *Sources and Effects of Ionizing Radiation.* New York: United Nations.

VIEL, J.F. (1993) Radon exposure and leukaemia in adulthood. *Int. J.Epidemiol.,* **22**, 627–31.

WALTON, A., KOLOGRIVOV, R. & KULP, J.L. (1959) The concentration and distribution of radium in the normal human skeleton, *Health Phys.,* **1**, 409.

WELFORD, G. & BAIRD, R. (1967) Uranium levels in human diet and biological materials, *Health Phys.,* **13,** 1321–4.

WHITEHOUSE, W.J. (1974) The quantitative morphology of anisotropic trabecular bone, *J. Microscopy,* **101,** 153–68.

WILSON, W.E. & PARETZKE, H.G. (1980) Calculation of ionising frequency distributions in small sites, *Radiat. Res.,* **81**, 326–35.

4
Response of Bone Marrow to Low LET Irradiation

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4
Response of Bone Marrow to Low LET Irradiation

4.1
Acute Irradiation

4.1.1
Haemopoietic Responses

The response of haemopoietic tissue to acute radiation doses follows the pattern expected of a hierarchical cell population comprising subpopulations with different sensitivities and modes of death. The hierarchy is shown in diagrammatic form in Figure 4.1 (see also Chapter 2). The sensitivity of the stem cells and their descendants, the committed precursor cells, is due to the induction of mitotic failure and/or their propensity to undergo apoptosis after irradiation. The mature non-dividing cells are either very resistant, e.g. red cells and platelets, or are very sensitive, e.g. some lymphocytes which become apoptotic. Hence, after whole-body irradiation, lymphocyte numbers decline rapidly whereas platelets have a short half-life (eight days in man) and their number declines more slowly in a dose-dependent fashion governed by the radiosensitivity of the precursor megakaryocytes. White cells have a short half-life (eight hours in man) and the number declines similarly to platelets because of the radiosensitivity of the white cell precursors. Red cells have a long half-life (four months in man), but after high doses their number may decline early because of haemorrhages. Some of these characteristics are shown in Figure 4.2 which is a summary of data derived from accident cases of human irradiation (Wald, 1971). The blood lymphocyte count is the most sensitive index of radiation injury, nadirs being reached earlier than for other cell types. The unusual initial pattern shown for neutrophils is considered to reflect an initial abortive rise due to a transient mobilisation of cells from marrow and/or extramedullary sites and to accelerated maturation of precursor cells (Bond *et al.,* 1965). The second abortive rise is considered to be due to recovery from precursor cell populations which are later exhausted because of a failure to renew them from earlier, more sensitive precursor cells.

The lack of granulocytes allows endogenous and exogenous organisms to flourish and this can lead to death of the individual. This can be exacerbated by concomitant damage to the immune system. The effects on the immune system are dose dependent and may result in an augmented or suppressed response depending on the dose and the time after irradiation (Anderson *et al.,* 1980). These differences in response relate to differences in the sensitivity of the cell populations involved in the response. Suppressor T-cells ($CD8^+$) are more radiosensitive than helper T-cells ($CD4^+$), and B-cells have an intermediate sensitivity (Anderson and Standefer, 1983; Stewart *et al.,* 1988). However, studies with large field irradiations in

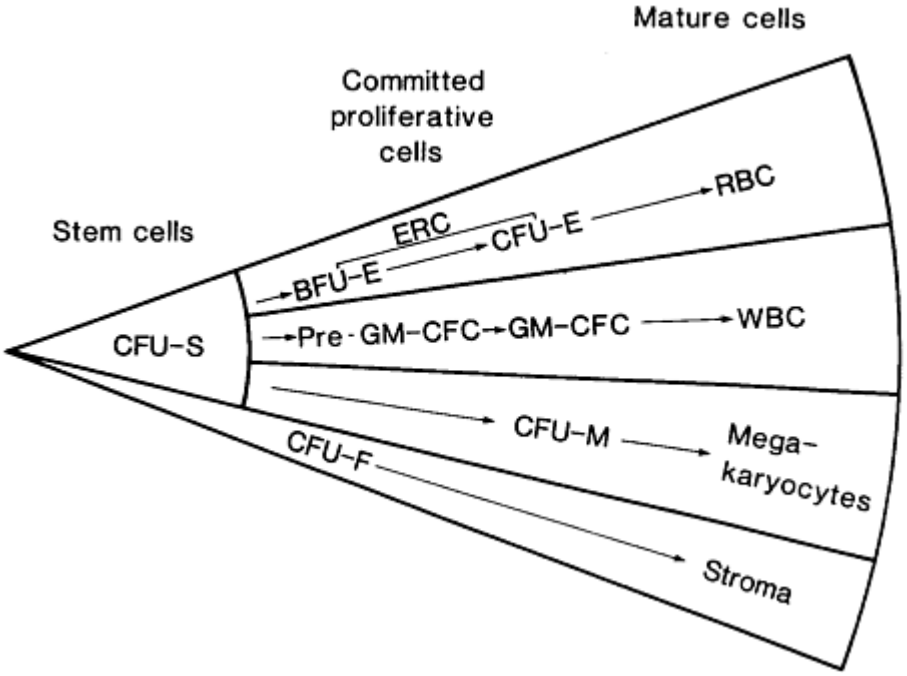


Figure 4.1 Diagrammatic representation of the haemopoietic cell hierarchy and the associated stroma

women have shown better recovery of T-cell numbers of the suppressor type (Rotstein *et al.,* 1985; Wasserman, 1986; Job *et al.,* 1984).

4.1.2
$LD_{50/30}$

Animals die from marrow failure within thirty days after doses between about 2 and 10 Gy depending on the species. The $LD_{50/30}$ (death of 50 per cent within 30 days) is related to body weight, as shown in Figure 4.3. There is an effect of age and, in mice, weanlings of around 4 weeks of age are more radiosensitive than 1 week old neonates or adults (Crosfill *et al.,* 1959). Death is associated variously among species with granulocytopenia, thrombocytopenia and lymphocytopenia (Bond *et al.,* 1965). In most species, anemia is less severe than neutropenia or thrombopenia, and it does not correlate well with the time of death (Bond *et al.,* 1965), due partly to the resistance and long lifespan of the red blood cells. After high doses, used in transplant situations, thrombocytopenia becomes increasingly important.

The differences in $LD_{50/30}$ between species have been interpreted in terms of differences in stem cell concentrations deduced from transplantation and rescue experiments (Vriesendorp and Van Bekkum, 1980, 1984). Perhaps, more appropriately, this should be termed target cell concentrations, because it is not known precisely which subpopulation in the hierarchy is responsible for rescue in the LD_{50} scenario. Indeed, a range of subpopulations may be capable of aiding rescue to varying extents. A complementary way of interpreting the LD_{50} differences is to consider different tolerances to similar levels of target cell depletion in the various species (Hendry and Roberts, 1990). This was supported by an analysis of dose-incidence data for six species using a mathematical model which assumed exponential target cell depletion over the

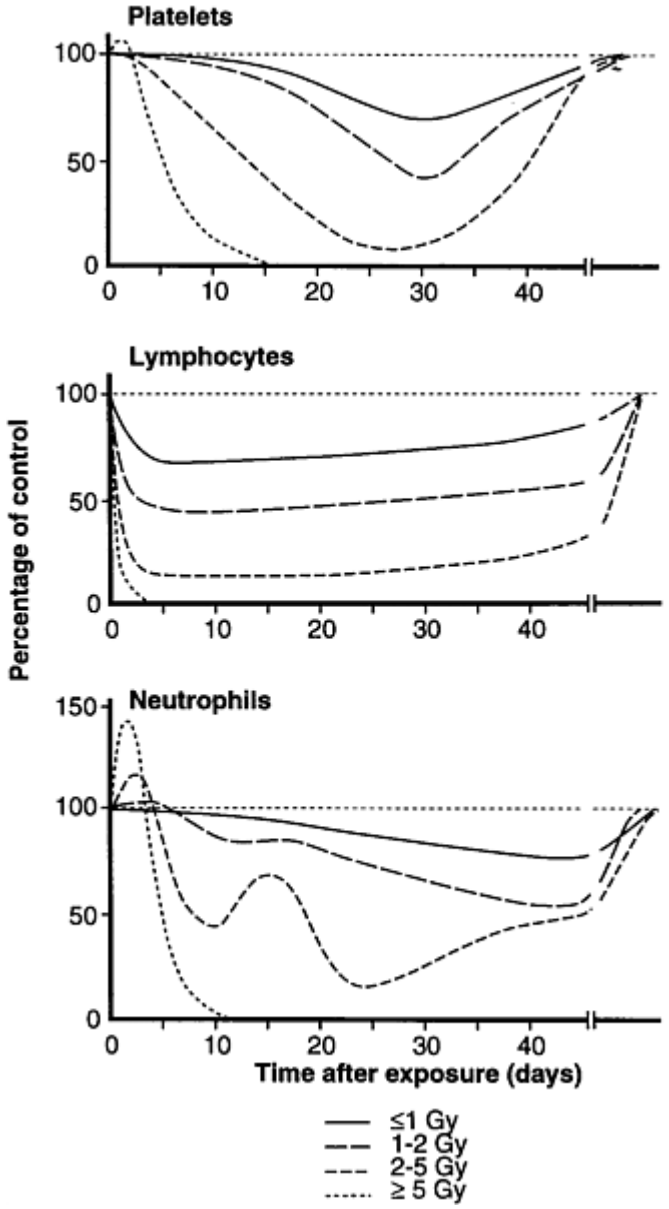


Figure 4.2 Schematic picture of average time courses for various cells in the blood, after various doses of radiation in man, derived from accident cases

dose range spanning the LD_{50} and survival of the animal if the irradiation resulted in survival of one or more tissue rescuing units (TRU). A TRU is the complement of target cells sufficient for rescue. These two premises led to the conclusion that at LD_{50} the depletion of target cells was to about 3×10^{-4} of normal in mice, and 10^{-2}–10^{-3} for larger species such as dogs, sheep, pigs, goats and monkeys. When CFU-S survival was used as an index of the ability of target cells to restore haemopoiesis in lethally irradiated mice, it was

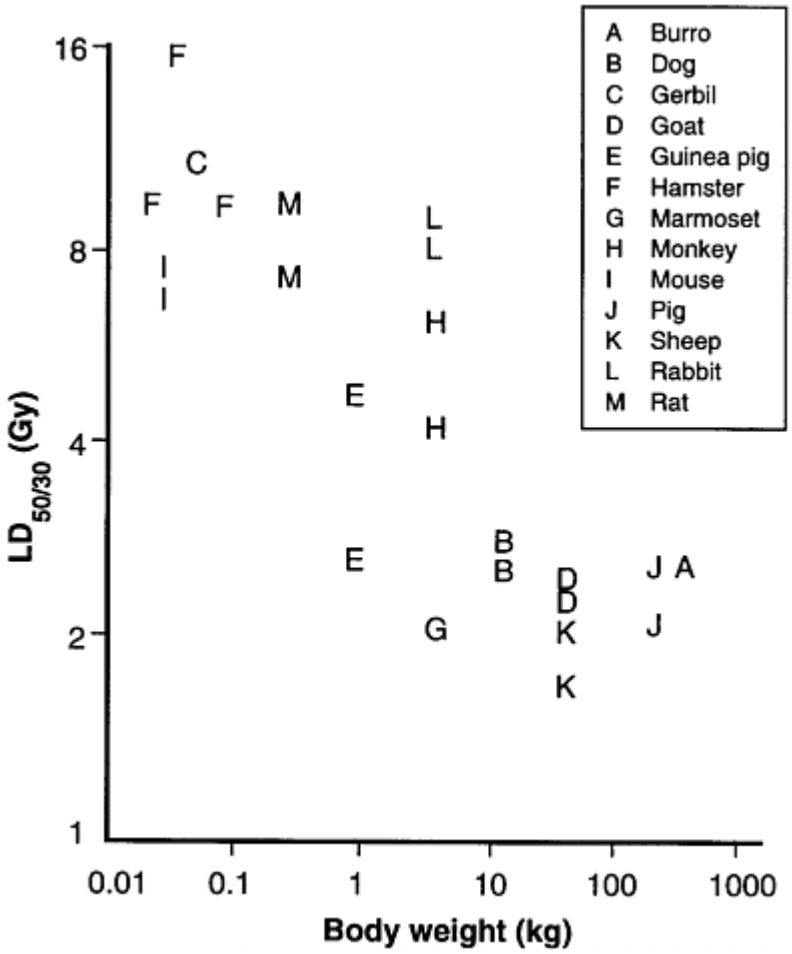


Figure 4.3 Relationship between $LD_{50/30}$ and body weight for various mammals

shown that this property of the surviving irradiated CFU-S declined with increasing radiation dose (Cronkite *et al.,* 1993). This effect would probably make the target cell sensitivity deduced from $LD_{50/30}$ data, higher than that measured directly for CFU-S.

A more recent and more complex mathematical model has invoked the importance of the stroma in determining the $LD_{50/30}$ (Jones *et al.,* 1993). This is because, in contrast to the deductions using the above model, the deduced sensitivity of the target cells was lower and more similar to that of the stromal precursor cells than that of the haemopoietic precursor cells. The plausibility of this later model has received criticism and it is, as yet, unresolved (Hendry *et al.,* 1994; Ruifrok and Thames, 1994).

The LD_{50} for man is usually taken to be sixty days because of later deaths compared with other species. For man, the LD_{50} has been estimated (UNSCEAR, 1988) from a number of data sources: (1) mortality following the atomic bombings in Japan in 1945; (2) the accident at Chernobyl in 1986; (3) various groups of cancer patients receiving whole-body irradiation, and (4) several other radiation accidents. The conclusion was that, with conventional medications, the $LD_{50/60}$ would probably be around 4 Gy. Lower

values would apply if there was a contribution to mortality from extensive burns, pre-existing illness, chronic nutritional deprivation or concurrent infection. Higher values would apply if the individuals were young and female, if there were good medical support and if the radiation were poorly penetrating, unilateral or protracted.

4.1.3
Haemopoietic Progenitor Cell Radiosensitivity

Cell sensitivity is quantified in several ways. The first way was devised on the basis that the relationship between levels of cell survival and increasing dose is approximately exponential at high doses. Hence, equal increments of dose kill equal proportions of cells. This is expected if the ionisations and their associated lethal events are distributed at random (stochastically) among the cell populations. The sensitivity parameter D_0 is the dose which will just kill the average cell in the population, so that by statistical probability, e^{-1} or 37 per cent of them will not suffer this lethal event. This applies at high dose when survival levels are exponentially related to increasing dose. If the latter applies also at low dose, then D_0=D_{37}, the dose depleting the initial population to 37 per cent. The equation which can be used to describe the dose survival relationship is:

$$S=\mathrm{n}.\exp(-D/D_0)$$

where n (the extrapolation number) allows for a threshold effect at low doses.

The second way was developed on the basis of a linear-quadratic exponential relationship, so that there was a finite sensitivity ( ) at low doses which increases gradually after high doses. The equation in this case is :

$$S=\exp(-\alpha D-\beta D^2)$$

where and are constants. These relationships are shown in Figure 4.4. Thus is the reciprocal of the 'initial D_0' and at a given high dose D, D_0=1/(+2 D). If doses are fractionated into N well separated increments d, D is replaced by $N\times d$.

A third way to quantify sensitivity, which is being used increasingly, is to calculate the average dose ( ), that will kill a cell over all dose ranges between zero and infinity. is the mean inactivation dose, and is calculated from the area under the curve of survival versus dose in linear coordinates. For a simple exponential curve, D_0=1/ = .

The sensitivity of many different types of haemopoietic progenitor cell has now been measured using colony assays. These have been listed in detail by Hendry and Lord (1983) and Nothdurft (1991), and a summary is shown in Figure 4.5 and in Table 4.1. All the haemopoietic progenitor cells are fairly radiosensitive compared with progenitor cells in most other tissues, and there is high sensitivity even to low doses. Also, there is an effect of age: CFU-S in weanling mice around 4 weeks of age are more radiosensitive than those in 1 week old neonates or adults (Fred and Smith, 1967; Gerber and Maes, 1981). CFU-S in foetal liver are more resistant than CFU-S in adult haemopoietic tissues (Siminovitch *et al.,* 1965). In recent years, it has become clear that the most primitive cells, cells with marrow repopulating ability (see Chapter 2) have a higher D_0 value than the 12-day CFU-S. This is higher again for 8-day CFU-S, but lower for the committed progenitors (Baird *et al.,* 1990; Meijne *et al.,* 1991; Ploemacher *et al.,* 1992). These studies confirm the previously reported tendency for CFU-S surviving irradiation to have a higher self-renewal ability than unirradiated CFU-S (Hendry and Lord, 1983). However, this is in contrast to data mentioned already showing that the quality of surviving CFU-S responsible for 30-day survival *decreases* with increasing radiation dose (Cronkite *et al.,* 1993). Also, at low doses there is some evidence for a threshold in effect up to doses around 0.2 Gy (Cronkite *et al.,* 1987), but other data do not support this (Hendry, 1988). Doses down to 0.05 Gy -rays produced a small but significant amount of killing of CFU-S, which is

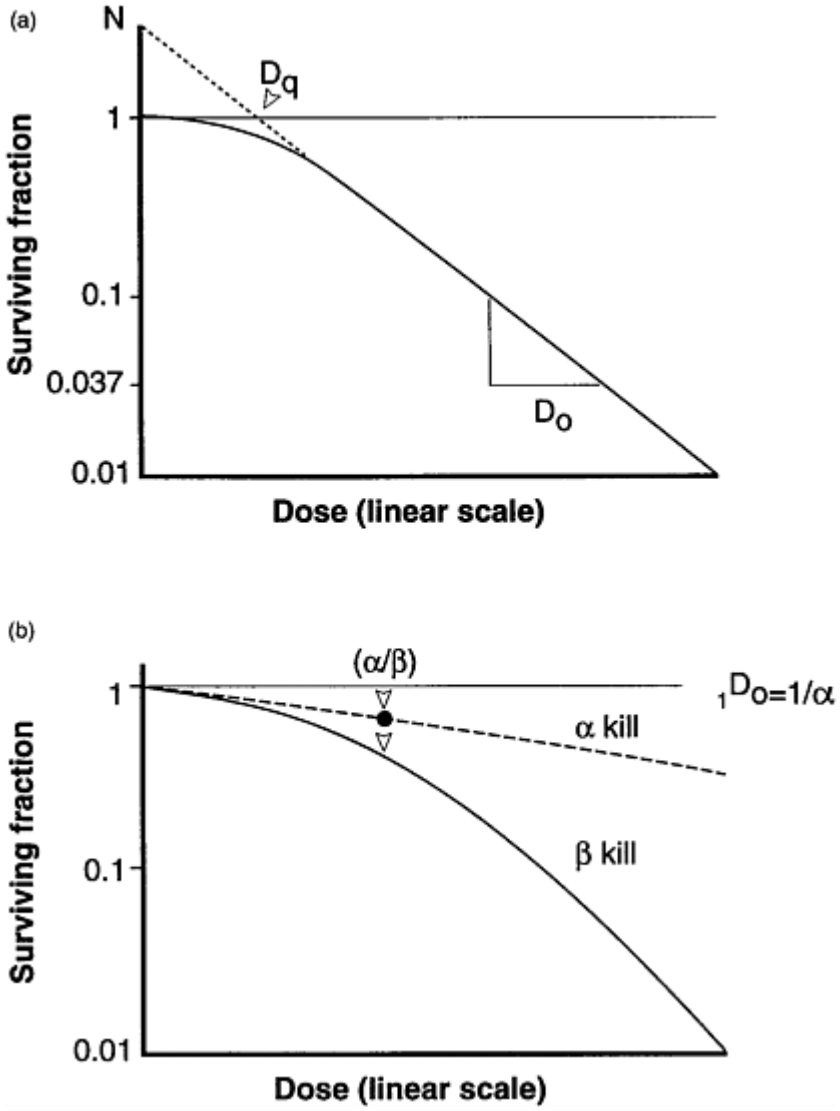


Figure 4.4 (a) Single-hit, multitarget survival curve, where D_q=D$_0$ln$N$; (b) linear-quadratic survival curve, showing that (/) is the dose at which the components of cell killing from the and modes are equal. Note also that =1/$_1D_0$. $_1D_0$ is the initial inverse slope of the survival curve

consistent with an exponential relationship down to zero dose (Hendry, 1988). GM-CFC (e.g. Nothdurft *et al.,* 1983) and BFU-E (e.g. Schwartz *et al.,* 1986) in the dog, have been reported to be particularly radiosensitive. It is still not known if technical factors are partly responsible for this, because culture conditions (e.g. Broxmeyer *et al.,* 1976) and growth factor concentrations (see below) are known to be able to produce different values of sensitivity. However, the high cellular sensitivities reported in the dog are consistent with the high slopes of dose incidence curves for marrow failure in the dog and other large

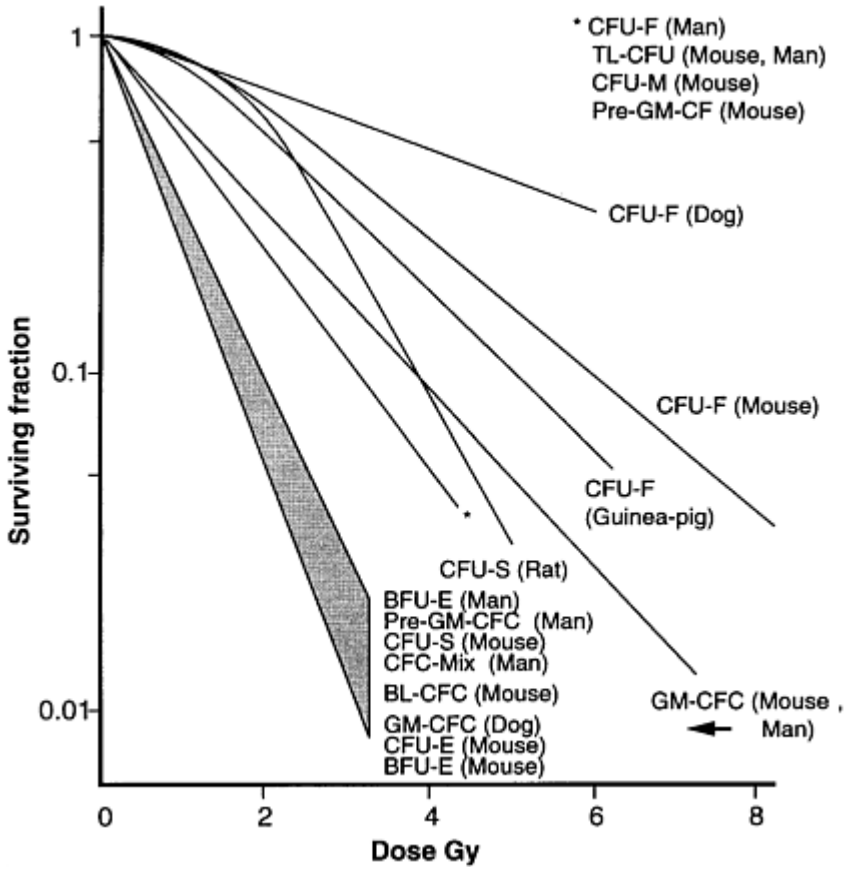


Figure 4.5 Survival curves for haemopoietic and stromal precursor cells in different species. (Redrawn from Hendry, 1988

Table 4.1 Average values of D_0(Gy) for different progenitor cells in mouse, rat, dog and human marrow

Cell type	Radiation[a]	Mouse	Rat	Dog	Man
MRA		1.25 (2)	–	–	–
	X	–	–	–	–
CFC_{mix}		–	–	–	–
	X	0.60 (1)	–	–	–
CFU-S_{12}		0.91 (2)	–	–	–
	X	–	–	–	–
CFU-$S_{7/9}$		0.98 (35)	0.96 (2)	–	–
	X	0.80 (29)	–	–	–
GM-CFC		1.54(11)	–	0.59 (4)	1.22 (5)
	X	1.04 (3)	–	0.61 (1)	1.17 (7)
ERC/ERA		0.97 (1)	–	–	–

Cell type	Radiation[a]	Mouse	Rat	Dog	Man
	X	0.76 (6)	0.60 (1)	–	–
CFU-E		0.66 (1)	–	–	–
	X	0.53 (1)	–	0.61 (1)	–
BFU-E		0.73 (1)	–	–	1.13 (1)
	X	0.69 (1)	–	0.21 (2)	1.27 (1)
Meg-CFC		1.28 (1)	–	–	–
	X	–	–	–	–
CFU-F		2.16 (1)	–	4 (1)	1.2 (2)
	X	2.32 (5)	–	3.4 (4)	1.2 (2)

[a] — -rays from ^{60}Co or ^{137}Cs; X—orthovoltage X-rays.

Note: The number of values averaged is given in parentheses. The dose rates used were greater than 0.03 Gy min^{-1}, and generally of the order of 1 Gy min^{-1}.

Sources: Summarised and updated from Hendry and Lord (1983) and Nothdurft (1991).

species (see above). Values of sensitivity for various progenitor cells in man are in line with values for mice, except for the erythroid progenitor cells which appear more resistant in man than in mouse (Table 4.1). Orthovoltage X-rays are more efficient than high energy -rays in killing cells by up to 20 per cent (Table 4.1).

Certain genetic disorders predispose towards high radiosensitivity. GM-CFC in severe combined immunodeficient (scid) mice, e.g. Hendry and Jiang (1994), or humans, Cavazzana-Calvo *et al.* (1993) have been found to be more sensitive than normal by a factor of 2–3. Similarly, GM-CFC from ataxia telangiectasia (AT) patients are also more sensitive than normal by about the same factor (Hart *et al.,* 1987). In the scid cells, there is a repair/recombination defect which results in more residual double strand breaks (Chang *et al.,* 1993). The gene responsible is on mouse chromosome 16 and on human chromosome 8 (Kirchgessner *et al.,* 1993). The AT gene is on human chromosome 11q22–23 (McConville *et al.,* 1994). The defect has not been fully elucidated but it is somehow related to DNA repair, and recently more residual double strand breaks have been detected in cells (Wurm *et al.,* 1994).

Comparison of cell sensitivities is made difficult by the fact that the constitution of the growth medium can alter radiosensitivity. This was observed in terms of the colony stimulating activity of the medium (Broxmeyer *et al.,* 1976) or just its age (Xu *et al.,* 1983a). Also, when bone marrow was irradiated and then cultured in medium containing different colony stimulating factors, different radiosensitivities resulted (Baird *et al.,* 1990). Although this occurred when normal marrow was cultured, suggesting preferential growth of particular subpopulations by different factors, it also occurred when semi-purified populations were used (Baird *et al.,* 1990). This suggested that sensitivity may be determined in part by the particular differentiation patterns imposed. Even when human marrow is preconditioned, prior to irradiation, with particular factors for only 24 hours, the radiosensitivity is variously modified (Uckun *et al.,* 1989). Those marrow cells that undergo apoptosis after irradiation are also very susceptible to the presence of growth factors. When marrow derived cells, dependent on IL-3 for growth were irradiated, IL-3 was necessary to prevent the accelerated onset of DNA cleavage and cell death from an irradiation induced G_2 arrest (Collins *et al.,* 1992).

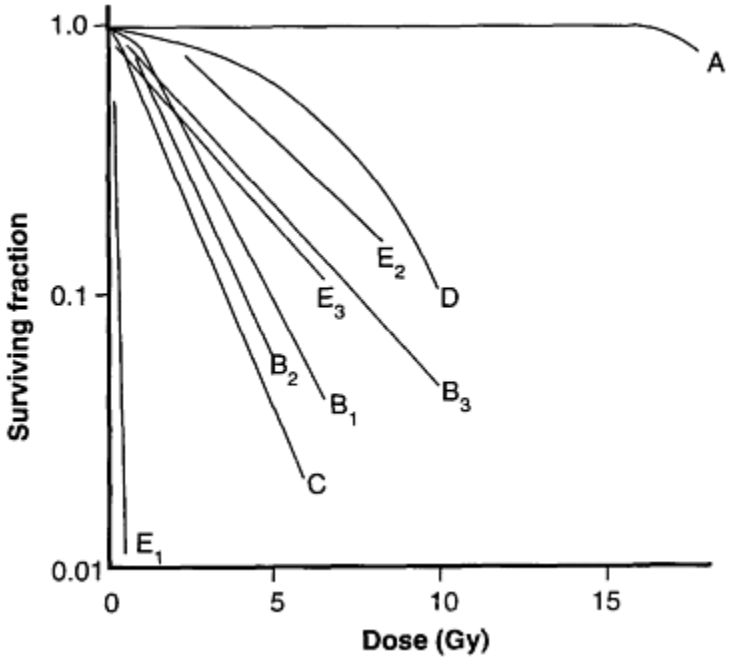


Figure 4.6 Radiosensitivity of the marrow stroma using different assays. A: loss of sinusoids at 1 year; B_1: CFU-F after *in vivo* irradiation (4.2 Gy min^{-1}); B_2 : CFU-F after *in vitro* irradiation; B_3 : CFU-F after *in vivo* low-dose rate (1.4 cGy min^{-1}) irradiation; C_1: CFU-S in kidney implants, using 4 Gy min^{-1}; D: CFU-S, GM-CFC and CFU-F content in subcutaneous femur implants; E_1: non-adherent CFU-S in type 1 long-term bone marrow cultures (LTBMCs) after 7 weeks, E_2 and E_3 : non-adherent and adherent CFU-S in type II LTBMCs after 5 weeks. (Redrawn from Bierkens *et al.,* 1989)

4.1.4 Marrow Stromal Radiosensitivity

The radiosensitivity of the stroma which nurtures the marrow depends to a large extent on its proliferation after irradiation (see Figure 4.6). Acute doses up to 15 Gy to intact stroma cause little morphological damage up to a year later (Knospe *et al.,* 1966). When irradiated femora are transplanted and their reconstitution by host marrow is measured, effects of a few gray can be measured (Piersma *et al.,* 1983). Greater sensitivity is observed when irradiated marrow is induced to form an ectopic ossicle harbouring host marrow under the kidney capsule (Molineux *et al.,* 1987). Radiosensitivity similar to the latter is found for the stromal precursor cells CFU-F (see Figure 4.6 and Table 4.2). CFU-F in the mouse are more resistant than the haemopoietic progenitor cells, are much more resistant in the case of the dog, but apparently they have a similar sensitivity in man (Table 4.1). In the rat, two populations of CFU-F have been detected (Kolesnikova *et al.,* 1995). One population forming diffuse colonies is more resistant (D_0=1.4 Gy) than the population forming compact colonies (D_0=0.8 Gy). Also clonal cell lines derived from human marrow which had similar morphological, functional and cytogenetic features were found to differ in radiosensitivity (Harigaya and Hamda, 1985; Fitzgerald *et al.,* 1988).

Adherent layers *in vitro* have been used extensively to study the effects of radiation on the stroma (reviewed by Bierkens *et al.,* 1989). When marrow is irradiated prior to its growth to establish a layer, it has

a sensitivity similar to that of CFU-F. Preformed competent layers are very radioresistant due to their non-proliferative state, unless they are inactive and require a second marrow inoculum to achieve prolonged haemopoiesis, when they appear very sensitive. Also, irradiation of established layers induces production of additional growth factors, predominantly GM-CSF (Naparstek *et al.,* 1985). Hence established stroma appears radioresistant until cell proliferation expresses the latent radiation injury.

Of possible importance for leukaemogenesis is the observation that irradiation of bone marrow stromal cell lines in plateau phase produces factor-independent growth of co-cultivated factor-dependent haemopoietic progenitor cell lines (Naparstek *et al.,* 1986). The process is associated with: (1) adherence of haemopoietic cells to stromal cells forming 'cobblestone islands'; (2) an intermediate stage during which the cells show proliferation in suspension in the presence of leukaemogenic stromal factor (LSF), a factor similar to M-CSF released by irradiated stromal cells, and (3) a third stage of factor-independence (Greenberger *et al.,* 1992).

Table 4.2 D_0 values (Gy) for normal and purified progenitor cells of the bone marrow

CSF	N-BM	FACS-BM	CCE-BM
rIL-3	1.15±0.09	1.03±0.04	1.18±0.06
rGM-CSF	1.29±0.12	–	1.35±0.07
pM-CSF	1.23±0.05	–	0.62±0.07
rG-CSF	0.42±0.03	–	–
rIL-3+rIL-1	1.06±0.08	1.31±0.04	0.46±0.04
pM-CSF+rIL-1	1.42±0.05	0.74±0.03	0.40±0.05
rIL-3+pM-CSF	1.36±0.07	1.27±0.04	1.03±0.05
rIL-3+pM-CSF+rIL-1	1.75±0.24 (biphasic)	1.71±0.18	1.31±0.10

Note: Normal mouse bone marrow cells (N-BM), or marrow enriched either for more primitive progenitor cells (fluorescence-activated cell sorted (FACS-BM) or more differentiated progenitor cells (counterflow centrifugal elutriated (CCE-BM). The cells were irradiated *in vitro* using ^{137}Cs -rays at 4.2 Gy min^{-1}, and then cultured with the various recombinant (r) or purified (p) factors for seven days before colonies were counted.

Sources: Summarised from Baird *et al.,* 1990, 1991.

4.1.5
Cell Population Recovery

After acute irradiation there is generally a delay of two to three days before regeneration of haemopoietic precursor cell populations begins in earnest. The delay can be abolished by small priming doses of cytotoxic agents (Millar *et al.,* 1978; Patel *et al.,* 1989). Repopulation includes an exponential phase of growth where the doubling time is dose-dependent, e.g. Coggle (1980). The minimum doubling time of CFU-S after high doses in the mouse is about 24 hours (reviewed by Hendry and Lord, 1983). From an analysis of colony growth, it was calculated that a doubling time of 24 hours could represent a cell cycle time as short as 6 hours with a concomitant 40 per cent loss of CFU-S at each division due to differentiation (Lajtha *et al.,* 1971). CFU-S recovery rates are similar in mice of different ages including 1 week old neonates (Gerber and Maes, 1981; Yang, Hendry and Testa, unpublished data). GM-CFC and the erythroid precursor cells follow the CFU-S recovery pattern quite closely (Testa *et al.,* 1974; Imai and Nakao, 1987). Some

investigators have found full recovery after near-lethal doses, others have not (reviewed by Hendry and Lord, 1983). A near-plateau in recovery occurs within two weeks in the mouse but longer, at around four weeks, in the dog (Baltschukat and Nothdurft, 1990). Stromal elements are not only more resistant, but also regenerate more slowly. Most repopulation is completed by three weeks in mice, but further recovery occurs to at least two months (Xu *et al.,* 1983a).

4.2
Protracted Irradiation

4.2.1
Low Dose Rates

Both haemopoiesis and stroma are spared injury when the dose rate is reduced to below about 0.1 Gy/min. The effect for haemopoietic progenitor cells is observed only after high doses because of the high - component (or initial D_0) which is dose rate independent. The maximum increase in $LD_{50/30}$ achieved by lowering the dose rate is about 40 per cent in mice (Nothdurft, 1991), and somewhat less in larger species (Baverstock *et al.,* 1985), probably because of the lower values of $LD_{50/30}$.

At very low dose rates (0.7 Gy/day), depopulation of CFU-S with increasing dose is similar to that after high dose rate irradiation, down to about 2 per cent CFU-S survival (Lajtha *et al.,* 1971). Then there is a break in the curve consistent with increased cycling in the population. The subsequent lower depopulation rate with further doses is dose rate dependent, as expected for a cycling cell population receiving different doses per cycle. A dose rate of 0.4–0.5 Gy/day will maintain the CFU-S level at 2–6 per cent of control for up to 30 days (Porteous and Lajtha, 1966; Wu and Lajtha, 1975). Even with this level of depopulation of CFU-S, 50 per cent of mice in these experiments survived to day 25 (an accumulated dose of 12.5 Gy) with haematocrits of 30–40 per cent of control. Also, nucleated cells per femur were maintained at 50 per cent of the control to at least day 12 (Wu and Lajtha, 1975). At 0.7 Gy/d there was no change in self-renewal ability of the CFU-S to day 11, but at 20–80 days after a 45-day period of chronic irradiation, the self-renewal ability was reduced (Schofield, 1978). The contrasting situation of low stem cell numbers and high mature cell numbers is maintained by extra amplification divisions of the transit cells, particularly ERC (Porteous and Lajtha, 1966, Reissmann and Udupa, 1972) and also in the maturing cell populations (Lord, 1964, 1965; Tarbutt and Blackett, 1968; Tarbutt, 1969). An extra five or six divisions in the transit cell series could compensate completely for a reduction in stem cells by 98 per cent. Stromal progenitor cells are spared more than haemopoietic progenitor cells at low dose rate. This has been demonstrated for cells forming ectopic ossicles (Molineux *et al.,* 1987), as well as for CFU-F in mice (Hendry *et al.,* 1985) and man (Fitzgerald *et al.,* 1986).

4.2.2
Fractionated Doses

Radiation damage of haemopoietic stem cells is reduced when doses are fractionated. For both $LD_{50/30}$ and CFU-S, the effects are characteristic of those expected for early reacting renewal tissues (reviewed by Hendry and Lord, 1983; Nothdurft, 1991; Thames and Hendry, 1987). The / ratio characterising the fractionation sensitivity is in the range 7–35 Gy for $LD_{50/30}$ in mice (Fowler, 1983). When doses are protracted over more than one week, there is evidence both for $LD_{50/30}$ (Mole, 1957) and for CFU-S (Hendry and Lajtha, 1972) of marked repopulation.

Greater reductions in damage when doses are fractionated, are observed for the stroma. In the rat, / ratios of 4.5 Gy for more-sensitive CFU-F forming compact colonies and 12.7 Gy for more-resistant CFU-F forming diffuse colonies have been deduced (Kolesnikova *et al.,* 1995). The former value, which would be important for regeneration after lower doses, is consistent with the marked sparing of stromal effects using low dose rates.

4.3
Inhomogeneous Irradiation

4.3.1
Marrow Distributions

The haemopoietic response to partial body irradiation depends primarily on the proportion of active marrow irradiated. The distribution of active marrow in the body is shown in Table 4.3 for different species. The sites with the greatest percentage of active marrow range from the head, ribs and vertebrae in mice to the ribs,

Table 4.3 Distribution of active marrow in different species

					Man	
	Mice	Rats	Dogs	Monkeys	A	B
Head	19.1	8.4	6.2	15.5	14.0	10.7
Upper limbs	5.7	8.6	11.1	12.2	–	3.7
Ribs	16.1	10.3	23.3	6.3	21.6	21.3
Vertebrae	38.1	19.9	39.3	31.5	36.3	24.4
Pelvis	8.2	15.5	12.2	14.5	28.1	28.6
Femora	6.0	16.9	7.2	13.3	–	10.6
Rest of lower limbs	6.8	22.5	0.7	6.7	–	–

Sources: Percentage content of different active marrow spaces in mice (Carsten, 1970), rats (Van Dyk *et al.,* 1964), dogs (Greenberg *et al.,* 1966), monkeys (Taketa *et al.,* 1970), and man (A: Woodard, 1984; B: Miyakawa *et al.,* 1970).

vertebrae and pelvis in man. The values are derived from histological and ^{59}Fe uptake measurements.

4.3.2
Partial Body Irradiation

The $LD_{50/30}$ can be markedly increased when small portions of the marrow are shielded during irradiation. Shielding one leg increased the $LD_{50/30}$ in mice from just less than 7 Gy to about 12 Gy (Carsten and Cronkite, 1971). In dogs, shielding the skull reduced lethality after 4–5 Gy from 100 per cent to 20 per cent, and shielding the sternum, pelvis or skull doubled the LD_{50} (Allen *et al.,* 1957). Shielding separately the head and neck, chest, abdomen or pelvis gave no deaths in dogs given 6 Gy, a 100 per cent lethal dose when given to the whole body (Li *et al.,* 1985). Even shielding one or two vertebrae was sufficient to protect dogs from an otherwise fatal radiation exposure (Shouse *et al.,* 1931).

With partial body irradiation, there is evidence of local control of CFU-S turnover and systemic control of CFU-S differentiation (Gidali and Lajtha, 1972). When the proportion of shielded marrow is small, say a femur in mice (5 per cent of the total), there is a dose-dependent initial decrease in CFU-S numbers within fifteen minutes to about 35 per cent after 15 Gy (Croizat *et al.,* 1976). This probably represents induced differentiation. It is followed by a release of CFU-S into the circulation, more rapidly during the next three hours than subsequently (Croizat *et al.,* 1980), when cycling of CFU-S increases markedly before reaching control levels by two to four days (Croizat *et al.,* 1970; Gidali and Lajtha, 1972). Hence after the initial perturbations, CFU-S numbers in the shielded region are restored and cycling decreases, even though the irradiated remaining marrow is still markedly deficient in CFU-S and those which are there continue to cycle rapidly. Correspondingly, if only one tibia is irradiated, mature cell production is insignificantly affected and there is increased cycling in the tibia. This implicates local control in the regulation and restoration of CFU-S numbers (Gidali and Lajtha, 1972; Ali *et al.,* 1989).

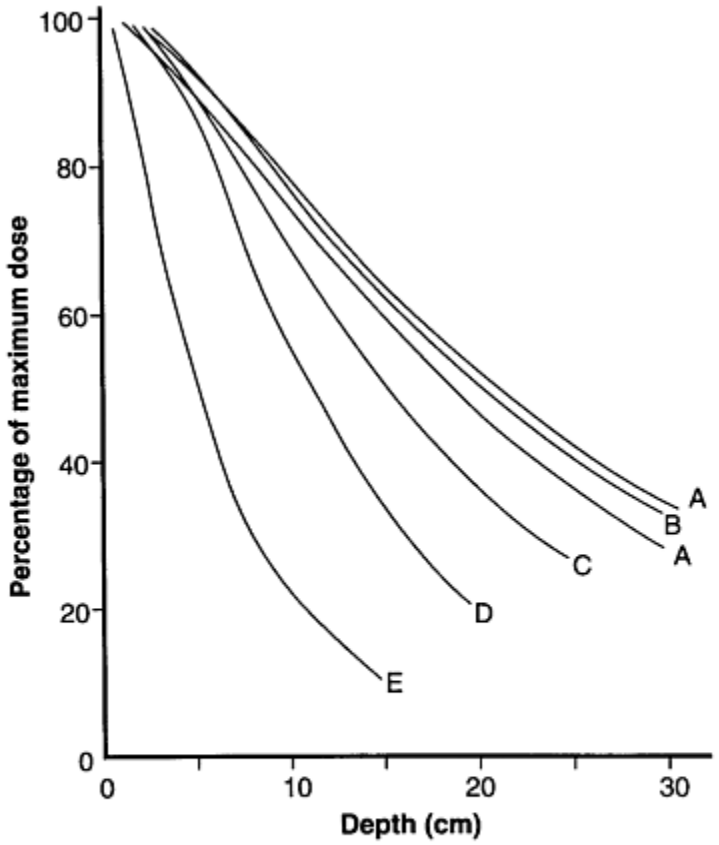
4.3.3
Exposure Geometry

For larger animals, exposure geometry is important because of the attenuation of the radiation in tissue. This is more important for lower energy photon beams and for neutrons. Typical depth dose curves are shown in Figure 4.7. This inhomogeneity of dose can be avoided if bilateral instead of unilateral irradiation is given. The influence of bilateral versus unilateral irradiation on LD_{50} for large animals is given in Table 4.4 (Mole, 1984). A higher value for unilateral than for bilateral irradiation was demonstrated, by about 0.5 Gy or by a factor of 1.2.

For low-energy X-rays there is an additional dose at bone surfaces due to the greater photoelectric effect with the high atomic number elements (e.g. calcium and phosphorus) in bone. The greater dose depends on the photon energy and on the thickness of the bone. The effect decays within a few hundred microns from the bone surface. The increase in dose can be as high as 50 per cent on the bone surface using 250 kVp X-rays (Epp *et al.,* 1959), and on average, about 20 per cent for a single layer of cells situated on the bone surface. This effect in the mouse would increase the dose to the marrow on average by about 9 per cent compared with the dose in soft tissue remote from bone, and hence this should be reflected in a slightly lower $LD_{50/30}$ unless it is taken into account. The decrease may be greater if the higher concentration (by a factor of about 2) of stem cells near bone surfaces is considered (Lord and Hendry, 1972). The latter may also apply in larger animals, but measurements are only available for human ribs where lower concentrations of GM-CFC exist close to bone surfaces as also is the case in mice (Testa *et al.,* 1985).

4.3.4
Radionuclides

Internal emitters have been used in large amounts to treat certain cancers in man. The dosimetry is complicated by tissue distribution, decay rates and clearance rates.



	Radiation type	*SSD (cm)*	*Field size (cm × cm)*
A	^{60}Co γ-rays	80	20 × 20 and 35 × 35
B	4 MV X-rays	infinite	20 × 20 or 35 × 35
C	^{137}Cs γ-rays	40	20 × 20
D	230 kVp X-rays	50	20 × 20
E	^{235}U fission neutrons	500	6 × 8

Figgure 4.7 Depth-dose curves for different radiation qualities. Data are tissue air ratios (corrected for the inverse-square law) expressed as a percentage of the maximum dose. (Redrawn form UNSCEAR, 1988)

Table 4.4 Difference in LD_{50} depending on unilateral or bilateral exposure of large animals

	Dog	Sheep	Pig	Goat
Body mass (kg)	7–13	32–57	average 62	60–95
Radiation	1 MV X-rays	1 MV X-rays	2 MV X-rays	2.5 MeV -rays
Source to animal midplane (m)	2.1	2.0	2.14	0.25
Trunk diameter (cm)	14	20–25	28	30
LD_{50}±SE (Gy) —unilateral	3.37±0.09	2.65±0.11	3.79±0.11	3.94±0.21
LD_{50}±SE (Gy) —bilateral	2.80±0.08	2.20±0.15	3.16±0.17	3.35±0.26

	Dog	Sheep	Pig	Goat

Source: Data from Mole, 1984.

Certain isotopes are taken up by specific organs, such as iodine by the thyroid and phosphorous in the marrow. A more uniform dose distribution is achieved with elements that are not taken up by specific organs. Haematological injury in man has been reported after the therapeutic use of colloidal gold, radio-iodine, radiophosphorus and radiosulphur.

Radiocolloids have been used to irradiate serosal surfaces following the accumulation of fluid and disseminated tumour cells. Mild radiation sickness and persistent leukopenia were reported following an intraperitoneal injection of 550 MBq of colloidal gold-198 (Hazra and Howell, 1978). One case of overdosage using 7400 MBq resulted in estimated average doses of 73 Gy to the liver and spleen and 4.4 Gy to the marrow, causing pancytopenia and subsequent death (Baron *et al.,* 1969; Scholman and Schwartz, 1956).

Bone marrow depression may be observed after doses of iodine-131 higher than 500 MBq, administered to treat metastatic thyroid cancer (Halnan and Pochin, 1957). The accumulated dose to the blood can be as high as 5 Gy (Silver, 1962; Benna *et al.,* 1962).

Single or multiple injections of 140–220 MBq phosphorus-32 have been given to reduce polycythemia vera, giving a cumulative dose to the marrow of about 1.4 Gy (Spiers *et al.,* 1976). The dose rate decays with a half-life of 6.7 days. Overdosage was reported with a patient who received 14.8 MBq/kg body weight and who showed a mild and reversible pancytopenia (Cobau *et al.,* 1967). Two other patients were given an overdosage of 1850–2220 MBq, delivering a cumulative dose to the marrow of about 10 Gy (Gmur *et al.,* 1983). Three weeks later there was agranulocytosis, thrombocytopenia and marrow aplasia, from which the patients later recovered.

A dose of about 10 Gy was delivered from sulphur-35 injected in sequential amounts to a total of 370–1780 MBq/kg of body weight in the treatment of chondrosarcoma and chordoma (Mayer *et al.,* 1978). Thrombocytopenia, leukopenia and later anaemia developed progressively and were dose-related.

Occupational exposure has also occasionally resulted in ingestion of large amounts of radionuclides. Two individuals developed anaemia and died after receiving doses from tritium estimated to be 3 Gy in 6 years and 10 Gy in 3 years (Minder, 1969; Seelentag, 1971). Another individual received about 1.5 Gy over 4 years and showed only a slight hypoplastic anemia.

Effects of radionuclides emitting high LET irradiations are discussed in Chapter 5.

4.4
Residual Injury

Recovery after single doses of irradiation is generally nearly complete, although there are reports of some persistent haemopoietic deficiencies, e.g. Lorimore and Wright (1990) and see Hendry and Lord (1983). The latter are reduced when the irradiation is delivered at low dose rate. For example, recovery of haemopoietic and stromal progenitor cells was almost complete by one year after 12.5 Gy delivered at 0.0005 Gy/min compared with incomplete recovery after only 6.5 Gy given at 0.7 Gy/min (Gallini *et al.,* 1988). Greater residual injury has been observed following repeated irradiations. After fifteen daily doses to a total of 7.5–10 Gy, the CFU-S population in mice recovered to about 50 per cent of control between two

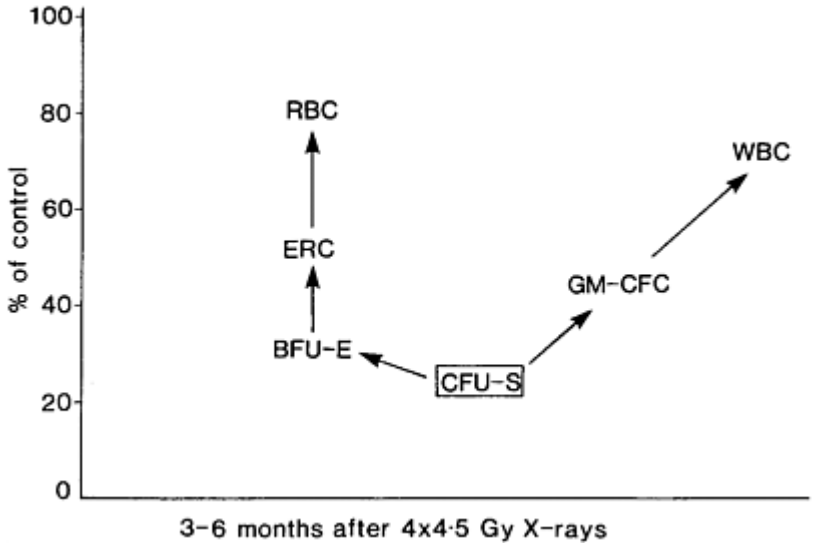


Figure 4.8 New steady-state achieved in the haemopoietic hierarchy at 3–6 months after 4×4.5 Gy X-rays

and twelve months later (Gallini *et al.,* 1989). Residual injury was also detected in the stroma, in the form of reduced numbers of CFU-S and the ability of ectopic ossicles to support haemopoiesis.

Repeated irradiation allowing time for recovery between doses is generally cumulative in producing residual injury. This was demonstrated for the erythropoietic system and for survival in rats and in dogs (Baum and Alpen, 1959; Baum *et al.,* 1961), and for the haemopoietic environment in mice (Fried *et al.,* 1976).

Following four doses of 4–4.5 Gy to mice, CFU-S numbers reach a suboptimal plateau of 10–20 per cent of control, which remains for at least one year (Hendry and Lajtha, 1972). The CFU-S continue to cycle more rapidly (Tejero *et al.,* 1988), and their self-renewal probability is reduced (Hendry *et al.,* 1983). The more-differentiated progenitor cells reach higher levels of recovery (Figure 4.8). This is probably due to extra amplification divisions in the transit cell series. Blood cell contents are near normal until past six months when the incidence of anemia increases (Hendry *et al.,* 1983). The reason for the suboptimal plateau is not known. It could relate to the long-term expression of lethal mutations (Seymour *et al.,* 1986; Hendry and West, 1995), or to the presence of stromal injury which, indeed, has been detected using long-term cultures derived from repeatedly irradiated marrow (Bierkens *et al.,* 1991), or both. Injections of unirradiated marrow serve to increase the levels somewhat, but not fully (Vos, 1972; Hendry *et al.,* 1983).

A consequence of this residual injury, which remains latent for a long time, is that further cytotoxic agents have a more dramatic effect than expected, because the population is already depleted. This has been studied in the mouse (Qi *et al.,* 1991) and has been observed in man regarding the tolerance to a second whole body irradiation (Miller *et al.,* 1958; Tubiana *et al.,* 1961a, b). The phenomenon is also observed in experimental and human systems with various chemotherapeutic agents (Testa *et al.,* 1985; Testa and Gale, 1988).

There is, in general, more residual injury following irradiation of young rather than adult animals. This might be expected both because natural growth is suppressed and because the tissue is recovering from radiation-induced damage. Such greater residual damage has been detected in neonatal mice regarding haemopoiesis (Weinberg, 1983; Yang, Hendry and Testa, unpublished data), as well as in the CFU-F content

(Yang, Hendry and Testa, unpublished data). Further, in dogs, persistent haemopoietic deficiencies were greater after irradiation of the foetus compared with the neonate (Nold *et al.,* 1987).

4.5 Conclusions

A large amount of knowledge has accrued over many decades concerning the response of bone marrow to ionising radiation. We now have some of the explanations for the sensitivity and the time course of haemopoietic responses in terms of the response of lineage-specific subpopulations of cells and their interactions. We now know how to avoid certain responses and how to modify others. The reasons for the various responses are currently being explored in terms of the genes responsible in particular cell types for damage recognition and its repair, and for the susceptibility of irradiated cells to differentiation, mitotic cell death and/or apoptosis. It is hoped that this will lead to better therapeutic strategies in cases of accidental irradiation and in the use of radiation in the treatment of disease.

References

ALI, A.M., WRIGHT, E.G. & RICHES, A.C. (1989) Local regulation of haemopoietic stem cell proliferation in mice following irradiation, *Cell and Tissue Kinetics,* **22**, 333–41.

ALLEN, J.G., EMERSON, D.M., LANDY, J.J. *et al.* (1957) The causes of death from total body irradiation. *Annals of Surgery,* **146,** 322–41.

ANDERSON, R.E. & STANDEFER, J.C. (1983) Radiation injury of the immune system. In C.S.Potten & J.H.Hendry (eds), *Cytotoxic Insult to Tissues: Effects on Cell Lineages.* Edinburgh: Churchill-Livingstone, pp. 67–104.

ANDERSON, R.E., LEFKOVITS, I. & TROUPS, G.M. (1980) Radiation-induced augmentation of the immune response, *Contemporary Topical Immunobiology,* **11**, 245–335.

BAIRD, M.C., HENDRY, J.H. & TESTA, N.G. (1990) Radiosensitivity increases with differentiation status of murine hemopoietic progenitor cells selected using enriched marrow subpopulations and recombinant growth factors, *Radiation Research,* **123**, 292–8.

BAIRD, M.C., HENDRY, J.H., DEXTER, T.M. & TESTA, N.G. (1991) The radiosensitivity of populations of murine hemopoietic colony-forming cells that respond to combinations of growth factors, *Experimental Haematology,* **19**, 282–7.

BALTSCHUKAT, K. & NOTHDURFT, W. (1990) Haematological effects of unilateral and bilateral exposures of dogs to 300 kVp x-rays, *Radiation Research,* **123**, 7–16.

BARON, J.M., VACHNIN, S., POLCYN, R. *et al.* (1969) Accidental radiogold (gold-198) liver scan overdose with fatal outcome. In *Handling of the Radiation Accidents,* IAEA Committee Report. Vienna: IAEA, pp. 399 ff.

BAUM, S.J. & ALPEN, E.L. (1959) Residual injury induced in the erythropoietic system of the rat by periodic exposures to X-irradiation, *Radiation Research,* **11**, 844–60.

BAUM, S.J., DAVIS, A.K. & ALPEN, E.L. (1961) Effect of repeated roentgen or neutron irradiation on the haematopoietic system, *Radiation Research,* **15**, 97–108.

BAVERSTOCK, K.F., PAPWORTH, D.G. & TOWNSEND, K.M.S. (1985) Man's sensitivity to bone marrow failure following whole body exposure to low LET ionising radiation: inferences to be drawn from animal experiments, *Int. J. Radiation Biology,* **47**, 397–411.

BENNA, R.A., CICALE, N.R., SORENBERG, M. *et al.* (1962) The relation of radioiodine dosimetry to results and complications in the treatment of metastatic thyroid cancer, *Am. J. Roentgenology,* **87**, 171–82.

BIERKENS, J.G., HENDRY, J.H. & TESTA, N.G. (1989) The radiation response and recovery of bone marrow stroma with particular reference to long-term bone marrow cultures, *Eur. J. Haematology,* **43**, 95–107.

BIERKENS, J.G., HENDRY, J.H. & TESTA, N.G. (1991) Recovery of the proliferative and functional integrity of mouse bone marrow in long-term cultures established after whole-body irradiation at different doses and dose rates, *Experimental Haematology,* **19**, 81–6.

BOND, V.P., FLIEDNER, T.M. & ARCHAMBEAU, J.O. (1965) *Mammalian Radiation Lethality: A Disturbance in Cellular Kinetics.* New York: Academic Press.

BROXMEYER, H.E., GALBRAITH, P.R. & BAKER, F.L. (1976) Relationship of colony-stimulating activity to apparent kill of human colony-forming cells by irradiation and hydroxyurea, *Blood,* **47**, 403–11.

CARSTEN, A.L. (1970) Active bone marrow distribution in the monkey, *Life Sciences,* **9**, 169–74.

CARSTEN, A.L. & CRONKITE, E.P. (1971) Comparison of autologous marrow injection to shielding in lethal irradiation of the mouse, *Proceedings of the Society of Experimental Biology and Medicine,* **137**, 948–51.

CAVAZZANO-CALVO, M., LE DEIST, F., DE SAINT BASILE, G., PAPADOPOULO, D., DE VILLARTY, J.P. & FISCHER, A. (1993) Increased radiosensitivity, of granulocyte macrophage colony-forming units and skin fibroblasts in human autosomal recessive severe combined immunodeficiency, *J. Clinical Investigation,* **91**, 1214–18.

CHANG, C., BIEDERMAN, K.A., MEZZINA, M. & BROWN, J.M. (1993) Characterisation of the DNA double strand break repair defect in scid mice, *Cancer Research,* **53**, 1244–18.

COBAU, C.D., SIMONS, C.S. & MEYERS, M.C. (1967) Accidental overdosage with radiophosphorous: therapy by induced phosphate diuresis, *Am. J. Medical Science,* **85**, 451–63.

COGGLE, J.E. (1980) Absence of late radiation effects on bone marrow stem cells, *Int. J. Radiation Biology,* **38**, 589–95.

COLLINS, M.K.L., MARVEL, J., MALDE, P. & LOPEZ-RIVAS, A. (1992) Interleukin 3 protects murine bone marrow cells from apoptosis induced by DNA damaging agents, *J. Experimental Medicine,* **176**, 1043–51.

CROIZAT, H., FRINDEL, E. & TUBIANA, M. (1970) Proliferative activity of the stem cells in the bone marrow of mice after single and multiple irradiation (total or body exposure), *Int. J. Radiation Biology,* **18**, 347–58.

CROIZAT, H., FRINDEL, E. & TUBIANA, M. (1976) Abscopal effect of irradiation on haemopoietic stem cells of shielded bone marrow: role of migration, *Int. J. Radiation Biology,* **30**, 347–58.

CROIZAT, H., FRINDEL, E. & TUBIANA, M. (1980) The effect of partial body irradiation on haemopoietic stem cell migration, *Cell Kinetics,* **13**, 319–25.

CRONKITE, E.P., BOND, V.P., CARSTEN, A.L., INOUE, I., MILLER, M.E. & BULLIS, J.E. (1987) Effects of low-level radiation upon haematopoietic stem cells: implications for leukaemogenesis, *Radiation and Environment Biophysics,* **26**, 103–14.

CRONKITE, E.P., INOUE, T., HIRABYASHI, Y. & BULLIS, J. (1993) Are stem cells exposed to ionising radiation *in vivo* as effective as non-irradiated transfused stem cells in restoring haematopoiesis? *Experimental Haematology,* **21**, 823–5.

CROSFILL, M.L., LINDOP, P.J. & ROTBLAT, J. (1959) Variation of sensitivity to ionising radiation with age, *Nature,* **183**, 1729–30.

EPP, E.R., WOODARD, H.Q. & WEISS, H. (1959) Energy absorption by the bone marrow of the mouse receiving whole-body irradiation with 250 kV x-rays or cobalt-60 gamma rays, *Radiation Research,* **11**, 184–98.

FITZGERALD, T.J., MCKENNA, M., ROTHSTEIN, L., DOUGHERTY, C., KASE, K. & GREENBERGER, J.S. (1986) Radiosensitivity of human bone marrow granulocyte-macrophage progenitor cells and stromal colony-forming cells: effect of dose-rate, *Radiation Research,* **107**, 205–15.

FITZGERALD, T.J., SANTUCCI, M.A., HARIGAYA, K. *et al.* (1988) Radiosensitivity of permanent human stroma cell lines: effect of dose rate, *Int. J. Radiation Oncology, Biology and Physics,* **15**, 1153–9.

FOWLER, J.F. (1983) Dose response curves for organ function or cell survival, *Br. J. Radiology,* **56**, 497–500.

FRED, S.S. & SMITH, W.W. (1967) Radiation sensitivity and proliferative recovery of hemopoietic stem cells in weanling as compared to adult mice, *Radiation Research,* **32**, 314–26.

FRIED, W., CHAMBERLAIN, W., KEDO, A. & BARONE, J. (1976) Effects of radiation on hemopoietic stroma, *Experimental Hematology,* **4**, 310–14.

GALLINI, R., HENDRY, J.H., MOLINEUX, G. & TESTA, N.G. (1988) The effect of low dose-rate on recovery of hemopoietic and stromal progenitor cells in mouse bone marrow, *Radiation Research,* **115**, 481–7.

GALLINI, R., HENDRY, J.H., MOLINEUX, G. & TESTA, N.G. (1989) Residual haemopoietic damage in the mouse after fractionated gamma-irradiation, down to 0.1 Gy per fraction, *Radiotherapy and Oncology,* **14**, 43–8.

GERBER, G.B. & MAES, J. (1981) The *in vitro* radiosensitivity of haemopoietic stem cells from control and pre-irradiated infant mice, *Radiation and Environmental Biophysics,* **19**, 173–9.

GIDALI, J. & LAJTHA, L.G. (1972) Regulation of haematopoietic stem cell turnover in partially irradiated mice, *Cell and Tissue Kinetics,* **5**, 147–57.

GMUR, J., BISCHOF, B., CONINX, S. *et al.* (1983) Spontaneous haematologic recovery from bone marrow aplasia after accidental tenfold overdosage with radiophosphorus, *Blood,* **61**, 746–50.

GREENBERG, M.L., ATKINS, H.L. & SCHIFFER, L.M. (1966) Erythropoietic and reticuloendothelial function in bone marrow in dogs, *Science,* **152**, 526–8.

GREENBERGER, J.S., SAKAKEENY, M.A. *et al.* (1992) Expression of M-CSF and its receptor (C-FMS) during factor-independent cell-line evolution from haematopoietic progenitor cells co-cultivated with gamma-irradiated marrow stromal cell lines, *Leukaemia,* **6***,* 626–33.

HALNAN, K.E. & POCHIN, E.E. (1957) Symposium on the thyroid. Part II: Aspects of the radioiodine treatment of thyroid carcinoma, *Metabolism,* **6**, 49–62.

HARIGAYA, K. & HAMDA, H. (1985) Generation of functional clonal cell lines from human marrow stroma, *Proc. National Academy of Science, USA,* **82**, 3477–80.

HART, R.M., KIMLER, B.F., EVANS, R.G. & PARK, C.H. (1987) Radiotherapeutic management of medulloblastoma in a paediatric patient with ataxia telangiectasia, *Int. J. Radiation Oncology, Biology and Physics ,* **13**, 1237–40.

HAZRA, T.A. & HOWELL, R. (1978) Uses of beta emitters for intra-cavitary therapy. In R. P.Spencer, (ed.), *Therapy in Nuclear Medicine.* New York: Grune and Stratton., pp. 307–12.

HENDRY, J.H. (1988) Survival of cells in mammalian tissues after low doses of irradiation: a short review, *Int. J. Radiation Biology,* **53**, 89–94.

HENDRY, J.H. & JIANG, T-N. (1994) Differential radiosensitising effect of the scid mutation among tissues, studied using high and low dose rates: implications for prognostic indicators in radiotherapy, *Radiotherapy and Oncology,* **33**, 209–16.

HENDRY, J.H. & LAJTHA, L.G. (1972) The response of haematopoietic colony-forming units to repeated doses of x-rays, *Radiation Research,* **52**, 309–15.

(1975) Response of mouse bone marrow to low dose rates, split acute doses, and multiple daily fractions, *Proceedings of 6th Gray Conference, London,* pp. 308–12.

HENDRY, J.H. & LORD, B.I. (1983) The analysis of the early and late response to cytotoxic insults in the haemopoietic cell hierarchy. In C.S.Potten & J.H.Hendry (eds), *Cytotoxic Insult to Tissue: Effects on Cell Lineages.* Edinburgh: Churchill-Livingstone, pp. 1–66.

HENDRY, J.H. & ROBERTS, S.A. (1990) Analysis of dose-incidence relationships for marrow failure in different species, in terms of radiosensitivity of tissue-rescuing units, *Radiation Research,* **122**, 155–60.

HENDRY, J.H. & WEST, C.M.L. (1995) The implications of delayed reproduction cell death (lethal mutations/ genomic instability) for the interpretation of tissue responses, *Int. J. Radiation Biology* (in press).

HENDRY, J.H., XU, C.X. & TESTA, N.G. (1983) A cellular analysis of residual haemopoietic deficiencies in mice after four repeated doses of 4.5 gray, *Int. J. Radiation Oncology, Biology and Physics,* **9**, 1641–6.

HENDRY J.H., WANG, S.B. & TESTA, N.G. (1985) Greater sparing of stromal progenitor cells than of haemopoietic stem cells in -irradiated mouse marrow using low dose-rates, *Biomedicine Express,* **38**, 356–8.

HENDRY, J.H., ROBERTS, S.A. & LORD, B.I. (1994) Is haematopoiesis or its regulatory microenvironment the rate-limiting factor for $LD_{50/30}$? *Experimental Hematology,* **22**, 2–4.

IMAI, Y. & NAKAO, I. (1987) *In vivo* radiosensitivity and recovery pattern of the haematopoietic precursor cells and stem cells in mouse bone marrow, *Experimental Hematology,* **15**, 890–5.

JOB, G., PFREUNDSCHUH, M., BAUER, M. *et al. (*1984) The influence of radiation therapy on T-lymphocyte subpopulation defined by monoclonal antibodies, *Int. J. Radiation Oncology Biology Physics,* **10**, 2077–81.

JONES, T.D., MORRIS, M.D., YOUNG, R.W. & KEHLET, R.A. (1993) A cell-kinetics model for radiation-induced myelopoiesis, *Experimental Hematology,* **21**, 816–22.

KIRCHGESSNER, C.U., TOSTO, L.M., BIEDERMANN, K.A. *et al.* (1993) Complementation of the radiosensitive phenotype in severe combined immunodeficient mice by human chromosome 8. *Cancer Research,* **53**, 6011–16.

KNOSPE, W.H., BLOM, J. & CROSBY, W.H. (1966) Local irradiation of bone marrow. I: Dose-dependent, long-term changes in the rat with particular emphasis upon vascular and stromal effect, *Blood,* **22**, 298–315.

KOLESNIKOVA, A.I., KONOPLYANNIKOV, A.G. & HENDRY, J.H. (1995) Differential sensitivity of two predominant stromal progenitor cell subpopulations in bone marrow to single and fractionated radiation doses, *Radiation Research* (in press).

LAJTHA, L.G., GILBERT, C.W. & GUZMAN, E.E. (1971) Kinetics of haemopoietic colony growth, *Br. J. Haematology,* **20,** 343–54.

LI, Y., JING, M., YANG, S. *et al.* (1985) Protective effect of partial shielding on 600 rad -irradiated dogs, *Chinese Journal of Radiology, Medicine and Protection,* **4**, 258–61.

LORD, B.E. & HENDRY, J.H. (1972) The distribution of haemopoietic colony-forming units in the mouse femur and its modification by x-rays, *British J. Radiology,* **45**, 110–15.

LORD, B.I. (1964) The effects of continuous irradiation on cell proliferation in rat bone marrow, *Br. J. Haematology,* **10,** 496–507.

(1965) Cellular proliferation in normal and continuously irradiated rat bone marrow studied by repeated labelling with tritiated thymidine, *Br. J. Haematology,* **11,** 130–43.

LORD, B.I. & HENDRY, J.H. (1972) The distribution of haemopoietic colony-forming units in the mouse femur and its modification by x-rays, *Br. J. Radiology,* **45**, 110–15.

LORIMORE, S.A. & WRIGHT, E.G. (1990) Late effect of X-irradiation in haemopoietic stem cells in CBA/H mice, *Int. J. Radiation Biology,* **57**, 385–93.

MAYER, K.M., PENTLOW, K.S., MARCOVE, R.C. *et al.* (1978) Sulphur-35 therapy for chondrosarcoma and chordoma . In R.P.Spencer (ed.) *Therapy in Nuclear Medicine,* New York: Grune & Stratton, pp. 185–92.

MCCONVILLE, C.M., BYRD, P.J., AMBROSE, H.J. & TAYLOR, A.M.R. (1994) Genetic and physical mapping of the ataxia-telangiectasia locus on chromosome 11q22– 23, *Int. J. Radiation Biology,* **66,** 545–56.

MEIJNE, E.I.M., VAN DER WINDEN-VAN GROENEWEGEN, A.J.M., PLOEMACHER, R.E., VOS, O., DAVIDS, J.A.G. & HUISKAMP, R. (1991) The effects of X-irradiation on haematopoietic stem cell compartments in the mouse, *Experimental Hematology,* **19,** 617–723.

MILLAR, J.L., BLACKETT, N.M. & HUDSPITH, B.N. (1978) Enhanced post-irradiation recovery of the haemopoietic system in animals pretreated with a variety of cybotoxic agents, *Cell & Tissue Kinetics,* **11,** 543–53.

MILLER, L.S., FLETCHER, G.H. & GERSTNER, H.B., (1958) Radiobiologic observations on cancer patients treated with whole-body irradiation, *Radiation Research,* **8**, 150–65.

MINDER, W. (1969) Interne kontamination mit tritium. *Strahlentherapie,* **137**, 700–4.

MIYAKAWA, T., ADACHI, T., ETO, H. *et al.* (1970) The bone marrow dose in teleradiotherapy in Japan, *Nippon Acta Radiologia,* **30,** 368–84.

MOLE, R.H. (1957) Quantitative observations on recovery from whole-body irradiation in mice. II: Recovery during and after daily irradiation, *Br. J. Radiology,* **30,** 40–6.

(1984) The LD_{50} for uniform low LET irradiation of man, *Br. J. Radiology,* **57**, 355–69.

MOLINEUX, G., TESTA, N.G., HENDRY, J.H. & SCHOFIELD, R. (1987) The radiation sensitivity of the haemopoietic microenvironment: effect of dose rate on ectopic ossicle formation. *Radiotherapy and Oncology,* **10,** 157–61.

NAPARSTEK, E., DONNELLY, T., KASE, K. & GREENBERGER, J.S. (1985) Biological effects of *in vitro* X-irradiation of murine long term bone marrow cultures on the production of granulocyte-macrophage colony-stimulaitng factors, *Experimental Hematology,* **13,** 701–8.

NAPARSTEK, E., PIERCE, J., METCALF, D. *et al.* (1986) Induction of growth alternations in factor-dependent haematopoietic progenitor cell lines by co-cultivation with irradiated bone marrow stromal cell lines, *Blood,* **67**, 1395–403.

NOLD, J.B., MILLER, G.K. & BENJAMIN, S.A. (1987) Prenatal and neonatal irradiation in dogs: haematologic and haematopoietic responses, *Radiation Research,* **112,** 490–9.

NOTHDURFT, W. (1991) Bone marrow. In E.Scherer, C.Streffer & K-R.Trott (eds), *Medical Radiology, Radiopathology of Organs and Tissues,* Heidelberg: Springer-Verlag, pp. 113–69.

NOTHDURFT, W., STEINBACH, K.H. & FLIEDNER, T.M. (1983) *In vitro* studies on the sensitivity of canine granulopoietic progenitor cells (GM-CFC) to ionizing radiation: differences between steady state GM-CFC from blood and bone marrow, *Int. J. Radiation Biology,* **43**, 133–40.

PANTEL, K. & NAKEFF, A. (1989) Cyclophosphamide-induced enhancement of stem cell recovery from whole-body irradiation: is radiation dose-dependent?, *Experimental Hematology,* **17**, 847–50.

PIERSMA, A.H., PLOEMACHER, R.E. & BROCKBANK, K.G.M. (1983) Transplantation of bone marrow fibroblastoid cells in mice via the intravenous route, *Br. J. Haematology,* **54**, 285–90.

PLOEMACHER, R.E., VAN OS, R., VAN BEURDEN, C.A.J. & DOWN, J.D. (1992) Murine haemopoietic stem cells with long term engraftment and marrow repopulating ability are less radiosensitive to gamma radiation than are spleen colony forming cells, *Int. J. Radiation Biology,* **61**, 489–99.

PORTEUS, D.D. & LAJTHA, L.G. (1966) On stem-cell recovery after irradiation, *Br. J. Haematology,* **12**, 177–88.

QI, D.Y. HENDRY, J.H. & TESTA, N.G. (1991) Interactions in recovery and in residual injury from sequential treatments of mouse haemopoietic and stromal marrow cell populations, using X-rays, cyclophosphamide and busulphan, *Radiotherapy and Oncology,* **20**, 46–52.

REISSMAN, K.R. & UDUPA, K.B. (1972) Effect of erythropoietin on proliferation of erythropoietic-responsive cells, *Cell and Tissue Kinetics,* **5**, 481.

ROTSTEIN, S., BLOMGREN, H., PETRINI, B. *et al.* (1985) Long-term effects on the immune system following local irradiation therapy for breast cancer. I: Cellular composition of the peripheral blood lymphocyte population, *Int. J. Radiation Oncology, Biology and Physics,* **11**, 921–25.

RUIFROK, A.C.C. & THAMES, H.D. (1994) Comparing cell-survival estimated from *in vivo* and *in vitro* data: beware of *in vivo* heterogeneity, *Experimental Hematology,* **22**, 535.

SCHOFIELD, R. (1978) The relationship between the spleen colony-forming cell and the haemopoietic stem cell: a hypothesis, *Blood Cells,* **4**, 7–25.

SCHOLMAN, H.H. & SCHWARTZ, S.O. (1956) Aplastic anemia secondary to intraveol therapy with radiogold. *J. Am. Med. Assoc.* **160**, 646.

SCHWARTZ, G.N., VIGNEULLE, R.M. & MACVITTIE, T.J. (1986) Survival of erythroid burst-forming units and erythroid colony-forming units in canine bone marrow cells exposed *in vitro* to 1 MeV neutron radiation or x-rays, *Radiation Research,* **108**, 336–47.

SEELENTAG, W. (1971) Two cases of tritium fatality. In A.Moghissi & M.W.Carter (eds), *Tritium. Proceedings of a Symposium, Las Vegas, 1971.* Messenger Graphics.

SEYMOUR, C.B., MOTHERSILL, C. & ALPER, T. (1986) High yields of lethal mutations in somatic mammalian cells that survive ionising radiation, *Int. J. Radiation Biology,* **50**, 167–79.

SHOUSE, S.S., WARREN, S.L. & WHIPPLE, G.H. (1931) Aplasia of the marrow and fatal intoxication in dogs produced by Roentgen radiation of all bones, *J. Experimental Medicine,* **53**, 421.

SILVER, S. (1962) *Radioactive Isotopes in Medicine and Biology,* vol. 2 (2nd edn). Philadelphia: Lea & Febiger.

SIMINOVITCH, L., TILL, J.E. & MCCULLOCH, E.A. (1965) Radiation response of hemopoietic colony-forming cells derived from different sources, *Radiation Research,* **24**, 482–93.

SPIERS, F.W., BEDDOE, A.H., KING, S.D. *et al.* (1976) The absorbed dose to bone marrow in the treatment of polycythaemia by ^{32}P, *Br. J. Radiology,* **49**, 133–40.

STEWART, C.C., STEPHENSON, A.P. & HABBERSETT, R.C. (1988) The effect of low-dose irradiation on unstimulated and PHA-stimulated human lymphocyte subsets, *Int. J. Radiation Biology,* **53**, 77–87.

TAKETA, S.T., CARSTEN, A.L., COHN, S.H., *et al.* (1970) Active bone marrow distribution in the monkey, *Life Sciences,* **9**, 169–74.

TARBUTT, R.G. (1969) Cell population kinetics of the erythroid system in the rat: the response to protracted anaemia and to continuous -irradiation, *Br. J. Haematology,* **16**, 9.

TARBUTT, R.G. & BLACKETT, N.M. (1968) Cell population kinetics of the recognisable erythroid cells in the rat, *Cell and Tissue Kinetics,* **1**, 65.

TEJERO, C., LORD, B.I., MASON, T.M. & HENDRY, J.H. (1988) Long-term haemopoietic injury in mice after repeated irradiation: precursor-cell cycling and its regulation, *Eur. J. Haematology,* 278–84.

TESTA, N.G. & GALE, R.P. (1988), (eds), Haemopoietics: long-term effects of chemotherapy and radiation. New York and Basel: Marcel Dekker, p. 413.

TESTA, N.G., HENDRY, J.H. & LAJTHA, L.G. (1974) The response of mouse haemopoietic colony forming units to repeated whole body X-irradiation, *Biomedicine,* **21,** 431–4.

TESTA, N.G., HENDRY, J.H. & MOLINEUX, G. (1985) Long-term bone marrow damage in experimental systems and in patients after radiation; or chemotherapy, *Anticancer Research,* **5**, 101–10.

THAMES, H.D. & HENDRY, J.H. (1987) *Fractionation in Radiotherapy.* London: Taylor & Francis, pp. 1–289.

TUBIANA, M., LALANNE, C.M. & SURMONT, J. (1961a) Whole-body irradiation for renal homotransplanation. In *Diagnosis and Treatment of Acute Radiation Injury,* Geneva: WHO, pp. 237–63.

TUBIANA, M., LALANNE, C.M. & SURMONT, J. (1961b) Total body irradiation for organ transplantation, *Proc. Royal Society of Medicine,* **54,** 31–8.

UCKUN, F.M., GILLIS, S., SOUZA, L. & SONG, C.W. (1989) Effects of recombinant growth factors on radiation survival of human bone marrow progenitor cells, *Int. J. Radiation Oncology, Biology and Physics,* **16,** 415–35.

UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation) (1988) *Early Effects in Man of High Doses of Radiation.* Report to the General Assembly. Appendix: Acute radiation effects in victims of the Chernobyl nuclear power plant accident, annex G of Sources, effects and risks of ionising radiation. New York: United Nations, pp. 545–647.

VAN DYK, D., ANDER, H. & POLLYCOVE, M. (1964) The effect of erythropoietic stimulation on marrow distribution in man, rabbit and rat as shown by Fe-59 and Fe-52, *Blood,* **24**, 356–71.

VOS, O. (1972) Stem cell renewal in spleen and bone marrow of mice after repeated total-body irradiation, *Int. J. Radiation Biology,* **22**, 41–50.

VRIESENDORP, H.M. & VAN BEKKUM, D.W. (1980) Role of total body irradiation in conditioning for bone marrow transplantation . In S.Thierfelder, H.Kobl & H.Y.Kolb (eds), *Immunobiology of Bone Marrow Transplanatation,* Heidelberg: Springer-Verlag, pp. 349–64.

(1984) Susceptibility to total body irradiation. In J.J.Broerse & T.J.MacVittie (eds), *Response of Different Species to Total Body Irradiation,* Amsterdam: Martinus Nijhoff, pp. 43–57.

WALD, N. (1971) Haematological parameters after acute radiation injury. In *Manual on Radiation Haematology*. Vienna: IAEA, pp. 253–64.

WASSERMAN, J. (1986) Immunological indicators. In A.Kaul, *et al.* (eds) *Biological indicators for radiation dose assessment.* Munich: MMV Medizin Verlag, pp. 85–103.

WEINBERG, S.R. (1983) Effects of prenatal irradiation of fetal, neonate, and young adult murine haemopoiesis, *Int. J. Radiation Oncology, Biology and Physics,* **9**, 1825–31.

WOODARD, H.Q. (1984) The relation of weight of haemopoietic marrow to body weight, *Br. J. Radiology,* **57**, 903–7.

WU, C. & LAJTHA, L.G. (1975) Haemopoietic stem cell kinetics during continuous irradiation, *Int. J. Radiation Biology,* **27**, 41–50.

WURM, R.E., BURNET, N.G., DUGGAL, N., YARNOLD, J. & PEACOCK, J.H. (1994) Cellular radiosensitivity and DNA damage in primary human fibroblasts, *Int. J. Radiation Oncology, Biology and Physics,* **30,** 625–33.

XU, C.X., HENDRY, J.H. & TESTA, N.G. (1983b) The response of stromal progenitor cells in mouse marrow to graded repeated doses of x-rays or neutrons, *Radiation Research,* **96,** 82–9.

XU, C.X., HENDRY, J.H., TESTA, N.G. & ALLEN, T.D. (1983a) Stromal colonies from mouse marrow: characterisation of cell types, optimisation of plating efficiency and its effect on radiosensitivity, *J. Cell Science,* **61,** 453–66.

5
Effects of High LET Irradiation on Haemopoiesis

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5
Effects of High LET Irradiation on Haemopoiesis

5.1
Introduction

Many of the pioneers of radiation research and development died from malignant diseases due to radiation exposure. Similarly, it was recognised that the use of atomic bombs would cause malignancies. More recently, there has been considerable concern over the consequences of nuclear accidents, culminating in the 1986 Chernobyl disaster, and the potential for any similar occurrences in the future. Much has been made of the apparent leukaemia clusters around Sellafield and Dounreay (Gardner and Winter, 1984; Darby and Doll, 1987; Forman *et al.,* 1987) although another report (Hill and Laplanche, 1990) found no comparable clusters in France. The importance of the skeleton as the major site of haemopoiesis inevitably, therefore, led research to focus on the bone-seeking radionuclides. For the most part, these are the -particle-emitting radionuclides with high LET and, furthermore, associated most closely with nuclear energy industry.

The incidence of leukaemias (and osteosarcomas—related to the osteogenic tissue in the marrow stroma, both of which are important to the proper functioning of haemopoietic tissue—see Chapter 2) are discussed in detail in later chapters. In short, human surveys have shown that leukaemia is less of a problem than osteosarcoma (Spiers *et al.,* 1983; Spiers, 1988; Baverstock and Papworth, 1985) though a continuing analysis of German ankylosing spondylitis patients treated with radium-224 is now showing a highly significant cohort of myeloid leukaemias (Wick *et al.,* 1995). Alpha-induced osteosarcomas are also evident in animals (Humphreys *et al.,* 1985, 1987, 1993; Sikov *et al.,* 1982; Lloyd *et al.,* 1975; Svoboda *et al.,* 1977, 1980; Moskalev *et al.,* 1989; Svoboda and Bubenikova, 1990; Schoeters *et al.,* 1991). On the other hand, it is clear that both radium and plutonium can also cause myeloid leukaemia (Humphreys *et al.,* 1985, 1987; Loutit and Carr, 1978; Svoboda and Bubenikova, 1990) and it is argued that potential leukaemogenesis may sometimes be masked by the earlier onset of osteosarcoma, thus explaining the unexpectedly low incidence of leukaemia in some of the human studies. For example, it was shown that mice are at greater risk from myeloid leukaemia than osteosarcoma in the region of administered ^{224}Ra dose below that which causes a maximum yield of osteosarcoma. Nevertheless, for both tumour types, direct relationships were shown to exist between the amount of ^{224}Ra administered and the incidence of tumours (Humphreys *et al.,* 1993).

Damage to haemopoiesis and/or its regulatory stroma clearly underlie any leukaemic or bone tumour development and for this reason, considerable effort has gone into investigating the effects of bone-seeking radionuclides on these tissues. Chronic myeloid leukaemia, the type most frequently induced in the murine

studies quoted above, is characterised as of haemopoietic stem cell origin. Much work has therefore been concentrated on induced changes in the progenitor cell populations. These studies are discussed below and it will be shown further that changes in the stem cell's regulatory microenvironment are not restricted to potential osteogenic damage but are also inextricably connected to perceived changes in haemopoiesis.

After some years of study on adult animals, the revelation that leukaemias in the Sellafield cluster were arising in young people caused the emphasis of research to switch to developing haemopoiesis. Here a different set of circumstances pertains. Bone marrow haemopoiesis is the final phase of blood production. During development, foetal and neonatal liver is the predominant haemopoietic organ (see Chapter 2) and it was known that, in addition to its bone-seeking properties, ingested plutonium also concentrates in the liver. This chapter therefore outlines the distribution of a radionuclide administered at different stages of embryonic, foetal and neonatal development and its ensuing effects on haemopoiesis. From these data we then attempt to ascribe some order to the relative radiation sensitivities of developing haemopoiesis to an incorporated -particle emitter.

An extension of this theme leads to a consideration of preconceptual parental contamination. Such studies were given credence by the report—recognisable as 'the Gardner hypothesis' (Gardner *et al.,* 1990)—implicating paternal occupational exposure to ionising radiation as a contributory factor in the Sellafield leukaemias. While a subsequent commentary (Doll *et al.,* 1994) concluded 'that the association between paternal irradiation and leukaemia is largely or wholly a chance finding', it will be seen that some haematological changes may be relevant.

While one might normally think of radiation-induced leukaemogenesis in terms of a potential -particle irradiation effect, it is clear that low LET radiation is also leukaemogenic (see Chapter 10). Mole (1986) induced myeloid leukaemias in CBA/H mice much more efficiently with 3 Gy X-rays than subsequent efforts with -emitters managed, i.e. about 4–5 per cent. It is clear, therefore, that one should be able to ascribe a relative biological efficiency (RBE) to the -particle damage. In experiments described below it will be shown that haemopoiesis can be exquisitely sensitive to -particle irradiation.

The interrelationship of haemopoietic tissue with its regulatory stromal microenvironment has been referred to above and stressed strongly in Chapter 2. It will be seen in our study of -particle irradiation how damage to the microenvironment has a large bearing on the performance of haemopoietic tissues. Hence, for a complete understanding of the nature, and ultimately the mechanisms, of high LET irradiation effects, it is necessary to define separately the effects in these two components. From such experiments it will become clear that, in most cases, damage to the stroma may be the dominant effect.

All these studies are set against the picture of normal haemopoiesis presented in Chapter 2. Techniques there were used liberally in these investigations and, to complete the cycle of this section, the implications of these experimental investigations with respect to leukaemogenesis can finally be entertained.

5.2
High LET Dose Distribution and its Relationship to Bone Marrow Cell Distribution

The first property to recognise about bone-seeking -particle-emitting radionuclides is that when in the bone they do not give a uniform radiation dose to the marrow contents. The radionuclides commonly used in experimental work, namely americium-241, radium-224 and 226 and plutonium-239, once associated with the bone, have a very short penetration range into the marrow space—approximately 40 μm maximum. In a mouse femur of 900 μm diameter this means that only about 17 per cent of the marrow cells—those nearest the bone—are irradiated. Further, these radionuclides are classified as bone surface-seekers (americium and plutonium) or bone volume-seekers (radium—although the short 3.7 days half-life of ^{224}Ra means that, in

practice, this should also be considered as a surface-seeker). Radium-226, however, becomes incorporated in the body of the bone and much of its -particle energy is spent getting through the bone, so that only about 4.5 per cent of the marrow nearest the bone is irradiated (see also Chapter 3).

Dosimetric considerations are also complicated by bone turnover. During skeletal growth and turnover, surface-seekers such as plutonium, become buried beneath the bone surface and then become more akin to a volume-seeker. The rate of apposition on growing human bone surfaces is about 1 μm/day (Frost, 1983). This means that, in about four weeks, surface plutonium would be buried to a depth which virtually prevents any penetration to the marrow. By contrast, in the adult human, 80 per cent of the bone surface may remain unchanged for a year (Parfitt, 1983). Different dosimetric models are therefore required for young and adult situations.

A final consideration must be the question of bone resorption by osteoclasts. This results in some of the surface radionuclides, e.g. ^{239}Pu, being transferred (Jee, 1972) where, in rats, the biological retention half-life of ^{239}Pu is three days (Priest and Giannola, 1980) and, therefore, of little dosimetric significance. Plutonium is released in particulate form which is then digested by macrophages where it is retained for long periods (Jee, 1972; Haines and Priest, 1984). As the macrophages are located in the bone marrow they may be of dosimetric importance in the longer term.

Clearly, extreme care must be exercised in radiation dose estimations from radionuclides incorporated in the bones. To this must be added the complications of non-uniform cell distributions in the marrow spaces as described in Chapter 2 (see also Lord, 1992). There it was demonstrated that the most primitive cells—those most likely associated with leukaemogenesis—are located close to the centre of the bone cavities and well outside the range of bone-associated -particle irradiation. Although some radiation may be delivered to these cells in the long term from bone marrow macrophages, the amount is relatively small—about 3 per cent of the skeletal plutonium is present in bone marrow macrophages (McInroy and Kathren, 1990) and perhaps one-third of these rest in the primitive cell locations. The biggest source of radiation remains the bone structures and probably, therefore, the most important factors are changes induced in the early haemopoietic progenitor cell populations as a result of damage to the more mature cell compartments or regulatory microenvironments which disrupt the normal dynamics of blood cell production. It is natural, therefore, that studies on the effects of administered radionuclides on haemopoiesis in different skeletal locations should always be included and that our starting point for discussion is of effects in adult animals.

5.2.1
Bone Marrow Cell Populations

Although described fully in Chapter 2, it is worth being reminded of the hierarchical structure of haemopoietic tissue in the bone marrow. Figure 5.1 illustrates the progression of cells from the small population of primitive multipotent cells with a high probability of self-renewal for the maintenance of the tissue—through the progenitors committed to specific lineage development to the bulk populations of the maturing, morphologically defined cells of those different lineages. Cell production is regulated by growth stimulatory and inhibitory factors which, in the main appear to be produced in the microenvironmental stromal cells. A major component of this stroma is the fibroblastoid colony-forming unit, CFU-F (Friedenstein *et al.,* 1967) which together with assay of the stroma's capacity to generate bone with a microenvironment hosting immigrant haemopoiesis (Friedenstein *et al.,* 1968) gives quantitative assessment of the quality and performance of the haemopoietic microenvironment. Haemopoiesis itself is generally assessed by the CFU-S technique (Till and McCulloch, 1961) measuring the multipotent population, and the *in vitro* colony-

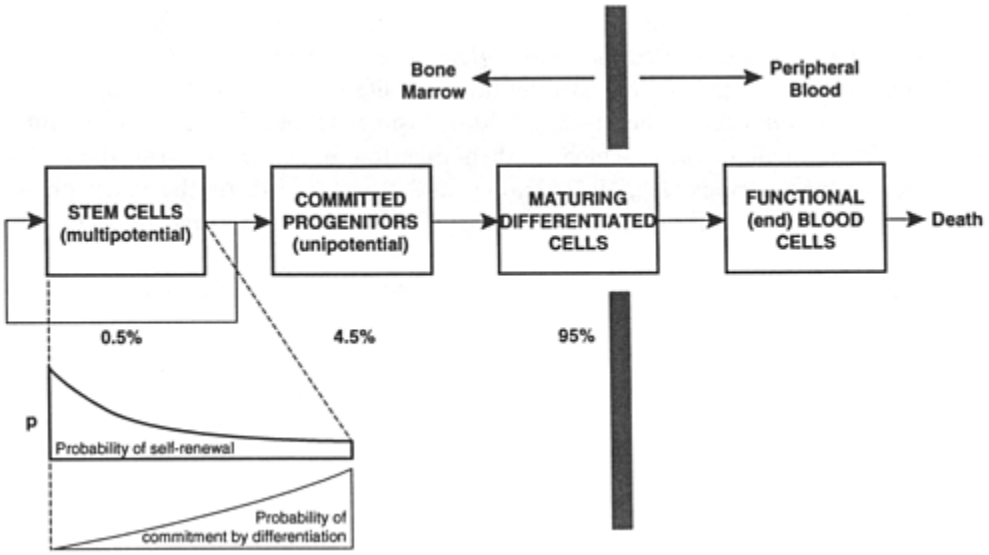

Figure 5.1 Haemopoietic cell hierarchy showing cell compartments in the bone marrow and peripheral blood. The numbers represent the approximate size—in cell number—of each compartment

forming cell (often taken as the granulocyte/macrophage colony forming cell, GM-CFC) technique (Heyworth and Spooncer, 1993) measuring the committed progenitor cells.

5.2.2
Radionuclides in Adult Bone Marrow

5.2.2.1
Haemopoietic tissue

Direct studies on the effects of high LET, -particle irradiation from incorporated bone-seeking radionuclides have been concentrated mainly in three centres, namely the Centre of Radiation Hygiene in Prague and working with plutonium-239 and a little with americium-241, the SCK/CEN Belgian Nuclear Centre in Mol and working with radium-226 and americium-241, and a collaborative programme in the UK between the MRC Radiobiology Unit at Chilton with the Paterson Institute in Manchester, and working with plutonium-239 and radium-224. All have considered the problem of inhomogeneity of radiation dose distribution and the consequent importance of bone morphometry. Hence most studies have measured effects in marrow located in parts of the skeleton representing different skeletal structure and dimensions. Administered amounts of radioactivity have varied over a wide scale, namely 230 or 660 kBq kg^{-1} for ^{226}Ra (Schoeters and Vanderborght, 1981, 1983); 138–768 kBq kg^{-1} for ^{241}Am (Schoeters and Vanderborght, 1983); 170–313 kBq kg^{-1} (Svoboda *et al.,* 1979, 1984, 1988; Svoboda and Kotaskova, 1982) or 13 kBq kg^{-1} (Svoboda *et al.,* 1987) or 35 kBq kg^{-1} (Schofield *et al.,* 1986; Lord *et al.,* 1991) for ^{239}Pu and 555 kBq kg^{-1} for ^{224}Ra (Lord *et al.,* 1991). The timescales of observation have been equally variable, from a few hours to 750 days after radionuclide administration in these same reports. Although differing in detail, the results presented in these various studies yielded good agreement in the principal effects on haemopoiesis. This

chapter therefore uses primarily the data of Schofield *et al.* (1986) and Lord *et al.* (1991) with ^{239}Pu and ^{224}Ra to illustrate these effects.

The amounts of ^{239}Pu and ^{224}Ra injected in these experiments—35 kBq kg^{-1} and 550 kBq kg^{-1} respectively—were chosen as being in the range where long-term myeloid leukaemogenesis might be expected (Humphreys *et al.,* 1985, 1987, 1993). It should be remembered that ^{239}Pu is a bone-surface seeker with a 24000 year half-life and, although subject to burial and reutilisation, it will give continuous irradiation throughout the experiments. By contrast, ^{224}Ra is a bone-volume seeker but its short half-life (3.7 days) means it should be treated as a surface-seeker and at the dose used will present little or no further radiation hazard in the marrow after about fifteen days.

This rapid loss of activity is the probable reason that the higher level of ^{224}Ra activity is required for long-term leukaemogenesis compared with the chronic radiation hazard of ^{239}Pu. The acute radiation dose delivered to the marrow, however, is correspondingly large and accounts for the considerably larger initial damage to multipotent haemopoietic cells, CFU-S, caused by ^{224}Ra compared with ^{239}Pu (Table 5.1). The lack of continuing chronic irradiation, however, does allow considerably better long-term recovery in many of the bone-associated CFU-S. In common with all the other reports cited, and as might be anticipated, there is considerably less initial damage in the axial zones of the marrow cavities than in regions close to bone. This is even more pronounced when looking at very early times (4–5 hours) after administration. Hyperconcentration of CFU-S to 140 per cent of control for ^{226}Ra (Schoeters *et al.,* 1991) and to 250 per cent of control for ^{224}Ra (Lord *et al.,* 1991) occurred in the axial region suggesting no direct damage; rather some migratory element. By contrast, marginal and bone associated CFU-S were immediately reduced by 40–50 per cent in both studies.

Table 5.1 CFU-S in different marrow locations at early and late times following administration of ^{239}Pu or ^{224}Ra

	CFU-S (% of age-matched control)			
	Early effects (4 days)		Late effects (2 yr)	
Bone marrow location[a]	^{239}Pu	^{224}Ra	^{239}Pu	^{224}Ra
Femur				
Axial	70	10	15	40
Marginal	25	5	35	30
Bone shaft	20	3	75	90
Proximal and distal ends	60	10	90	100
Lumbar vertebra	50	5	20	85

[a] Bone shaft refers to CFU-S associated with the bone of the femoral shaft after removal of the marrow. Ends of the femur contain marrow as do the lumbar vertebrae.

Source: Data from Lord *et al.,* 1991.

Table 5.2 Femoral cellularity and CFU-S content (percentage of age-matched control) following administration of plutonium-239 or radium-224

Time after ^{239}Pu/ ^{224}Ra administration[a] (days)	Plutonium-239		Radium-224	
Cells	CFU-S	Cells	CFU-S	
1	–	–	48	48
4	100	50	28	9
8	–	–	32	5
20/16	100	85	49	32
120/168	100	92	65	50
300/363	100	45	70	45
480	62	45	–	–
744/636	62	52	77	66

[a] Times of observation for the two series were not identical. Where two figures are given in the first column, the first is for the ^{239}Pu series and the second for the ^{224}Ra series.

Source: Data from Lord *et al.,* 1991.

Unlike with ^{224}Ra contamination where loss of activity allows moderately good long-term recovery of CFU-S, an initial recovery (in all but the axial zones) by 20 days following ^{239}Pu lasts only to 120 days before the chronic irradiation from the ^{239}Pu leads to a long-term depletion of the population (Table 5.2). By contrast, axial CFU-S continue to decline. These changes are explained, at least in part, by redistribution of ^{239}Pu initially deposited in the bone (Green *et al.,* 1978). As a result of bone resorption, ^{239}Pu originally deposited on the endosteal surface is removed and redistributed into marrow macrophages (Jee, 1972) causing more of the marrow to be irradiated and thus probably contributing to the prolonged decline in axial CFU-S numbers (Table 5.1). Although ^{239}Pu in macrophages is only about 3 per cent of the skeletal level (McInroy and Kathren, 1990), it is long-term and accrued long-term damage throughout the marrow is possible.

There are a number of manifestations of such damage. For example, following ^{239}Pu or ^{241}Am contamination, CFU-S become more radio-sensitive (Table 5.3). Svoboda and his colleagues (1983, 1984, 1987) measured a reduction in X-ray Do from 0.92 Gy to 0.57 after ^{239}Pu and 0.75 Gy after ^{241}Am. A parallel reduction in extrapolation number emphasises this increased radiosensitivity to X-rays. This same group (Svoboda *et al.,* 1987) reported increased chromosome aberrations a year after low level ^{239}Pu contamination, reduced ^{125}IUdR incorporation in bone marrow and spleen of these animals, and defective erythropoiesis in spleen colonies generated from contaminated marrow (Svoboda and Kotaskova, 1983).

The near normal numbers of CFU-S in the bone and bone ends (Lord *et al.,* 1991) and a fully maintained bone marrow cellularity for about one year following ^{239}Pu (Table 5.2 and Svoboda *et al.,* 1987) evidently require a high proliferation rate for their maintenance. This was seen in all regions of the bone (Table 5.4 and con

Table 5.3 Radiosensitivity of bone marrow CFU-S after treatment with ^{239}Pu or ^{241}Am

Amount ^{239}Pu/^{241}Am administered[a] (kBq kg^{-1})	Time (weeks)	Do[a] (Gy)			
Control	^{239}Pu	^{241}Am	Reference[c]		
200/210	10	0.89 (1.11)	0.58 (0.91)	0.64 (0.91)	1
200/210	10	1.02	0.60	0.86	2
13.3/–	52	0.84	0.52	–	3
Mean[b]		0.92±0.5	0.57±0.02	0.75+0.11	

[a] Where two figures are quoted, the first is for ^{239}Pu and the second is for ^{241}Am.
[b] Mean Do are quoted±standard error. Figures in parentheses are extrapolation numbers *(n)*.
[c] Reference 1: Svoboda and Kotaskova, 1983; 2: Svoboda *et al.,* 1984; 3: Svoboda *et al.,* 1987.

Table 5.4 Proliferative activity of CFU-S in different regions of the mouse femur following administration of plutonium-239

	CFU-S in DNA-synthesis (%)		
	Time following ^{239}Pu administration (days) location		
Femoral marrow	0 (11 wk old)	282 (12 month old)	467 (36 month old)
Axial (A)	5	6	30
Marginal (M)	17	28.5	26
Bone shaft	40	39.5	–
Distal and proximal ends	16	28	22

Mice 10–12 weeks old injected with ^{239}Pu. Controls for 6 month old mice 10±6% in DNA-synthesis; 14±5% for 36 month old mice. (Cf. averaged mean A+M at 282 d, % in S-phase=21: control=10% ; at 467 d, % in S-phase=27: control=14%.)
Source: Data from Lord *et al.,* 1991.

firming the earlier findings of Schoeters and Vanderborght (1981) for ^{226}Ra contamination) including eventually the axial CFU-S which are notably more resistant to stimulation than the marginal CFU-S (Lord, 1986). It would appear therefore that normality in the peripheral blood picture can be maintained only at the expense of increased proliferative activity in the marrow, a feature which makes the progenitors more vulnerable to additional late damage (e.g. increased radiosensitivity, drug damage, mutation, leukaemogenesis) and in the process may pressurise the self-renewal capacity of the stem cells. Although the CFU-S population did eventually recover its self-renewal capacity, it was severely reduced in the first four months (Table 5.5) when the marrow was struggling to maintain an abortive recovery phase (Lord *et al.,* 1991). Svoboda *et al.* (1985) showed similar changes in self-renewal of CFU-S and demonstrated that this loss in potential was more serious following ^{239}Pu than after ^{241}Am.

Like CFU-S, *in vitro* CFC are initially protected in the axial zones. Schoeters and Vanderborght (1981) showed no effect of ^{226}Ra in the first three hours after injection while marginal CFC were reduced by 33 per cent (Table 5.6). By three days, however, the *in vitro* CFC compartment was reaping the benefit of the earlier over-concentration of their precursors in the axial zones and generating more CFC as those precursors moved into the differentiation-inducing margins. These authors

Table 5.5 Self-renewal of CFU-S in different regions of the mouse femur at different times after administration of plutonium-239 (% of age-matched control)

	CFU-S per spleen colony		
	Time after plutonium-239 administration (days)		
Femoral marrow location	120	294	467
Axial	26	75	130
Marginal	34	94	83
Bone shaft	55	106	–

Source: Data from Lord *et al.,* 1991.

Table 5.6 Multipotent (CFU-S) and committed (i.v. CFC) progenitor cells in different marrow locations following administration of radium-226

Marrow zone and time (days)	CFU-S (% control)	i.v. CFC (% control)
Axial		
0.2	118	103
3	85	83
100	80	92
300	56	58
Marginal		
0.2	67	67
3	90	146
100	40	52
300	20	24

Source: Schoeters and Vanderborght, 1981.

suggested that the number and concentration of both CFU-S and *in vitro* CFC were most affected in sites which contained a large proportion of cells in S-phase. Since, for CFU-S, this also coincides with the penetration range of the bone-associated ^{226}Ra (Lord *et al.,* 1975; Lord, 1992, and see Chapter 2) it is difficult to confirm this conclusion.

Very few studies of direct effects on haemopoiesis from external -particle emitters exist. However, Materii and Maslova (1984) established that *microtus* living in territories with increased radiation background show myeloid hyperplasia in the bone marrow, increased maturation indices of the erythroid and myeloid lineages and increased mitotic activity of megakaryocytes. Although this was a combination of external and internal (by ingestion) radiation, they suggested that such activation of vole haemopoiesis is an adaptive reaction to the effects of the radioecological factor. Although the radiation levels were inevitably many times lower than those encountered in experimental situations—both in terms of radionuclide quantity and incorporation in the tissues—this reaction is undoubtedly similar to that which causes increased maturational population amplification in response to injected radionuclides or to external low dose rate - irradiation (Lord, 1964, 1965, and see Chapter 4), namely the need to maintain blood production.

5.2.2.2 Stromal tissue

Although considering the haemopoietic stem cell as the primary target for incorporated radionuclides, Schoeters and Vanderborght (1983) were the first to suggest that damage to the regulatory mechanisms may be important. As discussed in Chapter 2, stromal cells, of which a major component is the fibroblastoid CFU-F, form a microenvironment that regulates the performance of haemopoietic tissue as well as taking a major role in bone formation. As such, the stromal microenvironment could potentially also play a major role in the development of osteosarcoma and/or leukaemia. This property has been exploited by Van den Heuvel and her colleagues (1984) who, administering an amount of ^{241}Am known to induce bone tumours—fibroblasts are known as candidate target cells for these tumours—found its CFU-F sensitive to -particle irradiation. This same group further showed that the radio

Table 5.7 Cellularity and CFU-S content of renal ossicles (capsules) following administration of plutonium-239 or radium-224

Time following ^{239}Pu/^{224}Ra administration (days)	Renal ossicles		
Cells/ossicle ($\times 10^{-6}$)		CFU-S/ossicle($\times 10^{-3}$)	
^{239}Pu	^{224}Ra	^{224}Ra	
0	18	18	5.5
1	11	10	3.0
8	6	6	1.5
16	7.5	4.5	0.7
90	25	7.7	2.0
744/636[a]	6	3.5	0.8

[a] First figure is for ^{239}Pu and the second for ^{224}Ra administration.
Source: Data from Lord *et al.,* 1991.

sensitivity of CFU-F is directly related to their proliferative activity which also correlates with haemopoietic activity within the stroma (Versele *et al.,* 1987). Thus as haemopoiesis declines, so does CFU-F proliferative activity.

The capacity of the stromal tissue to form new bone and endosteum capable of hosting new haemopoiesis has also been studied in the renal capsule assay following ^{239}Pu or ^{224}Ra administration (Lord *et al.,* 1991). With both radionuclides, stromal damage, increasing with time, limited the capacity of the marrow to generate such capsules and correspondingly, less haemopoiesis was supported. Both the cellularity and CFU-S content of the capsule became increasingly limited (Table 5.7). The sensitivity of this osteogenic process to -particles (compared with X-rays) was reinforced in *in vitro* studies of the mineralising capacity of murine bone marrow by Schoeters *et al.* (1992) who showed that osteosarcomogenic doses of ^{241}Am impaired bone formation for at least a year—long enough to create a long-term hazard to bone-marrow haemopoiesis.

5.3
Radionuclides in Developmental Haemopoiesis

While it was recognised that -particle irradiation could lead to leukaemia in adult animals, of growing concern was the apparent clusters of childhood leukaemias in the vicinity of nuclear installations. Ingested radionuclides concentrate in the liver as well as in the skeleton. Since the foetal liver, in particular, is a major haemopoietic organ (see Chapter 2) developing haemopoietic tissue seemed potentially more sensitive than adult haemopoiesis. Studies involving contamination at various stages of development were undertaken primarily to investigate the relative sensitivity of haemopoiesis at those different stages and also to define the nature and extent of any radiation damage. Americium-241 (Schoeters *et al.,* 1990; Van den Heuvel, 1990; Van den Heuvel *et al.,* 1991) and ^{239}Pu (Mason *et al.,* 1991, 1992; Lord *et al.,* 1992) provided the main focus of these experiments.

Schoeters *et al.* (1990) showed that the foetal skeleton has higher or similar concentrations of ^{241} Am (injected) at 14 days gestation than the liver. By contrast, adult liver concentrates considerably more than the bones. These uptakes are considerably different from those reported for ^{239}Pu where the concentration in the neonatal marrow is only 20 per cent of that in the liver, and in adult marrow about twice that in liver (Mason *et al.,* 1991; Lord *et al.,* 1992). Much of this difference is probably related to the times of assay but there is no doubt that the kinetics of uptake of these radionuclides are different. In addition, incorporation of ^{241}Am continues to accrue after birth due to transfer from the mother via her milk (Schoeters *et al.,* 1990). This factor is minimal with ^{239}Pu since transfer and loss are about balanced (Mason *et al.,* 1991). In both cases, however, concentrations in the tissues were rapidly diluted after birth—in direct relation to organ growth. Liver incorporation was significant, particularly so in the case of ^{239}Pu.

Interestingly, pregnancy gave some protection to the maternal animal. Plutonium-239 concentrations in the liver and marrow were only 27 and 60 per cent respectively of those in virgin controls. Much of the excess was clearly transferred to the developing foetus.

As with adult haemopoiesis, stromal cell involvement was clearly going to be important. Versele *et al.* (1987) showed a very close correlation of CFU-F proliferation and haemopoietic activity, and in long-term cultures the stroma from haemopoietically active organs always gave the best support for *in vitro* CFC growth (Van den Heuvel *et al.,* 1991).

With ^{239}Pu, five specific developmental stages were chosen for comparing the following:

- Preimplantation at 4 days gestation when haemopoiesis is embryonic.
- Mid-term gestation (13 days) when foetal haemopoiesis is expanding.
- 17 days gestation—pre-birth.
- 4 days neonatal.
- 22 days weaning.

In the first three stages, contamination was via placental transfer and the last two were by direct injection.

5.3.1
Uptake, Transfer and Retention of ^{239}Pu

Incorporation is a sequential process with the placenta rapidly accumulating high levels of ^{239}Pu which then passes via the foetal membranes, building up in the foetal liver and ultimately into the skeleton. At its peak following an injection of 30 kBq kg^{-1} ^{239}Pu at 13 days gestation, the developing liver contains about 0.2 per cent of the injected activity at a concentration about 15 per cent of that in the mother. From the

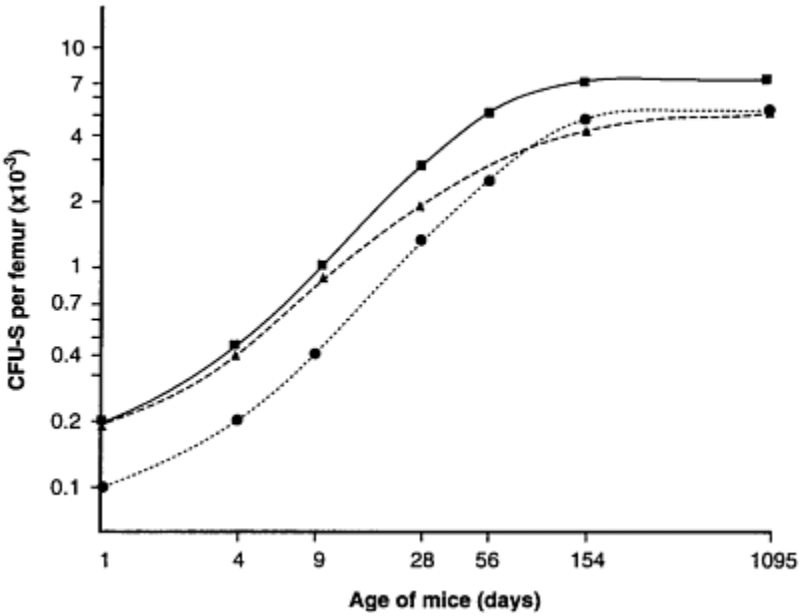

Figure 5.2 Growth of the haemopoietic spleen colony-forming unit population from birth. Control ( ), 30 kBq kg^{-1} ^{239}Pu injected to mother at 13 days gestation ( ); 30 kBq kg^{-1} ^{239}Pu injected to mother at 4 days gestation ( )

incorporation data, it was calculated that the foetal liver received a total average radiation dose of 10–14 mGy over the six days from injection to birth. Incorporation in the femur was only 0.01 per cent of the injected activity and, bearing in mind that murine marrow develops only after birth, bone growth rapidly dilutes even this concentration. Radiation dose to the marrow must, therefore, be considered many times lower than that to the foetal liver.

An average dose of radiation in the context of -particles is, however, misleading. High localised doses are the reality and this may be particularly important for contamination in the embryonic phase. Net uptake into the foetus following injection at 4 days gestation is 100-fold less than that at 13 days but high concentrations of activity exist in the foetal membranes and it is there that the embryonic stem cells reside.

Dose effect relationships are further complicated by retention after birth. It was calculated that the relative amounts of ^{239}Pu retained following injection at the five specific stages varied by a factor of 2500-fold in the liver at 4 weeks of age and by 500-fold in the femur at 1 year old. In both cases, the earlier the stage of injection, the lower the uptake and retention levels (Lord *et al.,* 1992). Since the ultimate haemopoietic damage was comparable in all cases, it means that the early stages of embryonic and foetal haemopoiesis are exquisitely sensitive to very low concentrations of incorporated radionuclides.

It is against this background of ^{239}Pu incorporation and retention that the effects on haemopoiesis must be judged.

5.3.2
Effects on Haemopoiesis

Using days 4 and 13 gestation contaminations as an illustration, Figure 5.2 shows that the CFU-S population fails to develop fully—a shortfall that lasted throughout life. Where does this shortfall arise? Probably not

from bone ^{239}Pu incorporation: so little of it got into or was retained by bone. More probably it resulted from incorporation during the embryonic and/or foetal liver development phases. However, there is an important difference in the mechanisms of the damage. After contamination at day 13, the foetal liver contained and transferred to the neonatal liver a normal number of CFU-S which then always grew more slowly than normal. By contrast, after contamination at day 4 the foetal liver contained and delivered only half its complement of CFU-S which then grew at a normal rate but always remained at a suboptimal level. Low marrow seeding, coupled with normal growth suggested direct damage to embryonic and/or foetal haemopoietic stem cell development for 4-day contamination. By contrast, poor growth of normal numbers for 13-day contamination suggested damage to the microenvironment (Lord *et al.,* 1992).

In spite of the reduced stem cell numbers, blood output was normal, reflecting normal compensatory, overproliferative activity in downstream haemopoietic cells, as with adult haemopoiesis. This was exemplified by complementary, increased production of *in vitro* CFC (Mason *et al.,* 1992) corroborating similar reports following 14-day gestation contamination with ^{241}Am (Van den Heuvel *et al.,* 1991).

5.3.3
Effects on the Microenvironment

Effects on the microenvironment were indicated when *in vitro* CFC were found to grow better in long-term cultures grown on stroma from 14-day ^{241}Am contaminated mice (Van den Heuvel *et al.,* 1991). Our experiments also assessed the overall capacity of the stromal tissue to generate bone and a new microenvironment capable of hosting bone marrow growth—using the renal capsule assay—and confirmed that the stroma from 13-day ^{239}Pu contaminated mice (at 4 and 8 weeks of age) were grossly deficient in this property. Stroma from 4-day contaminations fully supported bone marrow growth (Lord *et al.,* 1992).

Although the overall effect on stem cell development was similar in both contamination protocols, it is now clear that the one (4-day) affects primarily, and directly, the stem cells while the other (13-day) affects directly the regulatory microenvironment and only secondarily the stem cells.

5.4
Haemopoiesis *v*. the Microenvironment

Since 13-day contamination with ^{239}Pu indicates damage to the developing microenvironment, it becomes pertinent to ask whether there is any direct damage to the haemopoietic stem cells or whether their poor growth is entirely the result of an incompetent regulatory system. This was studied by a cross-transplantation technique (Yang *et al.,* 1995). A single acute dose of 1.8 Gy -rays was given on day 15 of gestation (see section 5.6) and CFU-S (in 8 week old offspring) growth capacity assayed. Yang *et al.* showed that CFU-S from normal or irradiated offspring grew equally well when transplanted into standard irradiated recipients. Normal cells, however, were unable to grow satisfactorily if the irradiated offspring were used as recipients. Thus, it is clearly the developing regulatory microenvironment which shows this unique sensitivity to irradiation, and not the stem cell population.

5.5
Preconceptual Paternal Contamination

In 1990, a report appeared which suggested that part of the perceived problems of a nuclear establishment lay in occupational, preconceptual exposure of the potential father (Gardner *et al.,* 1990). This has provoked

much discussion. There is some experimental evidence for increased lung tumour incidence following paternal X-irradiation (Nomura, 1982). On the other hand, a recent 'test case' in the British courts ruled against on the 'balance of probability' (see Doll *et al.,* 1994). Experimentally, 128 kBq kg^{-1} given to male mice twelve weeks before mating to uncontaminated females resulted in a reduction mainly in CFU-F, but also in CFU-S in their 6 week old offspring (Lord *et al.,* 1995). These preliminary experiments are currently being extended.

5.6
Radiation Dose Effectiveness of Alpha-particle Emitters

Since radiation toxicity is an important feature of -particle irradiation, there is a natural desire to ascribe values to radiation doses and to equate their effects with those of low LET, X- or -irradiation: i.e. their relative biological effectiveness or RBE. This first major attempt to ascribe equivalent radiation doses was for ^{226}Ra and ^{241}Am injected into adult mice by Schoeters and Vanderborght (1983). These researchers calculated absorbed doses (over 100 days) in different marrow locations (Table 5.8). Using a quality factor of 20 for -particles (ICRP, 1990) they further

Table 5.8 Absorbed dose (Gy) in different marrow locations to 100 days after injection of ^{241}Am or ^{226}Ra

Bone marrow location	^{241}Am 138–768 kBq kg^{-1}	^{226}Ra 230–660 kBq kg^{-1}
Axial	0	0–0.1
Marginal	4–22	1.4–3.9
Femur end	3–18	1.6–5.3
Vertebra	2–15	1.4–4.3

Note: Calculations assume that ^{241}Am is a pure bone-surface-seeker and ^{226}Ra is a pure bone-volume-seeker.
Source: Schoeters and Vanderborght, 1983.

calculated that the mean marrow dose needed to induce damage in haemopoietic stem cells over 100 days may be between 16 and 28 Sv.

A direct measurement of RBE for ^{239}Pu -particles on CFU-S, irradiated *in vitro* and compared with known CFU-S dose response data (see Chapter 4) gave a value of 2-to-4 (Lorimore *et al.,* 1993). In view of the inhomogeneity of -particle irradiation *in vivo,* however, it seemed more appropriate to consider the effects of contamination in terms of overall tissue or population sensitivities, rather than specific cell sensitivities. We chose the developing haemopoietic tissue with ^{239}Pu contamination at 13 days gestation when we could confine an -particle dose, calculated as 10–14 mGy, to the foetal liver and observe haemopoietic effects in the adult offspring.

Although largely dismissing irradiation from the bone, the spatial distribution of the CFU-S population in the adult bone marrow was distorted, presumably due to irreparable damage in the developing regulatory microenvironment. Accordingly, pregnant mice were irradiated for six days (from 13 days gestation) with chronic -irradiation and the spatial distribution of CFU-S mapped in the 8 week old offspring. The degree of damage manifest by ^{239}Pu was not matched with less than 3.6 Gy -rays (0.6 Gy/d) suggesting an effective RBE of 250–360 for -particles when a long-term tissue response is considered (Jiang *et al.,* 1994). A single acute dose of 1.8 Gy at 15 days gestation also matched the damage but either of these figures indicate a very high sensitivity of developing haemopoiesis to -particles compared to -rays.

5.7
Other Radionuclides

No detailed work on haemopoiesis has been carried out for other -particle-emitting radionuclides. Two are worth brief comment, however: radon and its daughter product, polonium-210, which have been dealt with in some detail in Chapter 3. Here it will suffice to comment that domestic radon exposure has been correlated with leukaemias in Britain (Eatough and Henshaw, 1993, 1994). It is of particular interest that radon is some sixteen times more soluble in fat than in blood (Nussbaum and Hursh, 1957, 1958) and the distribution of fat cells in the marrow could, therefore, give it an important impact on the more axially sited, primitive haemopoietic progenitor cells. In addition, ^{210}Po deposits in soft tissue and is known to be associated with marrow stromal cells. Schoeters *et al.* (1994) showed that differentiating osteogenic bone marrow cultures were adversely affected by the presence of ^{210}Po.

5.8
Other Sources of High LET Irradiation

5.8.1
Neutrons

In the early 1960s there was renewed interest in the possible use of fast neutrons in radiotherapy and this sparked a flurry of activity to assess their radiobiological effects in comparison with more conventional low LET X- or -radiotherapy. Unlike X-rays or -particles, which irradiate bone marrow close to the bone surfaces more heavily than axial marrow, neutron dose is reduced in the vicinity of bone because of the relative lack of hydrogenous matter which has the highest interaction cross-section. This, as expected, gave a relative sparing of CFU-S (in high concentration close to the bone) by neutrons compared to X-rays (Lord, 1978). Hendry (1972a) also showed that femoral bone created an overall dose reduction to marrow of about 10 per cent compared with that in spleen for 14 MeV neutrons. In general, RBE tends to depend on the energy of the neutron beam. Taken from D_0 values (37 per cent survival taken from the exponential phase of the dose response curves), neutron RBE values compared with 250 kVp X-rays were: reactor neutrons 1.58, ^{252}Cf neutrons 1.59 and from an accelerator they varied from 2.85 at 1.0 MeV to 0.85 at 13.4 MeV (Carsten *et al.,* 1976). Fission neutrons (0.8 MeV) compared well with an RBE value of 2.7 (Ainsworth *et al.,* 1989), as did values from a two-centre intercomparison giving an RBE of 1.1 for 14 MeV neutrons (Broerse *et al.,* 1971). The highest RBE reported was about 3 for low energy leakage neutrons from the Hiletron therapy unit (Chen *et al.,* 1983).

There is little or no dose rate effect for cell killing with neutrons (Ainsworth *et al.,* 1976; Chen *et al.,* 1983) and, unlike with -radiation, there is no split-dose sparing effect (Hendry and Howard, 1971; Hendry, 1972a). Ainsworth *et al.* (1976) came to the conclusion that these effects are not totally independent of dose rate or exposure time but that cell kill and division delay may vary independently. They showed a higher level of CFU-S survival and subsequent repopulation when equivalent doses, extended over twenty days, were given at 1.9 Gy d^{-1} compared with 1.3 Gy min^{-1}.

Residual damage to the haemopoietic stem cell population is also greater with neutrons than with low LET X-rays. Using 4.5 Gy X-rays or 3.5 Gy neutrons, chosen to give the same degree of initial CFU-S kill, Hendry (1972b) showed that the doubling time of the regenerating CFU-S was 50 per cent longer in both the femur and spleen. Repeated doses of X-rays (4×4.5 Gy) or neutrons (4×3.5 Gy) at 24-day intervals both produced increasing levels of residual damage which was always some 1.75-fold higher with neutrons

(Hendry *et al.,* 1974). Throughout these conditions of prolonged CFU-S suppression, Ainsworth *et al.* (1989) found, as for ^{239}Pu -particle irradiation (Lord *et al.,* 1991) and for 4×4.5 Gy X-rays (Tejero *et al.,* 1988) that CFU-S proliferation remains high—in order to maintain a normal output of mature cells.

Hendry (1972b) suggested that the poor regrowth of the CFU-S population could be the result of stromal cell damage. Xu *et al.* (1983) subsequently demonstrated that CFU-F were significantly more sensitive to neutrons than to X-rays (D_0=1.36 Gy compared with 1.64 Gy and extrapolation number of 1.0 compared with 1.8) and the maximum long-term reduction of about 50 per cent in CFU-F numbers was achieved with a total accumulated dose of only 9 Gy compared with 18 Gy for X-rays. Since the RBE for CFU-S was close to unity (Broerse *et al.,* 1971) this appeared to corroborate Hendry's hypothesis.

5.8.2
Pions

Negative pi mesons (pions) were at one time thought to be potentially useful in radiotherapy since the entrance dose of predominantly low LET events is followed by a sharply increased dose peak with a high LET component, thus giving a sharply localised radiation dose. CFU-S survival at a nominal dose of 2.4 Gy in a lucite chamber falls from 70 per cent at a depth of 2 cm, to 60 per cent at 12 cm and to 20 per cent at 18 cm, rising again to 70 per cent at 20 cm (Carlson and Thornton, 1976). However, although the CFU-S dose-survival curve was less steep than with -rays, the shoulder was significantly smaller. The combination of slope and shoulder thus in effect neutralised each other, giving an effective RBE of 1.0 for single doses between 1 and 4 Gy (Carlson and Thornton, 1976) and confirming that pions are not significantly more effective than -, X-rays or 14 MeV X-rays or electrons (Coggle *et al.,* 1975).

5.8.3
Heavy Charged Particles

Few data are available for heavy charged particles and those report measurements only of life-shortening. Thus, while fission neutrons and high energy silicon particles have similar dose-averaged LET values, the RBE for silicon is about 4: the value for neutrons is about 10 (Ainsworth *et al.,* 1989).

All these sources of high LET irradiation have been explored as potential means of radiotherapy. Unfortunately none has lived up to expectations: none has presented any real advantage over conventional low LET therapies.

5.9
Implications for Leukaemia (and Osteosarcoma)

The delay in appearance of leukaemia suggests that one is not dealing with a single event. Thus, damage to the regulatory, haemopoietic microenvironment, such as that already described, leads to compensated hyperproliferation in the surviving haemopoietic progenitor (stem) cells and, perhaps, an enhanced susceptibility to secondary damage. It is perhaps for this reason that only a proportion of exposed animals (and presumably humans) develop leukaemia (or osteosarcoma). Svoboda and Kotaskova (1982) suggested that there was a critical stage, presaging the onset of leukaemia. They observed 210 days following ^{239}Pu injection that, while 70 per cent of the mice continued to demonstrate serious haemopoietic damage, the other 30 per cent suddenly showed increased CFU-S numbers and proliferation, granulocytic cells and on transplantation gave colonies with increased ^{59}Fe incorporation and granulocytic cells in peripheral blood

and spleen. A possible viral intervention had already been suggested (Lloyd *et al.,* 1975) when 93 per cent of ^{226}Ra-treated female mice, known to carry a mammary tumour virus, developed osteosarcoma (only 40 per cent of the males did so) and 88 per cent of the female sarcomas contained virus particles.

Svoboda *et al.* (1987) reported chromosome aberrations in haemopoietic tissues twelve months after low-level (13.3 kBq kg^{-1}) ^{239}Pu contamination and, later, Bridges *et al.* (1991) showed a possible association between mutant frequency in peripheral lymphocytes and domestic radon concentrations. A possible explanation for the delayed development of leukaemia was now forthcoming in the intriguing finding that direct ^{238}Pu irradiation induced chromosome aberrations in haemopoietic CFC and their progeny, but only after the cells had progressed through a series of perfectly normal cell division cycles (Kadhim *et al.,* 1992). This concept of chromosome instability is discussed fully in Chapter 8. A cytogenetic connection is also possibly involved in childhood lymphoblastic leukaemia. The important B-lymphocyte precursors are particularly sensitive to ^{238}Pu -particle irradiation (Griffiths *et al.,* 1994a) showing a high level of apoptosis (Griffiths *et al.,* 1994b). Is it possible that among the surviving cells, some of the delayed damage shown by Kadhim and her colleagues may appear in the p53 gene, allowing uncontrolled proliferation? Mechanisms of leukaemogenesis are discussed, in detail, in Chapters 8 and 9.

References

AINSWORTH, E.J., JORDAN, D.L., MILLER, M., COOKE, E.M. & HULESCH, J.S. (1976) Dose rate studies with fission spectrum neutrons, *Radiation Research,* **67,** 30–45.

AINSWORTH, E.J., AFZAL, S.M.J., CROUSE, D.A., HANSON, W.R. & FRY R.J. M. (1989) Tissue responses to low protracted doses of high LET radiations or photons: early and late damage relevant to radio-protective countermeasures, *Advances in Space Research,* **9**, 299–313.

BAVERSTOCK, K.F. & PAPWORTH, D.G. (1985) The UK radium luminiser survey: significance of a lack of excess leukaemia, *Strahlentherapie Sonderbeit,* **80,** 22–6.

BRIDGES, B.A., COLE, J., ARLETT *et al.* (1991) Possible association between mutant frequency in peripheral lymphocytes and domestic radon concentrations, *Lancet,* **337**, 1187–9.

BROERSE, J.J., ENGELS, A.C., LELIEVELD, P. & VAN PUTTEN, L.M. (1971) The survival of colony forming units in mouse bone marrow after *in vivo* irradiation with D-T neutrons, X- and gamma-irradiation, *Int. J. Radiation Biology,* **19,** 101–10.

CARLSON, D.E. & THORNTON, J. (1976) Effects of negative pi mesons on mouse bone marrow cells, *Radiology,* **120,** 213–15.

CARSTEN, A.L., BOND, V.P. & THOMPSON, K. (1976) The RBE of different energy neutrons as measured by the haematopoietic spleen-colony technique, *Int. J. Radiation Biology,* **29,** 65–70.

CHEN, F., HENDRY, J.H., CHU, G. & GREENE, D. (1983) The RBE of the leakage radiation from the Hiletron neutron therapy unit, *Br. J. Radiololgy,* **56,** 551–8.

COGGLE, J.E., GORDON, M.Y., LINDOP, P.L., SHEWELL, J. & MILL, A.J. (1975) Some *in vivo* effects of $^{-}$ mesons in mice, *Br. J. Radiology* **49,** 161–5.

DARBY, S.C. & DOLL, R. (1987) Fallout, radiation doses near Dounreay and childhood leukaemia, *Br. Med. J.,* **294**, 603–7.

DOLL, R., EVANS, H.J. & DARBY, J.C. (1994) Paternal exposure not to blame, *Nature,* **367**, 678–80.

EATOUGH, J.P. & HENSHAW, D.L. (1993) Radon and monocytic leukaemia in England, *J. Epidemiology and Community Health,* **47**, 506–7.

(1994) Radon exposure and myeloid leukaemia, *Int. J. Epidemiology,* **23**, 430–1.

FORMAN, D., COOK-MOZAFFARI, P., DARBY, S. *et al.* (1987) Cancer near nuclear installations, *Nature,* **329**, 499–505.

FRIEDENSTEIN, A.J., CHAILAKHJAN, R.K., LATSINIK, N.V., PANASYNK, A.F. & KEILISS-BOROK, I.V. (1967) Stromal cell responsible for transferring the microenvironment of the haemopoietic tissue, *Transplantation,* **5**, 74–80.

FRIEDENSTEIN, A.J., PETRAKOVA, K.V., KURALESOVA, A.I. & FROLOVA, G.P. (1968) Heterotopic transplants of bone marrow: analysis of the precursor cells for osteogenic and haemopoietic tissues, *Transplantation,* **6**, 230–47.

FROST, H.M. (1983) Bone histomorphometry: analysis of trabecular bone dynamics. In R. R.Recker (ed.), *Bone Histomorphometry: Techniques and Interpretation.* Boca Raton: CRC Press, pp. 109–32.

GARDNER, M.J. & WINTER, P.D. (1984) Mortality in Cumberland during 1959–79 with reference to cancer in young people around Windscale, *Lancet,* i, 216–17.

GARDNER, M.J., SNEE, M.P., HALL, A.J., POWELL, C.A., DOWNES, S. & TERRELL, J.D. (1990) Results of case-control study of leukaemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria, *Br. Med. J.,* **300**, 423–9.

GREEN, D., HOWELLS, G.R. & THORNE, M.C. (1978) Plutonium-239 deposition in the skeleton of the mouse, *Int. J. Radiation Biology,* **34**, 27–36.

GRIFFITHS, S.D., MARSDEN, S.J., WRIGHT, E.G., GREAVES, M.F. & GOODHEAD, D.T. (1994a) Lethality and mutagenesis of B lymphocytes progenitor cells following exposure to alpha-particles and X-rays, *Int. J. Radiation Biology,* **66**, 197–205.

GRIFFITHS, S.D., GOODHEAD, D.T., MARSDEN, S.J. *et al.* (1994b) Interleukin-7-dependent B lymphocyte precursor cells are ultrasensitive to apoptosis, *J. Experimental Medicine,* **179**, 1789–97.

HAINES, J.W. & PRIEST, N.D. (1984) The loss of transportable plutonium deposits from the macrophages of the rat femoral bone marrow, *Radiation and Environmental Biophysics,* **23**, 133–5.

HENDRY, J.H. (1972a) The response of haemopoietic colony-forming units and lymphoma cells irradiated in soft tissue (spleen) or a bone cavity (femur) with single doses of X-rays, -rays or D-T neutrons, *Br. J. Radiology,* **45**, 923–32.

(1972b) A difference in haemopoietic stem cell repopulation after D-T neutron or X-irradiation, *Int. J. Radiation Biology,* **22**, 279–83.

HENDRY, J.H. & HOWARD, A. (1971) The response of haemopoietic colony-forming units to single and split doses of -rays or D-T neutrons, *Int. J. Radiation Biology,* **19**, 51–64.

HENDRY, J.H., TESTA, N.G. & LAJTHA, L.G. (1974) Effects of repeated doses of X-rays or 14 MeV neutrons on mouse bone marrow, *Radiation Research,* **59**, 645–52.

HEYWORTH, C.M. & SPOONCER, E. (1993) *In vitro* clonal assays for murine multipotential and lineage restricted myeloid progenitor cells. In N.G.Testa & G.Molineux (eds), *Haemopoiesis: A Practical Approach.* Oxford: IRL/ Oxford University Press, pp. 37–53.

HILL, C. & LAPLANCHE, A. (1990) Overall mortality and cancer mortality around French nuclear sites, *Nature,* **347**, 755–7.

HUMPHREYS, E.R., LOUTIT, J.F., MAJOR, I.R. & STONES, V.A. (1985) The induction by ^{224}Ra of myeloid leukaemia and osteosarcoma in male CBA mice, *Int. J. Radiation Biology,* **47**, 239–47.

HUMPHREYS, E.R., LOUTIT, J.F. & STONES, V.A. (1987) The induction by ^{239}Pu of myeloid leukaemia and osteosarcoma in female CBA mice, *Int. J. Radiation Biology,* **51**, 331–9.

HUMPHREYS, E.R., ISAACS, K.R., RAINE, T.A., SAUNDERS, J., STONES, V.A. & WOOD, D.L. (1993) Myeloid leukaemia and osteosarcoma in CBA/H mice given ^{224}Ra, *Int. J. Radiation Biology,* **64**, 231–5.

ICRP (1990) Recommendations of the International Commission on Radiological Protection, *Annals of the ICRP,* Publication 60. Oxford: Pergamon, p. 21.

JEE, W.S.S. (1972) Distribution and toxicity of plutonium in bone, *Health Physics,* **22**, 583–95.

JIANG, T-N., LORD, B.I. & HENDRY, J.H. (1994) Alpha particles are extemely damaging to developing haemopoiesis compared to gamma irradiation, *Radiation Research,* **137**, 380–4.

KADHIM, M.A., MACDONALD, B.A., GOODHEAD, D.T., LORIMORE, S.A., MARSDEN, S.J. & WRIGHT, E.G. (1992) Transmission of chromosome instability after plutonium alpha-particle irradiation, *Nature,* **357**, 548–50.

LLOYD, E.L., LOUTIT, J.F. & MACKEVICIUS, F. (1975) Viruses in osteosarcomas induced by ^{226}Ra: a study of the induction of bone tumours in mice, *Int. J. Radiation Biology,* **28**, 13–33.

LORD, B.I. (1964) The effects of continuous irradiation on cell proliferation in rat bone marrow, *Br. J. Haematology,* **10**, 496–507.

(1965) Cellular proliferation in normal and continuously irradiated rat bone marrow studied by repeated labelling with tritiated thymidine, *Br. J. Haematology,* **11**, 130–43.

(1978) Cellular and architectural factors influencing the proliferation of haematopoietic stem cells. In B.Clarkson, P.A.Marks & J.E.Till (eds), *Differentation of Normal and Neoplastic Hematopoietic Cells.* Cold Spring Harbor Conferences on Cell Proliferation, Vol. 5, pp. 775–88.

(1986) The sensitivity of G_0-state haemopoietic spleen colony-forming cells to a stimulus for proliferation, *Cell and Tissue Kinetics,* **19**, 305–10.

(1992) The architecture of bone marrow cell populations. In M.J.Murphy Jr (ed.), *Concise Reviews in Clinical and Experimental Haematology.* Dayton, OH: AlphaMed Press, pp. 225–34.

LORD, B.I., TESTA, N.G. & HENDRY, J.H. (1975) The relative spatial distribution of CFU-S and CFU-C in the normal mouse femur, *Blood,* **46**, 65–72.

LORD, B.I., MOLINEUX, G., HUMPHREYS, E.R. & STONES, V.A. (1991) Long-term effects of plutonium-239 and radium-224 on the distribution and performance of pluripotent haemopoietic progenitor cells and their regulatory microenvironment, *Int. J. Radiation Biology,* **59**, 211–27.

LORD, B.I., MASON, T.M. & HUMPHREYS, E.R. (1992) Age-dependent uptake and retention of ^{239}Pu: its relationship to haemopoietic damage, *Radiation Protection Dosimetry,* **41**, 163–9.

LORD, B.I., HUMPHREYS, E.R. & STONES, V.A. (1995) Preconceptual paternal plutonium-239 contamination: development of haemopoiesis in the offspring, In G.van Kaick, A.Karaoglou & A.M.Kellerer (eds), *Health Effects of Internally Deposited Radionuclides.* London: World Scientific, pp. 341–6.

LORIMORE, S.A., WRIGHT, E.G. & GOODHEAD, D.T. (1993) Inactivation of haemopoietic stem cells by slow - particles, *Int. J. Radiation Biology,* **63**, 655–60.

LOUTIT, J.F. & CARR, T.E. (1978) Lymphoid tumours and leukaemia induced in mice by bone seeking radionuclides, *Int. J. Radiation Biology,* **33**, 245–63.

MASON, T.M., HUMPHREYS, E.R. & LORD, B.I. (1991) Alpha-particle irradiation of haemopoietic tissue in pre- and postnatal mice. 1: Distribution of plutonium-239 after midterm contamination, *Int. J. Radiation Biology,* **59**, 467–78.

MASON, T.M., LORD, B.I. MOLINEUX, G. & HUMPHREYS, E.R. (1992) Alpha-irradiation of haemopoietic tissue in pre- and postnatal mice. 2: Effects of midterm contamination with ^{239}Pu *in utero, Int. J. Radiation Biology,* **61**, 393–403.

MATERII, L.D. & MASLOVA, K.I. (1984) Effect of increased natural radioactivity on bone marrow morphology of *Microtus oeconomus pall, Radiobiologiia,* **24**, 243–6.

MCINROY, J.F. & KATHREN, R.L. (1990) Plutonium content in marrow and mineralized bone in an occupationally exposed person, *Radiation Protection Dosimetry,* **32**, 245–52.

MOLE, R.H. (1986) Radiation-induced acute myeloid leukaemia in the mouse: experimental observations *in vivo* with implications for hypotheses about the basis of carcinogenesis, *Leukaemia Research,* **10,** 859–65.

MOSKALEV, YU. I., LYAGINSKAYA, A.M., ZALIKIN, G.A., NISIMOV, P.G., ROMANOVA, I.B. & KORNEEV, YE.YU. (1989) Carcinogenic effects in rat progeny exposed perinatally to radionuclides. In N.P.Napalkov, J.M.Rice, L.Tomatis & H. Yamasaki (eds.) *Perinatal and Multigeneration Carcinogenesis.* Lyon: International Agency for Research on Cancer (IARC), pp. 421–7.

NOMURA, T. (1982) Parental exposure to X-rays and chemicals induces heritable tumours and anomalies in mice, *Nature,* **296,** 575–7.

NUSSBAUM, E. & HURSH, J.B. (1957) Radon solubility in rat tissues, *Science,* **125,** 552–4.

(1958) Radon solubility in fatty acids and triglycerides. *J. Physical Chemistry,* **62,** 81–4.

PARFITT, A.M. (1983) Physiologic and clinical significance of bone histomorphometric data. In R.R.Recker (ed.), *Bone Histomorphometry: Techniques and Interpretation.* Boca Taton: CRC Press, pp. 143–224.

PRIEST, N.D. & GIANNOLA, S.J. (1980) Plutonium-241 deposition and redistribution in the rat rib, *Int. J. Radiation Biology,* **37**, 281–98.

SCHOETERS, G.E.R. & VANDERBORGHT, O.L. (1981) Temporal and spatial response of marrow colony-forming cells (CFU-S and CFU-C) after ^{226}Ra incorporation in BALB/c mice, *Radiation Research,* **88**, 251–65.

(1983), Relative effectiveness of ^{241}Am vs ^{226}Ra approached by haemopoietic stem cell studies in various bone marrow sites of contaminated mice, *Health Physics,* **44** (suppl. 1), 555–70.

SCHOETERS, G., VAN DEN HEUVEL, R., LEPPENS, H., VANDER PLAETSE, F. & VANDERBORGHT, O.L. (1990) Distribution of ^{241}Am on offspring from BALB/c mice injected with ^{241}Am at 14 days of gestation: relation to calcium and iron metabolism and comparison with distribution of ^{241}Am after injection of adults, *Int. J. Radiation Biology,* **58**, 371–82.

SCHOETERS, G.E., MAISIN, J.R. & VANDERBORGHT, O.L. (1991) Toxicity of ^{241}Am in male C57 B1 mice: relative risk versus ^{226}Ra, *Radiation Research,* **126,** 198–205.

SCHOETERS, G.E.R., VANDER PLAETSE, F. & VAN DEN HEUVEL, R.L. (1992) High radiosensitivity of the mineralization capacity of adult murine bone marrow *in vitro* to continuous -irradiation compared to acute X-irradiation, *Int. J. Radiation Biology,* **61,** 675–83.

SCHOETERS, G.E., VANDER PLAETSE, F. & VAN DEN HEUVEL, R.L. (1994) Effects of *in vitro* alpha-particle irradiation on osteogenic bone marrow cultures, *Int. J. Radiation Biology,* **66,** 207–14.

SCHOFIELD, R., LORD, B.I., HUMPHREYS, E.R. & STONES, V.A. (1986) Effects of plutonium-239 on haemopoiesis. I: Quantitative and qualitative changes in CFU-S in different regions of the mouse femur and vertebrae, *Int. J. Radiation Biology,* **49**, 1021–9.

SIKOV, M.R., ROMMEREIN, D.N. & MAHLUM, D.D. (1982) Correlations between age-related inhomogeneities in ^{239}Pu distribution and anatomic sites of bone tumour development, *Radiation Research,* **91,** 298–307.

SPIERS, F.W. (1988) Particle dosimetry in bone and the toxicity of bone seeking radionuclides, *Physics in Medicine and Biology,* **33**, 395–411.

SPIERS, F.W., LUCAS, H.F., RUNDO, H.J. & ANAST, G.A. (1983) Leukaemia incidence in the US dial workers, *Health Physics,* **44** (suppl. 1), 65–72.

SVOBODA, V. & BUBENIKOVA, D. (1990) Hemoblastoses in mice contaminated with low activities of ^{239}Pu, *Neoplasma,* **37**, 639–46.

SVOBODA, V. & KOTASKOVA, Z. (1982) Intensified proliferative activity of the CFU-S in vertebral bone marrow of ^{239}Pu-treated mice as one of the factors involved in the induction of granulocytic leukaemia, *Neoplasma,* **29,** 719–26.

SVOBODA, V. & KOTASKOVA, Z. (1983) Radiosensitivity and proliferative activity of vertebral CFU-S surviving in mice injected with oncogenic activities of ^{239}Pu or ^{241}Am, *J. Hygiene and Microbiological Immunology,* **27**, 329–35.

SVOBODA, V., KOFRANEK, V., KOTASKOVA, Z., BUBENIKOVA, D. & DVORAK, V. (1977) Planimetric evaluation and comparison of roentgenograms of osteogenic sarcomas induced by ^{226}Ra and ^{224}Ra in mice, *Neoplasma,* **24**, 311–18.

SVOBODA, V., KOTASKOVA, Z., LENGER, V. & THOMAS, J. (1979) Effect of ^{239}Pu on mouse haemopoietic stem cells in different types of bone marrow cavities, *Radiation and Environmental Biophysics,* **16,** 339–45.

SVOBODA, V., KLENER, V., BUBENIKOVA, D. & GLOBOCNIK, E. (1980) Bone sarcomas in ^{239}Pu treated mice, *Neoplasma,* **27**, 3–10.

SVOBODA, V., SEDLAK, A. & KOTASKOVA, Z. (1984) Radiosensitivity of bone marrow CFU-S surviving in mice internally contaminated with ^{239}Pu or ^{241}Am, *Radiation and Environmental Biophysics,* **23**, 41–50.

SVOBODA, V., SEDLAK, A., BUBENIKOVA, D., KOTASKOVA, Z. & TRUXOVA, O. (1985) Self-renewal capacity of murine haemopoietic stem cells under internal contamination with p^{239}Pu and ^{241}Am, *Radiation and Environmental Biophysics,* **24**, 203–9.

SVOBODA, V., SEDLAK, A., KYPENOVA, H. & BUBENIKOVA, D. (1987) Long-term effects of low-level ^{239}Pu contamination on murine bone-marrow stem cells and their progeny, *Int. J. Radiation Biology,* **52**, 517–26.

SVOBODA, V., BUBENIKOVA, D. & SEDLAK, A. (1988) Effect of plutonium-239 on the mitotic activity of the mouse bone marrow cells. *Radiation and Environmental Biophysics,* **27**, 79–85.

TEJERO, C., LORD, B.I., MASON, T.M. & HENDRY, J.H. (1988) Long-term haemopoietic injury in mice after repeated irradiation: precursor-cell cycling and its regulation, *Eur. J. Haematology,* **41,** 278–84.

TILL, J.E. & MCCULLOCH, E.A. (1961) A direct measurement of the radiation sensitivity of normal mouse bone marrow cells, *Radiation Research,* **14,** 213–22.

VAN DEN HEUVEL, R.V. (1990) Bone marrow from BALB/c mice radiocontaminated with ^{241} Am *in utero* shows a deficiency in *in vitro* haemopoiesis, *Int. J. Radiation Biology,* **57**, 103–15.

VAN DEN HEUVEL, R.L., SCHOETERS, G.E. & VANDERBORGHT, O.L. (1984) Effects of ^{241}Am on bone fibroblasts, *Radiation and Environmental Biophysics,* **23**, 137–40.

VAN DEN HEUVEL, R.L., SCHOETERS, G., LEPPENS, H. & VANDERBORGHT, O.L. (1991) Stromal cells in long-term cultures of liver, spleen and bone marrow at different developmental ages have different capacities to maintain GM-CFC proliferation, *Experimental Haematology,* **19,** 115–21.

VERSELE, S.R.M., VAN DEN HEUVEL, R.L., SCHOETERS, G.E.R. & VANDERBORGHT, O.L.J. (1987) Proliferative activity of stromal stem cells (CFU-F) from haemopoietic organs of pre- and postanal mice, *Radiation Research,* **111,** 185–91.

WICK, R.R., CHMELEVSKY, D. & GOSSNER, W. (1995) Current status of the follow-up of radium-224 treated ankylosing spondylitis patients. Paper presented at the International Seminar on Health Effects of Internally Deposited Radionuclides: Emphasis on Radium and Thorium, Heidelberg.

XU, C.X., HENDRY, J.H. & TESTA, N.G. (1983) The response of stromal progenitor cells in mouse marrow to graded repeated doses of X-rays or neutrons, *Radiation Research,* **96**, 82–9.

YANG, F-T., LORD, B.I. & HENDRY, J.H. (1995) Gamma irradiation of the fetus damages the developing haemopoietic microenviornment rather than the haemopoietic progenitor cells, *Radiation Research,* **141**, 305–9.

6
Experimental Approaches for Therapeutic Treatment of Radiation-induced Haemopoietic Injury

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6
Experimental Approaches for Therapeutic Treatment of Radiation-induced Haemopoietic Injury

6.1
Introduction: Radiation, Cytokines, Stem Cells, Treatment

6.1.1
Critical Factors in Survival

Recovery from the lethal effects of irradiation across the haemopoietic syndrome requires at least three key events. First is the self-renewal of several populations of pluripotential, multipotential and committed stem and progenitor cells that will eventually lead to both short-term recovery from the immediate consequences of irradiation and long-term reconstitution of the haemopoietic system. Second is the generation of functional end cells, i.e. the neutrophils and platelets which will prevent the morbidity and mortality associated with the consequent haemorrhage and sepsis. Third is the production of these functional end cells within a critical, clinically manageable period of time, defined by the capacity to support the irradiated host with antibiotics and platelet or whole-blood transfusions. Until recently, we could not, with the exception of bone marrow transplantation, manipulate haemopoietic reconstitution therapeutically except by providing clinical support as mentioned above. Such replacement and substitution therapy is essential but severely hampered by transfusion limitations and bacterial and fungal resistance to antibiotics.

The past decade has witnessed the identification, cloning and production of novel proteins (cytokines) that serve as growth factors for maintaining the viability and/or inducing self-renewal, proliferation and differentiation of haemopoietic stem cells and progenitor cells. In addition, there has been significant expansion in the knowledge of stem cell biology to include their identification, separation, purification, heterogeneity, assay, microenvironmental influence, *in vivo* mobilisation, *ex vivo* manipulation and genetic and transcriptional regulation. This continuously expanding repertoire of proteins or cytokines has been shown to regulate cellular interactions in probably every organ and tissue in the body. It is most likely that every cell type in the body can produce and respond to several different cytokines. This cytokine network is the common denominator involved in the regeneration of haemopoietic tissue, the production and function of platelets and neutrophils as well as the initiation, progression and control of the pathogenesis associated with infection and acute and chronic inflammation.

6.1.2
Stem Cell Heterogeneity

Over the past decade considerable evidence has accumulated in favour of extensive heterogeneity in the pluripotent haemopoietic stem cell population (PHSC). The splenic colony forming cell (CFU) assay, the CFU-S day 8, had long been regarded as the footprint of the HSC. Subsequent evidence indicated that the later-forming CFU-S assayed at day 12–14 represented a subpopulation of CFU-S that were thought to correlate with true stem cell activity (Magli *et al.,* 1982; Molineux *et al.,* 1986). Serial transplantation of reconstituted bone marrow to secondary recipients revealed a population of bone marrow cells that were unable in the long term to repopulate the secondary hosts, although the presence of short-term, early-survival-promoting cells was observed. These results suggested that the donor marrow population lacked self-renewing pluripotent stem cells and that the transplanted marrow did not contain PHSC. Engraftment of the aplastic marrow space was thus considered to occur in two phases. An early phase in which short-term reconstitutional, multipotent, progenitor cells with limited self-renewal capacity repopulate the marrow, followed by a stage of engraftment by pluripotent stem cells capable of stable long-term reconstitution (van Zant *et al.* 1991; Jordan and Lemischka, 1990). Further distinction between CFU-S and PHSC or long-term reconstituting cells (LTRC) emerged from studies involving 5-fluorouracil (FU). It was shown that within one or two days after a single 5-FU injection, 99.5 per cent of all CFU-S were killed, whereas the LTRC were spared (Hodgson and Bradley, 1979; van Zant, 1984; Lerner and Harrison, 1990; Harrison and Lerner, 1991). These results emphasise that: (1) LTRC are most likely in a quiescent state and therefore not susceptible to the toxic effects of 5-FU for dividing cells, and (2) short-term *in vivo* experiments quantify multipotent progenitor cells with limited self-renewal and that long-term *in vivo* reconstitution assays measure the PHSC activity. The applicability of the CFU-S assays for measuring PHSC activity has also been challenged since physical, antigenic and metabolic separation of short-term reconstitutional cells and long-term reconstitutional cells can be achieved (Jones *et al.,* 1989, 1990; Orlic *et al.,* 1993; Ploemacher and Brons, 1988; Ploemacher *et al.,* 1992; Bertoncello *et al.* 1985).

The early concept of the PHSC being a relatively radiosensitive subpopulation of haemopoietic cells (Hendry and Lord, 1983; Till and McCulloch 1961) has also been successfully challenged (Ploemacher *et al.,* 1992; Meinje *et al.,* 1991). Ploemacher and colleagues determined the radiation sensitivity of various subsets of the haemopoietic stem cell hierarchy. It was determined that PHSC with long-term reconstitution and marrow repopulating ability were more radioresistant than the CFU-S d8 or d12–14. This has significant implications in that the fractional survival of more pluripotent HSC may be greater than that of the more committed HSC and progenitor cells following an acute or fractionated dose of ionising radiation. This fact also underscores the variable of time, in recovery from high doses of radiation. The LTRC are characterised as not being capable of promoting survival of mice through 30 days after lethal radiation exposure, that is, when they are susceptible to infection and haemorrhage. The time required for self-renewal, proliferation and differentiation to generate mature and functional neutrophils and platelets cannot occur within that critical, clinically manageable, finite period of time defined by severe neutropenia and thrombocytopenia.

The success of new treatment modalities will be defined by their ability to modulate the viability, renewal, proliferation and/or differentiation processes required to produce mature cells for prevention of sepsis and haemorrhage and yet provide for stem cell renewal and differentiation leading to long-term reconstitution of the haemopoietic tissue. The following sections in this chapter discuss current experimental therapeutic approaches designed either to replace haemopoietic stem cells and/or committed progenitor cells through transplantation, or to influence renewal and recovery of surviving haemopoietic cells through cytokine therapy. The techniques of stem cell mobilisation, cord blood stem cell isolation and *ex vivo* expansion of selected stem and progenitor cell subsets in combination with cytokines have provided

increased potential for successful modulation of both early and late phases of engraftment. Also, the discovery of new growth factors (GF) such as the c-*mpl* ligand (thrombopoietin) and foetal liver tyrosine kinase (*flt*-3/*flk*-2) ligand coupled with the use of therapeutic combination cytokine protocols offer potential for increasing recovery of endogenous haemopoietic tissue as well as that of transplanted cells.

6.2
Clinical Support and Modulation of the Radiation Response

6.2.1
Modification of Morbidity and Mortality through the use of Antibiotics, Platelet and Whole-blood Transfusions

Modification of survival throughout the haemopoietic dose range is achievable using a clinical support regimen designed to replace or substitute for the function of those mature cells, neutrophils and platelets, depleted as a consequence of lethal radiation exposure to haemopoietic stem and progenitor cells. Experimental work performed over twenty years ago demonstrated the efficacy of good supportive care centred on systemic antibiotics against gram negative bacteria and fresh, irradiated, platelet transfusions (Sorensen *et al.,* 1960; Perman *et al.,* 1962; Jackson *et al.,* 1959; Bagdasarov *et al.,* 1959; Furth *et al.,* 1953). Several studies indicated that antibiotics given singly or in combination were somewhat effective in reducing mortality of irradiated dogs in the $LD_{50/30}$ range (Sorensen *et al.,* 1960; Perman *et al.,* 1962; Jackson *et al.,* 1959). Sorensen *et al.* reported on the effectiveness of several antibiotics in conjunction with fresh whole blood transfusions and parenteral fluids to control dehydration in reducing lethality from an LD_{99} (4 Gy air dose at skin surface) to an LD_{20}. Perman *et al.* extended this study over a dose range from 4 to 5.50 Gy well into the LD_{100}, and achieved a significant increase in survival. The Sorensen regimen consisted of several antibiotics, fresh platelet-rich plasma and fluid therapy (isotonic saline or 5 per cent dextrose) given during the period of inappetence. Success with these regimens supports the concept that infection and haemorrhage are the two primary factors in the lethal consequences of radiation exposure in the haemopoietic syndrome (Miller, 1956; Carter and Collins, 1974; Collins, 1956). While fresh platelets or whole blood transfusions may suffice for early control of inopportune haemorrhage, choice of prophylactic antibiotics should focus on elimination of opportunistic *enterobacteriaceae*. Lethal doses of irradiation to mice induced dose-related reductions in both aerobic and anaerobic bacteria from $10^{10–12}$ to $10^{4–6}$ per gramme of stool within four days (Brook *et al.,* 1986). While the number of anaerobic bacteria stays low, the number of *enterobacteriaceae* significantly increases by several log intervals by the twelfth day following radiation. This increase is associated with bacterial translocation and fatal bacteremia (Collins, 1956; Carter and Collins, 1974; Miller *et al.,* 1956). Brook *et al.* (1986) demonstrated that antimicrobials that suppress anaerobic flora have a deleterious effect on survival by promoting earlier enterobacterial sepsis. In contrast, antimicrobials that inhibit gram-negative enteric bacteria and preserve the anaerobic flora have shown a beneficial effect by preventing bacterial translocation, in studies of animals (Hathorn *et al.,* 1987; Berg 1981; Van Der Waaij *et al.,* 1982) and immunocompromised patients (Bodey *et al.,* 1966). Perman *et al.* (1962) reiterated that controlling infection during the critical neutropenic and thrombocytopenic phase is the limiting factor in successful treatment. MacVittie *et al.* (1991) extended the evaluation of clinical support studies in the irradiated dog over a complete haemopoietic syndrome dose range capable of determining the shift in $LD_{50/30}$ due to clinical support. The shift in the canine $LD_{50/30}$ from 2.6 Gy to approximately 3.39 Gy measured as midline tissue dose (Figure 6.1). The treatment regimen consisted of a combination of amnioglycoside (gentamicin) and third-generation cephalosporin (cephotaxime SO_4) plus

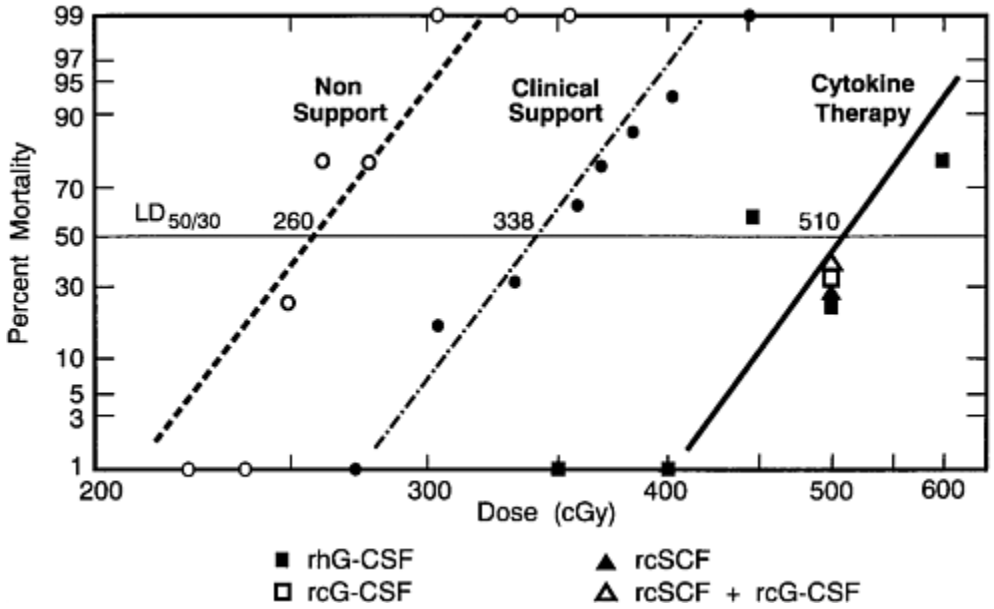

Figure 6.1 Influence of clinical support and cytokine therapy on survival ($LD_{50/30}$) of irradiated dogs. Dogs were exposed to bilateral, whole-body, ^{60}Co radiation at doses ranging from 2 to 6 Gy. Cohorts of animals were divided into three groups: no support, clinical support (MacVittie et al., 1990) or cytokine support which was administered subcutaneously, once daily for 23 days beginning on day 1 after irradiation. Animals received either recombinant human-G-CSF (10 μg/kg/d), recombinant canine (rc) SCF (100 μg/kg/d), rcG-CSF (5 μg/kg/d) or rcSCF (100 μg/kg/d) and rcG-CSF (5 μg/kg/d)

fluids and fresh platelets. It is worth noting that the mean survival time of decedent animals in this study increased from 14 to 22 days in the non-supported and supported groups respectively. These collective data indicate that modest clinical support is capable of shifting the $LD_{50/30}$ by a factor of 1.3. A more intensive regimen of support to include sterile barriers and selective, prophylactic intestinal aerobic decontamination should allow an even greater shift towards reduced morbidity and mortality. It must be emphasised that successful administration of clinical support in the non-transplant scenario is dependent upon two facts. First, the haemopoietic tissue damage is reversible and, secondly, the surviving fractions of haemopoietic stem cells are capable of spontaneous regeneration. Haemopoietic regeneration must result in the production of functional neutrophils and platelets within the critical, clinically manageable period of time that is dictated by success of the selected support and duration of cytopenia. The significant shift in the $LD_{50/30}$ noted in the canine study (MacVittie *et al.,* 1991) could be attributed to the additional time afforded to the supported animals for regeneration of the haemopoietic progenitors and timely production of functional neutrophils. The occurrence of bacterial antibiotic resistance and transfusion limitations underscores the utility of new therapeutic approaches that focus on enhancing restoration of haemopoietic tissue with consequent benefits in host defence and haemostasis.

6.2.2
Immunomodulators

Treatment of radiation-induced marrow aplasia through use of immunomodulators is based on the concept that such agents as endotoxin (lipopolysaccharides) or bacterial cell wall components are capable of significantly increasing non-specific host defence, thereby acting as broad-spectrum prophylaxis against infectious challenge as well as stimulating haemopoietic recovery with a single injection shortly before or after radiation exposure (reviews by Ainsworth, 1988; Patchen, 1995). Immunomodulators have generally been used in prophylactic protocols as radioprotectants. The best immunomodulators, represented by glucan, trehalose dimycolate or monophophoryl lipid A, have produced DRFs of around 1.2 when administered 18–20 hours prior to exposure, relative to 3.0 for traditional aminothiol radioprotectants (Davidson *et al.,* 1980). The mechanism of action for the immunomodulators is complex and undoubtedly involves activation of macrophages for host defence and induction of cytokines capable of stimulating haemopoietic recovery and enhancing cellular host defence.

The use of immunomodulators in therapeutic protocols has shown somewhat limited, albeit efficacious, utility relative to the therapeutic use of recombinant cytokines or the prophylactic administration of immunomodulators. Injecting synthetic trehalose dicorynomycolate (S-TDCM) into mice 1 day before, 1 hour after or 1 day after irradiation significantly increased survival by stimulating cellular defences (Madonna *et al.,* 1989). Further study showed that injecting S-TDCM after sublethal irradiation increased survival following a lethal, bacterial challenge with *K. pneumoniae* 4 days later. Combination of such treatment with antibiotic therapy synergistically increased survival in irradiated and neutropenic mice infected with *K. pneumoniae* (Madonna *et al.,* 1989). Carbohydrate-based immunopharmaceuticals continue to be evaluated in preclinical models and clinical trials for prevention of sepsis and septic shock, although therapeutic utility after high dose of radiation has been dampened by the demonstrated potential of cytokines.

6.3
Reconstitution of the Haemopoietic System after Radiation-induced Marrow Aplasia: Potential of Cytokine Therapy

6.3.1
Current Generation: Single Cytokine Protocols

The haemopoietic system is responsive to a number of cytokines and/or growth factors. At least fifteen interleukins (IL) alone, in addition to the colony stimulating factors (CSF), granulocyte (G)-CSF, granulocyte-macrophage (GM)-CSF and macrophage (M)-CSF, and other cytokines such as the ligands to c-*kit* receptor, *kit* ligand (KL) otherwise known as stem cell factor (SCF) and *flt*-3/*flk*-2 receptor, the *flt*-3 ligand (FL) and transforming growth factor (TGF)- and macrophage inhibitory protein (MIP)-1 have shown specific or pleotropic effects on various aspects of the haemopoietic system and its mature progeny. Three of the most studied from the laboratory to the clinic are G-CSF, GM-CSF and IL-3 (multi-CSF). The cytokines G-CSF and GM-CSF are more lineage-restricted in activity and have been effective in enhancing recovery of neutrophils (PMN) in preclinical models of radiation (Farese *et al.* 1993a,b; Fushiki *et al.,* 1990; Kobayashi *et al.,* 1987; MacVittie *et al.,* 1990; Monroy *et al.,* 1987, 1988; Patchen *et al.,* 1990; Schuening *et al.,* 1989; Tanikawa *et al.,* 1989, 1990) or drug-induced (Welte *et al.,* 1987; Shimamura *et al.,*

1987; Moore and Warren, 1987; Meisenberg *et al.,* 1992; Matsumoto *et al.,* 1987) marrow aplasia. In addition to G-CSF and GM-CSF, seven other cytokines, IL-1 (Morrissey *et al.,* 1988; Moore and Warren, 1987; Moore, 1989; Castelli *et al.,* 1988; Gasparetto *et al.,* 1989; Benjamin *et al.,* 1989), IL-6 (Patchen *et al.,* 1991; Takatsuki *et al.,* 1990; MacVittie *et al.,* 1994; Burstein *et al.,* 1992a; Herodin *et al.,* 1992; Zeidler *et al.,* 1992; Winton *et al.,* 1994; Farese *et al.,* 1994a), IL-11 (Leonard *et al.,* 1994), KL (Patchen *et al.,* 1994a,b; Schuening *et al.,* 1993; Farese *et al.,* 1993a), LIF (Farese *et al.,* 1994b) and the IL-3/GM-CSF fusion protein PIXY-321 (Williams *et al.,* 1993a, b) have been shown to be therapeutically effective in enhancing recovery of irradiated or cytotoxic drug-treated mice, dogs or non-human primates. These successes were dependent upon the ability of the cytokine protocol to accelerate the recovery from radiation or drug-induced neutropenia and/or thrombocytopenia significantly. The mature neutrophils and platelets provided for competent host defence against opportunistic pathogens and reduced risk of haemorrhage respectively.

6.3.1.1
Cell viability and survival

Haemopoietic growth factors promote proliferation, differentiation and survival of haemopoietic progenitor cells (Koury and Bondurant, 1990; Williams, G.T. *et al.,* 1990; Bodine *et al.,* 1992; Brandt *et al.,* 1994; Katayama *et al.,* 1993) and mature cells (Lopez *et al.,* 1986; Colotta *et al.,* 1992; Cox *et al.,* 1992; Brach *et al.,* 1992). Dexter and colleagues suggested that haemopoietic growth factors suppress apoptosis and that their availability *in vivo* may regulate the population sizes of the colony forming cells (Fairbairn *et al.,* 1993). The lineage-specific cytokines G-CSF and GM-CSF increase the viability of neutrophils and eosinophils (Lopez *et al.,* 1986; Colotta *et al.,* 1992; Cox *et al.,* 1992; Brach *et al.,* 1992; Begley *et al.,* 1986). GM-CSF greatly increases the lifespan of the neutrophil (Lord *et al.,* 1992) and increases that of the eosinophil by 9 hours. *In vitro* incubation of neutrophils with G-CSF or GM-CSF increased their survival and decreased the numbers of apoptotic cells as estimated by morphological criteria and DNA fragmentation assays. Increased production of neutrophils in concert with increased lifespan can offer significant advantages to the neutropenic host at risk for infection. It has also been shown that late-stage erythroid progenitors require erythropoietin for survival and that this effect is achieved through retardation of apoptotic DNA breakdown.

This ability of growth factors to suppress apoptosis and thereby promote cell survival is fundamental to their ability to enhance cell proliferation and differentiation. The pleiotropic action of growth factors may be mediated through their cognate receptors which may possess intrinsic, protein tyrosine kinase activity essential for triggering distinct signal transduction pathways (Fairbairn *et al.,* 1993; Thèze, 1994). A number of haemopoietic growth factors have been shown to promote survival of haemopoietic precursor cell lines by suppressing apotosis (Williams, G.T. *et al.,* 1990). These results suggest that a primary role of some growth factors or cytokines may be enabling rather than inductive. Two cytokines, IL-3 and KL/SCF that exhibit modest colony-forming ability by themselves have marked synergistic activity when combined with other cytokines or with each other. Several experiments have demonstrated the survival enhancing properties of IL-3 and KL (Caceres-Cortes *et al.,* 1994; Bodine *et al.,* 1992; Katayama *et al.,* 1993; Brandt *et al.,* 1994; Collins *et al.,* 1992; Yee *et al.,* 1994). Bodine *et al.* (1992) reported that IL-3 and KL each promote the survival of primitive haemopoietic progenitor cells that possess bone marrow repopulating ability. Katayama *et al.* (1993) also showed that survival of dormant murine lympho-haemopoietic progenitor cells is dependent on the presence of IL-3 or KL. It is of interest that Brandt *et al.* (1994) further analysed the effects of IL-3 and KL on the survival of various classes of human haemopoietic cells. The addition of KL to serum-free suspension cultures of $CD34^+$, $HLA\text{-}DR^-$, c-kit^+ cells delayed the appearance

of the DNA fragmentation patterns associated with apoptosis to a greater extent than did IL-3. The addition of IL-3, but not KL, to $CD34^+$, $HLA\text{-}DR^+$ cell populations delayed the appearance of morphological changes and DNA fragmentation patterns associated with apoptotic cell death. Their results suggested that IL-3, but not KL, promotes haemopoietic progenitor cell survival whereas KL plays a greater role in sustaining more primitive pre-CFC haemopoietic progenitor cells than IL-3. Yee *et al.* (1994) used an *in vitro* model of bone-marrow-derived mast cells to evaluate the role of KL and IL-3 in cell proliferation and survival under conditions of growth factor deprivation and -irradiation. Whereas both IL-3 and KL suppressed apoptosis induced by irradiation or growth factor deprivation, IL-3 (but not KL) induced expression of *bcl*-2, a proto-oncogene associated with the regulation of cell survival (Korsmeyer, 1992). The fact that KL mediated suppression of radiation-induced apoptosis in the mast cells is in agreement with KL described as a radioprotectant (Zsebo *et al.,* 1992). *In vitro,* KL must be added within 1 hour of irradiation to be effective; this is consistent with the *in vivo* finding that KL administered 4 hours after irradiation was ineffective (Zsebo *et al.,* 1992).

One of the more recent cytokines to be identified is the ligand for the tyrosine kinase receptor *flk*-2/*flt*-3 (Lyman *et al.,* 1993; Hannum *et al.,* 1994; Lyman *et al.,* 1994). The *flt*-3 ligand (FL) has been shown to stimulate proliferation in cultures of human bone marrow and foetal liver cells but only modest colony formation in $CD34^+$ bone marrow cell cultures in the presence of serum. Muench *et al.* (1995) recently demonstrated that growth of the most primitive subset of human foetal liver cells ($CD34^{++}$ $CD38^-Lin^-$, light density) required the interaction of at least two cytokines such as FL with GM-CSF, IL-3 or KL. The results of delayed cytokine addition experiments also suggested that, individually, these cytokines promote the survival of the primitive foetal liver cell subset but not their proliferation.

Understanding the regulatory roles of cytokines such as IL-3, KL/SCF and FL in stem cell survival, proliferation and/or differentiation within the haemopoietic microenvironment may provide clues to their most efficacious use in the design of therapeutic protocols to enhance survival and/or proliferation of stem cells following severe radiation damage. It may be more desirable, early after irradiation, to utilise a cytokine with low potential for inducing colony formation but high potential to promote survival and prevention of radiation-induced apoptosis (Table 6.1).

6.3.1.2 Granulopoiesis: G-CSF, GM-CSF

The neutrophil (PMN) is the key cellular component in non-specific host defence against opportunistic pathogens. Infection in the neutropenic patient or animal carries a poor prognosis, particularly when the precise nature of the infection cannot be determined. Neutropenia increases risk of life-threatening infection and the magnitude of the risk is closely correlated with the severity and duration of neutropenia (Bodey *et al.,* 1966, 1986; Pizzo *et al.* 1984, 1987; Trowbridge, 1977). Survival of

Table 6.1 Cytokine-based therapy for treatment of radiation-induced bone marrow aplasia

Single cytokines	Combined cytokines
Current generation	
IL-1, G-CSF	IL-1+G-CSF
IL-3, GM-CSF	IL-3+GM-CSF
IL-6, LIF[a]	IL-3+IL-6
IL-11, KL[a]	IL-6+G-CSF, GM-CSF

Single cytokines	Combined cytokines
Fusion protein IL-3/GM-CSF[a]	IL-3+G-CSF
	KL+G-CSF, GM-CSF
New generation	
Synthokine[a], IL-3 variant	IL-3+IL-11
flt-3/*flk*-2 ligand[a]	IL-11+G-CSF, GM-CSF
c-*mpl* ligand[a]	IL-3v+G-CSF
	c-*mpl* ligand+G-CSF, GM-CSF
	PIXY321+G-CSF
	flt-3/*flk*-2 ligand+PIXY321
	Others? IL-7[b], IL-12[c], IL-13[d]

[a] Synthokine—synthetic cytokine IL-3 (G.D.Searle); KL—c-*kit* ligand, stem cell factor (SCF), steel factor, mast cell growth factor (MGF); fusion protein—IL-3/GM -CSF (PIXY321, (Immunex); LIF— leukemia inhibitory factor; c-mpl ligand=thrombopoietin (TPO, Genentech, ZymoGenetics), megakaryocyte growth and development factor (MGDF, Amgen); *flt*-3/*flk*— 2-foetal liver tyrosine kinase ligand (Immunex)
[b] Jacobsen, F.W. *et al.,* 1993
[c] Jacobsen, S.E.W. *et al.,* 1993
[d] Jacobsen, S.E.W. *et al.,* 1994

neutropenic patients with presumed bacterial infection is greatest following resolution of neutropenia and restoration of neutrophil numbers and function (Bodey *et al.,* 1966; Pizzo *et al.,* 1984, 1993).

The value of GM-CSF and G-CSF as therapy for the treatment of severe neutropenia extends beyond their ability to increase the survival, proliferation, differentiation and amplification of granulocyte CFCs to produce mature neutrophils. Receptors for both G- and GM-CSF exist on all cells of the lineage, from CFC to the mature neutrophil, eosinophil, monocyte and macrophage. Both cytokines can activate or prime neutrophils for enhanced function such as microbicidal activity (Weisbart *et al.,* 1985, 1989; Arnaout *et al.,* 1986; Gasson *et al.,* 1984; Mayer *et al.,* 1987, 1991b; Cohen *et al.,* 1988; Kitagawa *et al.,* 1987) which is critical to host defence against micro-organisms.

GM-CSF has been shown to enhance phagocytic activity of neutrophils against opsonized *S. aureus* and *Candida albicans* (Fleischmann *et al.,* 1986; Weisbart *et al.,* 1989; Lopez *et al.,* 1983), to increase the superoxide anion production in response to stimulation by FMLP (Weisbart *et al.,* 1985) and to augment the microbicidal activity of neutrophils against *S. aureus* and *E. coli*. Preclinical studies have demonstrated the efficacy of GM-CSF administered prophylactically in protecting normal and neutropenic mice from lethal infections by *P. aeruginosa, S. aureus* and *C. albicans* (Mayer *et al.,* 1991b; Tanaka *et al.,* 1989; Bermudez *et al.,* 1990; Bleiberg *et al.,* 1990; Frenck *et al.,* 1990). The data suggest that protection against lethal infections was conferred by the augmentation of neutrophil production in normal or myelosuppressed animals, the timely induction of appropriate cytokines or mediators, and the activation of mature cells for efficient host defence. The timing of these events was underscored in a recent study by Toda *et al.* (1994) in which GM-CSF was administered after the onset of infection, as peritonitis-induced sepsis. In this case, GM-CSF failed to increase the survival rate.

G-CSF enhances the phagocytic activity of PMNs was well as the oxidative metabolism in response to FMLP stimulation. Microbicidal activity against *S. aureus* is increased but not the fungicidal activity against *C. albicans* blastoconidia (Rollides *et al.,* 1991). Preclinical activity of G-CSF has been evaluated in a number of models of both normal and neutropenic animals. Shinomiya *et al.* (1991) demonstrated G-CSF

induced increased phagocytosis, hydrogen peroxide formation and *in vivo* elimination of *L. monocytogenes*. Matsumoto *et al.* (1991) showed the protective effect of G-CSF in cyclophosphamide-induced neutropenic mice from systemic challenge with *S.marcescens, E.coli, S.aureus* and *C.albicans.* Wakiyama *et al.* (1993) using a similar model of neutropenia demonstrated the prophylactic efficacy of G-CSF in protecting the mice against the lethal infection by *P. aeruginosa, S. aureus,* or *K. pneumoniae*. Although G-CSF monotherapy was ineffective when administered after infection and when combined with antibiotics, survival was significantly improved. O'Reilly *et al.* (1992) demonstrated the treatment efficacy of G-CSF in a very stringent model of intra-abdominal infection, caecal ligation and puncture (CLP). G-CSF was evaluated using several treatment protocols including a combination with antibiotic at the time of sepsis induction. Toda *et al.* (1993) demonstrated the therapeutic efficacy of G-CSF in a rat model of CLP in which G-CSF increased the chemotactic and bactericidal activity of neutrophils at the inflammatory site. In another study, antibiotics did not reduce mortality from bacterial and fungal sepsis in mice rendered neutropenic by cyclophosphamide. However, restoration of neutrophil counts by G-CSF administration resulted in 100 per cent survival and return of antibiotic efficacy (Matsumoto *et al.,* 1991). Ono *et al.* (1988) demonstrated that resolution of neutropenia with G-CSF treatment lowered the effective dose of antibiotics in mice challenged with bacterial infections. The efficacy of treatment with cytokines such as G-CSF or GM-CSF may also depend upon modulation of proinflammatory cytokines in addition to augmentation of neutrophil function and number. It is of interest that Görgen *et al.* (1992) recently reported that administration of G-CSF to LPS-challenged, galactosamine-sensitized mice protected against the consequent hepatitis and serum tumour necrosis factor (TNF) increases whereas administration of GM-CSF decreased neither TNF release nor mortality.

New information relative to the induction of gene expression in the PMN, and the action of specific cytokines and their natural antagonists, compel us to revise the role of the PMN in host defence and the inflammatory process (Lloyd and Oppenheim, 1992). The ability of the PMN to migrate to the infectious site and kill bacteria, as well as release toxic oxygen radicals and enzymes is well known. As previously discussed, G-CSF and GM-CSF can significantly modulate both the production and function of the PMN. Recent evidence documents the more interactive, regulatory nature of the PMN. This cell has the capacity to produce not only cytokines, but also cytokine antagonists or binding proteins (soluble receptors) in response to stimulatory cytokines and bacterial products such as endotoxin. The PMN can produce IL-1 , (Lindemann *et al.,* 1988; Marucha, *et al.,* 1990; Lloyd and Oppennheim, 1992), tumour necrosis factor (TNF), G-CSF, M-CSF, GM-CSF (Lindemann *et al.,* 1989; Djeu *et al.,* 1990), IL-3, Il-6 (Cicco *et al.,* 1990), GRO- , interferon- (Shirafuji *et al.,* 1990) and IL-8 (Bazzoni *et al.,* 1991; Cassatella *et al.,* 1992) in response to endotoxin or cytokines such as IL-1, TNF and GM-CSF. The PMN also produces and sheds both TNF receptors (TNFR p60, p80) (Porteu and Nathan, 1990) which act as binding proteins for TNF in addition to an IL-1 receptor (Fasano *et al.,* 1991) and the IL-1 receptor antagonist (IL-1ra) (McColl *et al.,* 1992; Re *et al.,* 1993), a protein that binds to IL-1 receptors thus blocking the receptors for IL-1 occupancy. These properties emphasise the autocrine and paracrine roles of the PMN and stress the ability of the PMN to orchestrate host defence and inflammation.

The value of G-CSF and GM-CSF in the treatment of radiation-induced marrow aplasia lies in their ability to modulate both the production and function of the neutrophil, the key cell in the host's nonspecific response to opportunistic infection. Both growth factors effectively decrease the duration of neutropenia and recovery time of neutrophils to normal levels in irradiated rodents (Kobayashi *et al.,* 1987; Tanikawa *et al.* 1989, 1990; Patchen *et al.,* 1990, 1993, 1994a; Fushiki *et al.,* 1990), dogs (Schuening *et al.,* 1989, 1993; MacVittie *et al.,* 1990; Nothdurft *et al.,* 1992; Farese *et al.,* 1993a), and non-human primates (Monroy *et al.,* 1987, 1988; Farese *et al.,* 1993b, 1994a). G-CSF has been particularly effective in restoration of

neutrophil production in otherwise lethally irradiated dogs (MacVittie *et al.,* 1990; Schuening *et al.,* 1989, 1993). Experiments in large animals such as dogs and primates allow proper utilisation of clinical support with antibiotics, whole-blood or platelet transfusion, fluids and selective gut decontamination. As mentioned earlier, clinical support as replacement/substitution therapy for platelets and neutrophils provides the host with additional time required for adjunctive cytokine therapy to stimulate surviving stem and progenitor cells or nascent target cells to produce mature cells earlier than normal. These experiments emphasise the fact that sufficient numbers of stem cells survive lethal doses of radiation. Lethality ensues in control animals because the surviving stem cells cannot generate mature cells within the critical, clinically manageable period of time dictated by the efficacy of clinical support.

Our laboratory has extended the therapeutic administration of G-CSF in the whole-body irradiated dog over the range of radiation doses from 3 to 6 Gy. All animals received clinical support according to a prescribed protocol plus G-CSF administered from day 1 to day 23 after irradiation. The respective $LD_{50/60}$ and $LD_{95/60}$ with clinical support alone were 3.38 Gy and 4 Gy (MacVittie *et al.,* 1991). Treatment with G-CSF plus clinical support, increased the $LD_{50/60}$ to 5.1 Gy representing a dose reduction factor of 2 (unpublished data) over the 2.6 Gy $LD_{50/60}$ of unsupported dogs (Figure 6.1). Schuening *et al.* (1989) used rcG-CSF and clinical support in a similar canine model to promote survival of four out of five dogs irradiated at their laboratory '$LD_{99/60}$', 4 Gy.

The therapeutic protocols using G-CSF or GM-CSF are based on their *in vitro* and *in vivo* biology, pharmacokinetics and assumptions that a fraction of target cells survive the radiation or drug exposure or that an adequate number of CFCs will spontaneously regenerate soon after exposure and be available for further stimulation by CSF administration. The common protocol is to initiate the CSFs early after exposure and to continue consecutive daily administration to ensure regeneration of CFCs and early production of neutrophils. Testing this protocol by delaying administration of G-CSF or GM-CSF for at least seven to nine days in large animal models of lethal and sublethal radiation or drug exposure has led to conflicting results. Schuening *et al.* (1989) delayed the administration of G-CSF for seven days to otherwise lethally irradiated dogs ($LD_{99/60}$) as compared with initiating daily therapy within two hours of exposure. All dogs in the delayed protocol died consequent to haemopoietic aplasia compared with one out of five in the early protocol. In an unpublished study MacVittie *et al.,* using a similar lethally irradiated canine model ($LD_{60/30}$), confirmed the loss of therapeutic efficacy following a delayed administration schedule of G-CSF. Recombinant canine G-CSF delayed for 9 or 10 days did not decrease the morbidity or mortality associated with this lethal dose of irradiation, whereas initiating G-CSF therapy on day 1 post exposure significantly decreased the neutropenic duration, time to neutrophil recovery and survival ($LD_{30/30}$). In contrast, several other studies in dogs and primates (Meisenberg *et al.,* 1992, Farese *et al.,* 1993b) have shown that a similarly delayed protocol did not alter the haemopoietic response as compared with the early protocol. Khwaja *et al.* (1993) examined the effect of delaying G-CSF administration until 8 days after autologous bone marrow transplant in seventeen patients. They found the delayed G-CSF protocol to be as effective in accelerating neutrophil recovery when compared with historical controls and this translated into a clinical benefit with a reduction in antibiotic therapy.

It is of interest that a study by Sureda *et al.* (1993) showed that a single dose (1 mg/kg) of G-CSF, 2 hours or 24 hours after an $LD_{95/30}$ exposure to mice, increased survival to 78 per cent and 65 per cent respectively. This high dose, single injection therapeutic protocol stands alone, but the above-mentioned studies in dogs (Schuening *et al.,* 1989; MacVittie unpublished) and results obtained in rodents by Tanikawa *et al.* (1990) and Patchen *et al.* (1990) suggest that the earlier and more frequently CSF is administered, the earlier the recovery of haemopoiesis. The results by Sureda *et al.* may involve the pleiotropic action of G-CSF to enhance survival of irradiated HPCs by suppressing apoptosis (Williams,

G.T. *et al.,* 1990). Preservation of an increased survival fraction may allow for early regeneration of CFCs and adequate production of neutrophils necessary for short-term survival.

6.3.1.3
Megakaryocytopoiesis and thrombocytopoiesis: IL-3, IL-6, IL-11, LIF, OSM

The production of platelets via megakaryocytopoiesis and thrombocytopoiesis follows a complex developmental pathway that appeared to be regulated by several haemopoietic growth factors (Hoffman, 1989; Gordon and Hoffman, 1992). IL-3 (Emerson *et al.,* 1988; Kavnoudias *et al.,* 1992; Koike *et al.,* 1986a,b; Quesenberry *et al.,* 1985, 1991; Bruno *et al.,* 1989; Ikebuchi *et al.,* 1987; Yonemura *et al.,* 1992; Stahl *et al.,* 1992; Mayer *et al.,* 1989; Monroy *et al.,* 1991), GM-CSF (Emerson *et al.,* 1988; Quesenberry *et al,,* 1985; Stahl *et al.,* 1991, 1992; Mayer *et al.,* 1987), IL-6 (Burstein *et al.,* 1992a, Mayer *et al.,* 1991a; Asano *et al.,* 1990; Stahl *et al.,* 1991b, Hill *et al.,* 1991; Ishibashi *et al.,* 1989a,b; Leary *et al.,* 1988; Imai *et al.,* 1991; Lotem *et al.,* 1989; Quesenberry *et al.,* 1991; Ziedler *et al.,* 1992; Suda *et al.,* 1988), IL-11 (Burstein *et al.,* 1992b; Teramura *et al.,* 1992; Yonemura *et al.,* 1992; Schlerman *et al.* 1992; Musashi *et al.,* 1991), Leukemia inhibitory factor (LIF) (Metcalf *et al.,* 1990, 1991; Burstein *et al.,* 1992b; Mayer *et al.,* 1993; Leary *et al.,* 1990) and oncostatin-M (OSM) (Wallace *et al.,* 1995) have all been demonstrated to affect megakaryocytopoiesis and platelet production at different stages of development both *in vitro* and *in vivo.* IL-3 is a pivotal T-cell derived cytokine in the stimulation of haemopoiesis. *In vitro* studies have shown that IL-3 stimulated the proliferation of early multipotential progenitors as well as the growth and differentiation of lineage restricted colony forming cells (Quesenberry *et al.,* 1985; Koike *et al.,* 1986a,b; Ikebuchi *et al.,* 1987; Emerson *et al.,* 1988; Leary *et al.,* 1988; Bruno *et al.,* 1989; Kavoudias *et al.,* 1992). Thus IL-3 has been reported to stimulate both megakaryocyte progenitor cells and megakaryocytes. Variable responses however to native IL-3 in normal monkeys (Kavnoudias *et al.,* 1992; Mayer *et al.,* 1989; Krumwieh, 1990; Wagemaker *et al.,* 1990; Monroy *et al.,* 1991; Stahl *et al.,* 1992; Geissler *et al.,* 1992) predicted an equivocal response in preclinical and clinical situations of marrow failure syndromes and radiation or chemotherapy-induced marrow aplasia (Kavnoudias *et al.,* 1992; Mayer *et al.,* 1989; Krumwieh, 1990; Wagemaker *et al.,* 1990; Monroy *et al.,* 1991, Stahl *et al.,* 1992; Geissler *et al.,* 1992; Gillio *et al.,* 1990; Farese *et al.,* 1993b; MacVittie *et al.,* 1994; Winton *et al.,* 1994; Ganser *et al.,* 1990; Kurzrock *et al.,* 1991; Biesma *et al.,* 1992; Nemunaitis *et al.,* 1992; Gerhartz *et al.,* 1992; Fibbe *et al.,* 1992; Vallenga *et al.,* 1992). Gillio *et al.* (1990) showed that whereas IL-3 enhanced myeloid recovery and abrogated the predicted period of neutropenia in cyclophosphamide or 5-FU-treated non-human primates, platelet recovery could not be demonstrated. In contrast, it has been shown that IL-3 enhanced regeneration of platelets, and reduced the duration of thrombocytopenia while showing no effect on recovery of neutrophils in a non-human primate model of radiation-induced marrow aplasia (Farese *et al.,* 1993b; MacVittie *et al.,* 1994). Recently Winton *et al.* (1994) showed that once-daily administration of IL-3 in a non-human primate model of hepsulfam-induced pancytopenia had no demonstrable effect on regeneration of platelets or neutrophils. Therefore, despite its therapeutic potential, native IL-3 is characterised by a relatively narrow therapeutic index.

IL-6, LIF, OSM and IL-11 are structurally related pleiotropic cytokines that share several biological activities (Taga and Kishimoto, 1992). Recent elucidation of the receptors for these cytokines demonstrated a common receptor subunit, the 130 kDa glycoprotein known as gp 130. This is a similar situation to that noted for IL-3, GM-CSF and IL-5 which share a common subunit that is essential for signal transduction and provides the basis for common biological activities (Lopez *et al.,* 1992; Thèze, 1994). As noted for these

cytokines, cross-competition in binding between LIF, IL-6 OSM and IL-11 has been demonstrated and suggested as the basis for shared biological activities (Yang and Yin, 1992; Rose and Bruce, 1991).

Haemopoietic cells that respond to IL-11 range from stem cells to lineage committed-cells of erythroid, megakaryocyte and monocyte lineages. *In vitro,* IL-11 has been shown to promote megakaryocyte colony formation and in combination with IL-3, to mediate megakaryocyte endoreduplication and maturation (Yonemura *et al.,* 1992; Teramura *et al.,* 1992). The pleiotropic action of IL-11 is evidenced when combined with KL or IL-3 and early stem-cell-enriched cell populations. These combinations support the growth of primitive 'blast' colonies that can yield all of the haemopoietic cell lineages. In cultures of megakaryocyte progenitors, IL-11 alone has little effect on colony formation. When combined with IL-3, the number and size of megakaryocyte colonies significantly increases (Yonemura *et al.,* 1992; Teramura *et al.,* 1992; Paul *et al.,* 1990; Burstein *et al.,* 1992b).

In vivo studies in normal mice have shown that administration IL-11 resulted in a marked stimulation of megakaryocytopoiesis and a corresponding increase in peripheral platelet counts (Neben *et al.,* 1993). Further evaluation in lethally irradiated, bone marrow transplanted mice demonstrated that IL-11 can accelerate both neutrophil and platelet recovery (Du *et al.,* 1993). In mice exposed to a combined modality regimen of sublethal irradiation and carboplatin followed by IL-11 administration, recovery of both peripheral platelets and haematocrit was accelerated while leukocyte recovery was unaffected (Leonard *et al.,* 1992). Stimulation of bone marrow and splenic-derived GM-CFC, however, suggested the potential for combined cytokine protocols with G-CSF or GM-CSF.

The therapeutic efficacy of LIF was suggested by *in vitro* studies with murine and human bone marrow cell cultures (Metcalf *et al.,* 1991; Burstein *et al.,* 1992b). Metcalf *et al.* showed that LIF increased megakaryocyte colony formation and megakaryocytes when used in combination with IL-3. LIF alone had no demonstrable direct effect on these parameters despite the presence of LIF receptors on both immature and mature murine megakaryocytes. Burstein *et al.* (1992b) compared LIF with IL-6 and IL-11, and found that LIF promoted megakaryocyte maturation in both murine and human liquid marrow cultures but did not induce an increase in megakaryocyte number in the absence of IL-3. Leary *et al.* (1990) also showed that LIF in combination with IL-3 enhanced human-marrow-derived blast cell colony formation.

Recombinant murine LIF administered to normal mice increased both megakaryocyte progenitor cells in marrow and spleen and resulted in an almost two-fold increase in the circulating platelet count (Metcalf *et al.,* 1990). LIF administered to normal primates for 14 days significantly increased circulating platelets at the end of this time (Mayer *et al.,* 1993). Daily injections of LIF in normal mice or primates had no influence on total and differential leukocyte counts. Farese *et al.* (1994b) examined the therapeutic efficacy of LIF in a non-human primate model of radiation-induced marrow aplasia. Administration of LIF significantly decreased the duration of thrombocytopenia (platelet count at 30000 or 20000 μl^{-1}), i.e. 9.3 days or 6.3 days respectively versus the HSA-treated control monkeys, 12.2 days or 10.2 days respectively. Treatment with LIF did not alter the duration of neutropenia (absolute neutrophil count <1000 μl^{-1}) as compared with controls.

IL-6 has numerous biological activities and appears to play important roles in the regulation of the immune response, the acute phase reaction, and haemopoiesis (Hirano *et al.,* 1990; Kishimoto, 1989; Mayer *et al.,* 1991a). *In vitro s*tudies have shown that IL-6 alone or combined, either sequentially or simultaneously, with IL-3 enhanced the proliferation of myeloid multipotential haemopoietic progenitor cells (Ikebuchi *et al.,* 1987; Imai *et al.,* 1991; Ishibashi *et al.,* 1989b; Quesenberry *et al.,* 1991; Leary *et al.,* 1988). Ikebuchi *et al.* (1988) proposed that IL-6 shifted haemopoietic stem cells from the G_0 to the G_1 stage of the cell cycle where they became more responsive to the effects of additional haemopoietic growth factors. Furthermore, numerous preclinical studies using IL-6 have shown accelerated thrombopoietic

effects in normal mice, dogs and non-human primates (Burstein *et al.,* 1992a; Mayer *et al.,* 1991a; Asano *et al.,* 1990; Stahl *et al.* 1991b; Carrington *et al.,* 1991; Hill *et al.,* 1991, Ishibashi *et al.,* 1989a).

Use of IL-6 in animal models of either drug or radiation-induced marrow aplasia has repeatedly shown its efficacy in the reduction of both the severity and duration of thrombocytopenia (Patchen *et al.,* 1991; Selig *et al.,* 1994; Inoue *et al.,* 1994; Herodin *et al.,* 1992; Takasuki *et al.* 1990; Burstein *et al.,* 1992a; Winton *et al.,* 1994; Zeidler *et al.,* 1992; MacVittie *et al.,* 1994; Farese *et al.,* 1994a). Moreover, the use of IL-6 in rodent models of radiation-induced marrow aplasia effectively accelerates multilineage haemopoietic recovery (Patchen *et al.,* 1991; Inoue *et al.,* 1994) and enhances recovery of platelets. Efficacy of IL-6 in a 5-FU model of myelosuppression has been variable (Takatsuki *et al.,* 1990; Carrington *et al.,* 1992; Inoue *et al.,* 1994). Whereas Takatsuki *et al.,* showed accelerated recovery of CFU-S, GM-CFCs and platelets without a concomitant increase in leukocytes, Carrington *et al.* demonstrated that neither IL-3 nor IL-6 alone could shorten the 5-FU induced thrombocytopenic period. Inoue *et al.* (1994) recently showed the increased therapeutic efficacy of a pegylated (PEG) form of IL-6 relative to native IL-6 in rodent models of both radiation and 5-FU-induced myelosuppression. PEG-IL-6 increased the rate of platelet recovery and completely abrogated the platelet nadir in the X-irradiated animals. No mention was made of the leukocyte recovery kinetics in these IL-6 treated animals.

Recent studies of IL-6 therapeutic efficacy in larger animal models such as dogs and non-human primates confirm the lineage-specific stimulatory effect on megakaryocytopoiesis and platelet production (Selig *et al.,* 1994; Bursten *et al.,* 1992a; Herodin *et al.,* 1992; Zeidler *et al.,* 1992; MacVittie *et al.,* 1994; Farese *et al.,* 1994a). Burstein *et al.* and Selig *et al.* showed IL-6 induced a significantly enhanced recovery from thrombocytopenia in sublethally irradiated dogs. However, a marked increase in anaemia was noted in both models, relative to the non-IL-6 treated controls. In addition both models showed no influence of IL-6 on recovery of blood granulocytes. Additional studies in non-human primates confirmed the efficacy of IL-6 in enhancing recovery from radiation-induced thrombocytopenia although doses of IL-6 and irradiation, injection protocol and form of IL-6 varied (Zeidler *et al.,* 1992; Herodin *et al.,* 1992; MacVittie *et al.,* 1994; Farese *et al.,* 1994a). Zeidler *et al.* compared PEG-IL-6 with the native molecule. He found that 100 μg/kg/day (sc, qd) PEG-IL-6 was required to demonstrate efficacy relative to no effect at 10 μg/kg doses of either native or PEG-modified 11–6. They utilised cynomolgus primates, total body irradiated with 3.8 Gy of 10 MV X-rays. Herodin *et al.* (1992) showed significant efficacy in baboons unilaterally exposed to 5 Gy mixed neutron- radiations with IL-6 administered at 10 μg/kg/d (sc, bid) from day 1 to day 13 after exposure. MacVittie *et al.* (1994) administered IL-6 at 15 μg/kg/d (sc, qd) to 4.5 Gy total-body, bilaterally, mixed neutron- irradiated, rhesus primates also demonstrating marked efficacy in enhancing platelet production. In this protocol IL-6 treatment of irradiated primates improved the platelet nadir, and reduced the duration of thrombocytopenia and time to recovery of platelet levels to baseline. Lineage-specificity in the IL-6 response was also noted in that parameters of neutrophil recovery were unaffected with the exception of significantly modifying the neutrophil nadir. This effect however did not translate into sustained production of neutrophils required to modify the neutropenic duration significantly.

IL-6 induced anaemia has been reported by several groups (Bursten *et al*., 1992a; Geissler *et al.,* 1992; Asano *et al.,* 1990). Burstein *et al.* noted a dose-dependent decrease in haematocrit in normal and irradiated IL-6-treated dogs relative to controls. Geissler *et al.* and Asano *et al.* noted significant decreases in red blood counts and haematocrit in IL-6-treated primates. Our data in normal primates would concur with these observations (unpublished). However, Herodin *et al.* (1992) reported that RBC parameters in irradiated and IL-6-treated baboons were not worsened, whereas our study (MacVittie *et al.,* 1994) actually showed that the radiation-induced anaemia was ameliorated by IL-6 treatment. Treatment with IL-6 alone decreased the requirement for transfusions, lessened the nadir of radiation-induced anaemia and induced an

earlier recovery to baseline values. Although the pathophysiology of the IL-6-induced anaemia remains to be determined, the cytokine-induced production of platelets in the irradiated animals probably contributed to the prevention of bleeding.

Current-generation cytokines evaluated in preclinical models of sublethal radiation or drug-induced myelosuppression as single agent therapeutic protocols have shown significant efficacy in enhancing recovery of haemopoiesis and production of neutrophils and platelets. In addition, several cytokines, G-CSF, GM-CSF, KL and PIXY321 significantly increased the survival of otherwise lethally irradiated animals. These data collectively demonstrate the potential of cytokine therapy to significantly lessen the morbidity and mortality associated with the acute radiation syndrome. Preclinical models in dogs and primates allow the further combination of clinical support regimens and analysis of parameters such as transfusions and antibiotic requirements, durations of neutropenia, thrombocytopenia and anaemia as well as systemic toxicities. Our laboratory experience with a number of cytokines in a single animal model, the sublethally irradiated rhesus monkey, has allowed a cautious comparison of therapeutic efficacy defined as the ability to enhance production of neutrophils and platelets and thereby reduce the respective durations of cytopenia (Table 6.2). Platelet production was enhanced most by IL-6 followed by IL-3 and than LIF. All three cytokines, IL-6, IL-3 and LIF, were more efficacious than either GM-CSF or G-CSF, both of which increased platelet production relative to the controls. The noted platelet effect of G-CSF and GM-CSF in preclinical models has, however, not translated into the observed clinical experience. Such 'false positives' underscore the need for realistic interpretation and extrapolation from animals to the clinic. It is also worth noting that native human IL-3 does not bind efficiently to rhesus monkey target cells and therefore its efficacy may be underestimated, although, as stated in the text, the equivocal preclinical response to IL-3 forecasts its variable clinical efficacy.

The cytokines G-CSF and GM-CSF were not significantly different from each other and both were more efficacious in producing neutrophils than IL-3, IL-6 or LIF relative to the controls. These results forecast similar efficacy in the clinical situation.

Table 6.2 Efficacy of cytokine therapy in a primate model of radiation-induced (4.5 Gy neutron:) marrow aplasia

A.	*Single-agent therapy*
	• Platelet production
	IL-6>IL-3>LIF=GM-CSF=G-CSF>HSA
	• Neutrophil production
	G-CSF=GM-CSF IL-3>IL-6=LIF=HSA
B.	*Combination therapy*
	• Platelet production
	IL-3+GM-CSF$_{CO-AD}$ PIXY321=IL-3/IL-6$_{SEQ}$>IL-3/GM-CSF$_{SEQ}$ = IL-3/IL-6$_{CO-AD}$
	• Neutrophil production
	IL-3/GM-CSF$_{CO-AD}$>PIXY321>IL-3/GM-CSF$_{SEQ}$>IL-3/IL-6$_{CO-AD, SEQ}$
C.	*Cautionary note*
	Results in NORMAL animals may not predict the best protocol for use in animals with MARROW APLASIA.

Note: All cytokine protocols were evaluated in the same primate model of radiation-induced marrow aplasia (Farese *et al.,* 1993, 1994a, b; Williams *et al.,* 1993; MacVittie *et al.,* 1994). Cytokine efficacy described here was evaluated using one dose and therefore may not represent the optimal responses. Doses were chosen based on haematological, toxicity and pharmacokinetic data in normal primates.

Our experience evaluating a delayed administration protocol for G-CSF or GM-CSF is consistent with observations of Meisenberg *et al.* (1992) and Khwaja *et al.* (1993) and in contrast to that noted by Schuening *et al.,* (1989). We have also evaluated IL-6 in a delayed protocol (Farese *et al.,* unpublished) and found that all efficacy for platelet production was lost if IL-6 administration was delayed for seven days relative to starting daily therapy one day after irradiation. Efficacy of selected cytokine therapy after acute, severe radiation exposure will depend upon cytokine biology, dose and protocol as well as the availability of haemopoietic target cells.

6.3.2
Current Generation: Combined Cytokine Protocols

The complications resulting from high dose radiation-induced marrow aplasia are two-fold and require the successful management of both infection and haemorrhage. The haemopoietic growth factors just described are fairly lineage-specific in their ability to accelerate haemopoietic regeneration following radiation or cytotoxic drug exposure. Therefore, the regeneration of the two critical cell lineages, neutrophils and platelets, may require a cytokine with multilineage stimulatory properties or the combination of single lineage-specific cytokines (Table 6.1). *In vitro* analysis of cytokine interactions promote the concept that early stem cells and progenitors require multiple cytokines to trigger proliferation of quiescent populations that would otherwise fail to respond to single cytokines. IL-1, IL-6, IL-7, IL-11, IL-12, IL-13, KL and FL all appear to enhance synergistically their proliferative response to colony-stimulating factors while having little or no effect on growth of haemopoietic progenitor cells when acting alone (Stanley *et al.* 1986; Ikebuchi *et al.,* 1988; Jacobsen S.E.W. *et al.,* 1993, 1994; Jacobsen F.W. *et al.,* 1993; Musashi *et al.,* 1991; Metcalf and Nicola, 1991; Williams *et al.,* 1992; Li and Johnson, 1992; Zsebo *et al.,* 1990b). IL-3 as a multilineage CSF also promotes the survival, growth and differentiation of various progenitor cells including multipotential CFCs and in this capacity may act as a synergistic cytokine used to prime or expand CFCs responsive to other lineage-restricted CSFs. Other cytokines such as KL, Fl, IL-6, IL-7, IL-11, IL-12 and IL-13 have no clonogenic activity of their own but have demonstrated synergistic activity when combined with IL-3 and other cytokines such as G-CSF or GM-CSF. KL, FL and IL-3 have also been shown to increase survival of primitive haemopoietic progenitor cells and may thus play important roles in modulating early radiation-induced apoptosis (Aglietta *et al.,* 1993). Identification of the multiple stimulatory signals for survival, proliferation and differentiation of surviving haemopoietic stem cells consequent to severe depletion by radiation exposure will be essential to the continued development of efficacious combination cytokine protocols (Tables 6.1 and 6.3). Combination cytokine protocols evaluated in preclinical models of radiation or drug-induced marrow aplasia include, IL-1 plus G-CSF or GM-CSF (Moore and Warren, 1987; Moore, 1989), IL-3 and GM-CSF (Farese *et al.,* 1993b; Gonter *et al.,* 1993a; Patchen *et al.,* 1994a), IL-3 plus IL-6 (MacVittie *et al.,* 1994; Winton *et al.,* 1994), IL-6 plus G-CSF or GM-CSF (Farese *et al.,* 1994a; Gonter *et al.,* 1993b; Patchen *et al.,* 1993) and KL plus G-CSF (Molineux *et al.,* 1991; Schuening *et al.,* 1993; Farese *et al.,* 1993a) or GM-CSF (Patchen *et al.,* 1994a).

6.3.2.1 IL-3 plus GM-CSF

IL-3 is conceptually a key cytokine for use in combination protocols based on its capacity for multilineage haemopoietic proliferation and generation of megakaryocytes. Studies in normal primates indicated that the sequential combination of IL-3 plus GM-CSF promoted a synergistic rise in peripheral white blood cells and platelet levels when IL-3 was administered several days before GM-CSF (Donahue *et al.,* 1988; Krumweih *et al.,* 1990; Stahl *et al.,* 1992). The same effect was evident in humans (Lord *et al.,* 1994). Co-administration of IL-3/GM-CSF to normal primates, however, resulted in diminished neutrophil production relative to GM-CSF alone (Farese *et al.,* 1991, unpublished). The sequential combination of IL-3 is based on the concept that IL-3 will expand the pool of GM-CSF sensitive target CFCs for more efficient synergistic generation of neutrophils and megakaryocytes. By con

Table 6.3 Potential combination cytokine protocols: administration schedule v. cytokine activity

Administration schedule	Early brief		Early to full		Delayed to full
Cytokine activity	Survival/viability	plus	Multilineage proliferation, differentiation	plus	Lineage specific proliferation, differentiation
Cytokines	KL, *flt*-3/*flt*-2 G-CSF IL-3, IL-6, IL-7, IL-11, IL-12, IL-13		IL-3, IL-6, IL-11 GM-CSF, G-CSF		G-CSF, GM-CSF EPO

Note: Concept of administration protocol based on *in vitro* and *in vivo* database emphasising survival (apotosis-inhibiting), proliferation and differentiating properties of various cytokines.

trast, the combined administration may result in down modulation of GM-CSF receptors on CFCs and thus dampen the potential for increased production of mature cells.

Our laboratory evaluated the therapeutic efficacy of two combination protocols, the sequential and co-administration of IL-3 and GM-CSF relative to respective single cytokine therapy and delayed GM-CSF administration in sublethally irradiated rhesus monkeys (Farese *et al.,* 1993b) (Figure 6.2) as well as the concomitant IL-3/GM-CSF protocol in sublethally irradiated mice (Patchen *et al.,* 1994a). In the primate study, IL-3/GM-CSF was co-administered from day 1 to day 21 (sc, bid) after exposure while the sequential protocol used IL-3 from day 1 to day 7, and GM-CSF from day 7 to day 21 after exposure. Co-administration of IL-3/GM-CSF reduced the average 16-day period of neutropenia and antibiotic support to 6 days as well as eliminating the need for transfusions. This was also significantly better than GM-CSF alone which had an 11-day duration of neutropenia. Similarly the average 10-day period of thrombocytopenia, which necessitated platelet transfusions in the control animals, was reduced to 3 days. The IL-3 alone group had a 6-day thrombocytopenic period. The sequential IL-3/GM-CSF protocol had no greater effect on PMN production than GM-CSF alone and was less effective than IL-3 alone in reducing thrombocytopenia. This response would not have been predicted from the results noted in normal primates by Donahue *et al.* (1988), Krumweih *et al.* (1990) and Stahl *et al.* (1992) which favoured sequential administration of IL-3 followed by GM-CSF. The therapeutic efficacy of co-administered IL-3/GM-CSF protocol was also confirmed in a rhesus monkey model of hepsulfam-induced myelosuppression (Gonter *et al.,* 1993a). Thus the concomitant IL-3/GM-CSF protocol was capable of enhancing recovery of both

neutrophils and platelets and its efficacy was noted by a lower nadir, a reduced cytopenic duration and recovery time of both cell types relative to respective cytokine control proteins (Figure 6.2).

It is worth noting that the obligatory control of using delayed GM-CSF administration without IL-3 priming in these experiments, accelerated neutrophil recovery in a kinetic fashion similar to that observed in the animals administered the full 21-day course of GM-CSF monotherapy. These results are similar to those of Meisenberg *et al.* (1992) who demonstrated that neutropenia, consequent to a single dose of mechloroethamine, can be equally reduced with either early or delayed G-CSF monotherapy. These results suggest that G-CSF and GM-CSF sensitive CFCs are not available early after this dose of radiation or drug and that it requires at least seven days for generation of responsive CFCs. In addition, early monotherapy with IL-3 did little for the generation of such responsive target cells.

6.3.2.2
IL-6 plus IL-3

In addition to promoting thrombocytopoiesis, IL-6 *in vitro* has been shown to prime stem cells for IL-3-induced proliferation (Ikebuchi *et al.,* 1987). Megakaryocytopoiesis was also enhanced *in vitro* when IL-6 was combined with other cytokines such as IL-3 and GM-CSF (Bruno *et al.,* 1989; Quesenberry *et al.,* 1991; Carrington *et al.,* 1991; Lotem *et al.,* 1989). IL-6 has also been demonstrated to synergise with IL-3, GM-CSF and IL-4 to regulate growth and differentiation of colonies representing all myeloid lineages (Ikebuchi *et al.,* 1988; Leary *et al.,* 1988; Caracciolo *et al.,* 1989; Rennick *et al.,* 1989). When used alone, IL-6 has been shown to stimulate the *in vitro* proliferation of murine GM-CFC (Wong *et al.,* 1988; Suda *et al.,* 1988). The demonstrated *in vitro* pleiotropic action of IL-6 when combined with the multilineage effects of IL-3 have prompted the combination cytokine protocols of IL-3 plus IL-6 and IL-6 plus G-CSF or GM-CSF.

Geissler *et al.* (1992) demonstrated the synergistic effect of sequential administration of IL-3 and IL-6 on platelet production in normal rhesus primates relative to IL-3 or IL-6 alone. The combination protocol did not elicit an increase in leukocytes or GM-CFC progenitors. Mayer *et al.* (1991a) showed in normal primates that IL-6 followed by GM-CSF did not significantly increase the production of platelets or neutrophils relative to the respective effects of each cytokine alone.

The therapeutic efficacy of IL-3 plus IL-6, both sequential and co-administration relative to the respective single cytokine protocols was evaluated in our laboratory using a high dose, sublethally irradiated, non-human primate. With respect to the combination of IL-3/IL-6, the sequential protocol significantly increased production of platelets when compared with HSA-treated controls and primates receiving the concomitant IL-3/IL-6, but the protocol did not enhance platelet recovery over IL-6 alone. However, neutrophil recovery was improved over IL-6 alone and controls. With regard to thrombocytopoiesis, the sequential IL-3/IL-6 protocol was not significantly different in effect from either cytokine alone although platelet recovery was enhanced. It is of interest that Winton *et al.* (1994) showed that while concomitant administration of IL-3/IL-6 was no better than IL-6 alone in reducing the duration of hepsulfam-induced thrombocytopenia, both protocols significantly reduced the frequency of neutrophils $<500\ \mu l^{-1}$ relative to the control-treated groups. It should be noted that Winton's protocol utilised IL-3 at a lower dose, 15 μg kg^{-1}, and a once per day, sc protocol relative to our 25 μg kg^{-1} dose administered sc, twice a day.

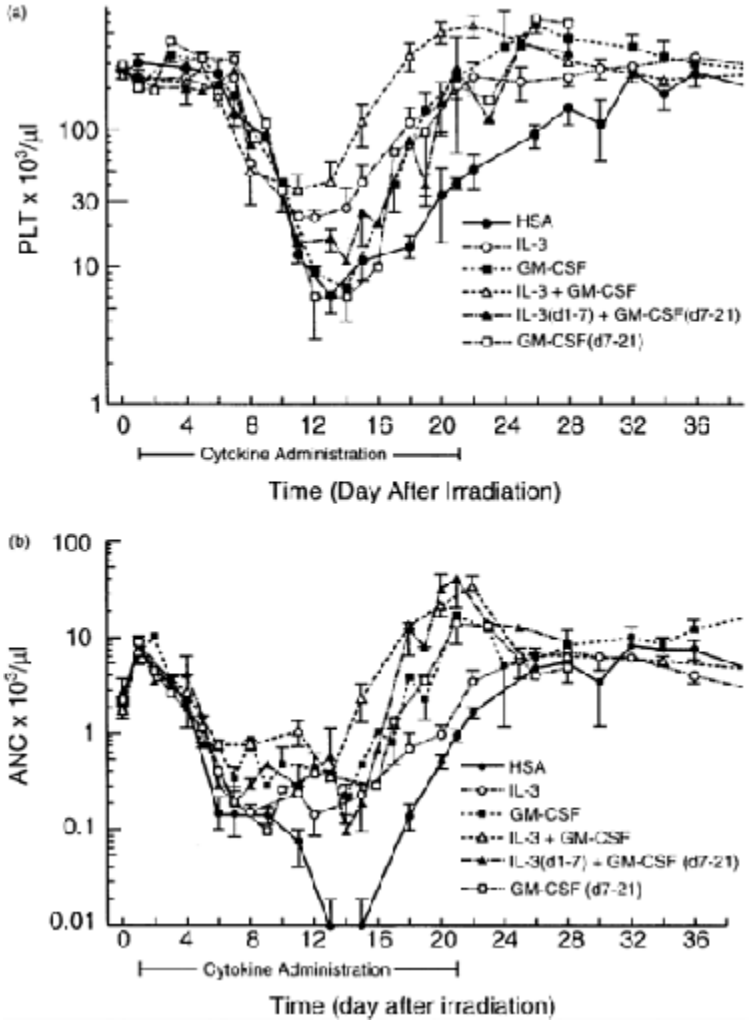

Figure 6.2 Regeneration of the (a) circulating platelet (PLT) and (b) absolute neutrophil counts (ANC) following sublethal irradiation of rhesus monkies treated with HSA or IL3/GM-CSF cytokine protocols. Counts are reported as the mean values±SEM. Animals were administered twice daily with subcutaneous injections rhIL-3 (25 μg/kg/d, n=4), rhGM-CSF (25 μg/kg/d, n=4), rhIL-3 and rhGM-CSF (25 μg/kg/d each, n=4) for 21 days beginning on day 1 following irradiation (4.5 Gy, mixed fission neutron: gamma). In studies of sequential cytokine administration, IL-3 (25 μg/kg/d) was administered for 7 days (beginning on day 1) followed by 14 days of GM-CSF (25 μg/kg/d, n=4). Another cohort of animals received HSA for days 1–7, followed by 14 days of GM-CSF (25 μg/kg/d)

6.3.2.3
IL-6 plus GM-CSF, G-CSF

Recent studies have evaluated the therapeutic efficacy of IL-6 and G-CSF or GM-CSF (Patchen *et al.,* 1993; Gonter *et al.,* 1993b; Farese *et al.,* 1994a). IL-6 plus G-CSF, combined as a therapeutic protocol in a rodent model of radiation-induced aplasia, showed an accelerated recovery of haemopoiesis to include

neutrophils and platelets over each cytokine as monotherapy. Gonter *et al.* (1993b) using the primate model of hepsulfam-induced myelosuppression reported that the concomitant IL-6/ GM-CSF protocol rendered animals transfusion-independent and had the most sustained effect on platelet regeneration. Neutrophil effects in this model appeared to be dominated by the use of GM-CSF or G-CSF. Farese *et al.*'s recent study (1994a) demonstrated that the combination of IL-6 with either G-CSF or GM-CSF can accelerate the production and enhance recovery of both platelets and neutrophils following radiation-induced marrow aplasia. The cumulative data to date suggest that IL-6 combined with G-CSF or GM-CSF will be therapeutically effective in enhancing the recovery of both platelets and neutrophils whereas the sequential use of IL-3 and IL-6 may improve platelet recovery relative to concomitant IL-3/IL-6 or monotherapy with IL-3 or IL-6.

6.3.2.4
IL-11 plus IL-3, GM-CSF

Schlerman *et al.* (1992) have evaluated the combination of IL-11 with either IL-3 or GM-CSF in normal primates. The IL-11/IL-3 or IL-11/GM-CSF concomitant protocols resulted in increased peripheral platelets and neutrophils relative to the administration of IL-11, IL-3 or GM-CSF as single agents. These data taken together with the reported efficacy of IL-11 as monotherapy in myelosuppressed rodents suggest that IL-11 would be useful in monotherapeutic or combined cytokine therapeutic protocols for the management of thrombocytopenia and neutropenia.

6.3.2.5
Fusion proteins

Another approach toward modifying growth factors in an attempt to utilise their different biological characteristics is to form fusion proteins of selected cytokines. The *in vitro* and preclinical literature suggests that in the case of expanding production of CFCs and mature cells, optimal production is achieved when the earlier, more immature pool of progenitor cells is enlarged (Donahue *et al.,* 1988; Krumweih *et al.,* 1990). In this regard IL-3 is a likely candidate based on demonstrated *in vitro* efficacy as a growth factor for stimulation of multipotent and committed myeloid progenitors as well as its synergistic effect on colony formation when combined with G-CSF, GM-CSF, IL-6 (Emerson *et al.,* 1988; Koike *et al.,* 1986; Leary *et al.,* 1988; Quesenberry *et al.,* 1985; Kavnoudias *et al.,* 1992), IL-11 (Paul *et al.,* 1990; Yonemura *et al.,* 1992; Teramura *et al.,* 1992) and erythropoietin (Migliacchio *et al.,* 1988).

In an effort to combine the preclinical and clinical efficacies of IL-3 and GM-CSF, a GM-CSF/IL-3 fusion protein (PIXY321) was developed that exhibited enhanced receptor affinity and biological activity against normal human haemopoietic progenitor cells (Curtis *et al.,* 1991). PIXY321 stimulated more CFU-GEMM, BFU-E and CFU-GM than GM-CSF and/or IL-3. Preclinical efficacy of the GM-CSF/IL-3 fusion protein was demonstrated in a non-human primate model of radiation-induced marrow aplasia (Williams *et al.* 1993a). PIXY321 was shown to accelerate regeneration of both platelet and neutrophil compartments with consequent reduction in days on antibiotics and a significant reduction in the need for platelet transfusions than the animals treated with GM-CSF or IL-3 alone. These results were comparable in preclinical efficacy for enhancing recovery of both neutrophils and platelets as the concomitant administration of IL-3 and GM-CSF in the same model of radiation-induced myelosuppression (Farese *et al.,* 1993b; see Table 6.2). Further evaluation of PIXY321 has been conducted in a lethal model of high dose rate (8 Gy min^{-1}) cobalt-60 irradiation of non-human primates (Williams *et al.,* 1993b). In this

stringent model of radiation damage, all control, non-cytokine-supported animals succumbed as a result of haemopoietic insufficiency. Interestingly, all animals treated with GM-CSF or concomitant IL-3/GM-CSF died, while three of four PIXY321 treated animals survived with full haemopoietic recovery. These preclinical models provide evidence for the clinical efficacy of PIXY321 for the enhanced recovery of both platelets and neutrophils. Clinical trials are currently under way (Vadhan-Raj *et al.,* 1994; Weinthal *et al.,* 1994).

Another fusion protein consisting of IL-3 and erythropoietin (EPO) has been evaluated for *in vitro* activity (Weich *et al.,* 1993). *In vitro* proliferative activity was evaluated on cell lines responsive to the fusion protein or the IL-3, EPO cytokines alone. The results demonstrated that although the IL-3/EPO fusion protein was active, enhancement of erythropoiesis over that observed with a mixture of IL-3 and EPO could not be achieved. This is in contrast to the enhanced *in vitro* activity noted for the GM-CSF/IL-3 fusion protein (Curtis *et al.,* 1991) and emphasises the significance of the variables involved in receptor biology and haemopoietic cell function. The full biological activity of such fusion proteins also underscores their potential for enhancing therapeutic utility. In addition, fusion proteins offer the advantage of single agent administration.

6.3.3
New Generation Cytokines

6.3.3.1
MGDF/TPO

Recent efforts by four different research groups have culminated in the discovery of the elusive growth factor, thrombopoietin (TPO) or megakaryocyte growth and development factor (MGDF), that is lineage-specific for the production of megakaryocytes and platelets (Lok *et al.,* 1994; Kaushansky *et al.,* 1994a,b; Bartley *et al.,* 1994; Wendling *et al.,* 1994; de Sauvage *et al.,* 1994). A seminal piece of the puzzle was the discovery of a novel cellular oncogene, c-*mpl,* by French scientists in 1990 (Souyri *et al.,* 1990). Further confirmation that the ligand for c-*mpl* is responsible for the production of both megakaryocytes and platelets came in 1994 when Wendling *et al.* (1994) demonstrated that both megakaryocytopoietic and thrombopoietic activity in thrombocytopenic plasma could be removed by recombinant c-*mpl*. Methia *et al.* (1994) had previously shown that c-*mpl* antisense oligonucleotides could specifically inhibit megakaryocyte colony formation. The isolation, cloning and purification of the c-*mpl* ligand has been accomplished and the *in vitro* and *in vivo* data accumulated to support the belief that this ligand is indeed the elusive thrombopoietin. The potential clinical impact of this protein is great but there is a significant amount of preclinical biology yet to be performed.

To this end we have recently completed the evaluation of recombinant human (r-Hu) MGDF in normal rhesus monkeys (Farese *et al.,* 1995). The magnitude of the megakaryocytic/thrombopoietic response attained in normal rhesus monkeys to r-HuMGDF is several times greater than the platelet increase observed in primates administered variable doses of either IL-3 (Monroy *et al.,* 1991; Stahl *et al.,* 1992; Geissler *et al.,* 1992; Donahue *et al.,* 1988), IL-6 (Zeidler *et al.,* 1992; Asano *et al.,* 1990; Geissler *et al.,* 1992; Mayer *et al.,* 1991; Herodin *et al.,* 1992), IL-11 (Bree *et al.,* 1991; Schlerman *et al.,* 1992) or LIF (Mayer *et al.,* 1993). These cytokines elicited maximal two-fold responses in platelet counts over the first to second week of treatment. In contrast, the administration of MGDF for 10 consecutive days significantly increased circulating platelet levels within 5 days and reached maximal levels, approximately 600 per cent of baseline values at 12–14 days post cytokine administration (3 days after cessation of treatment)

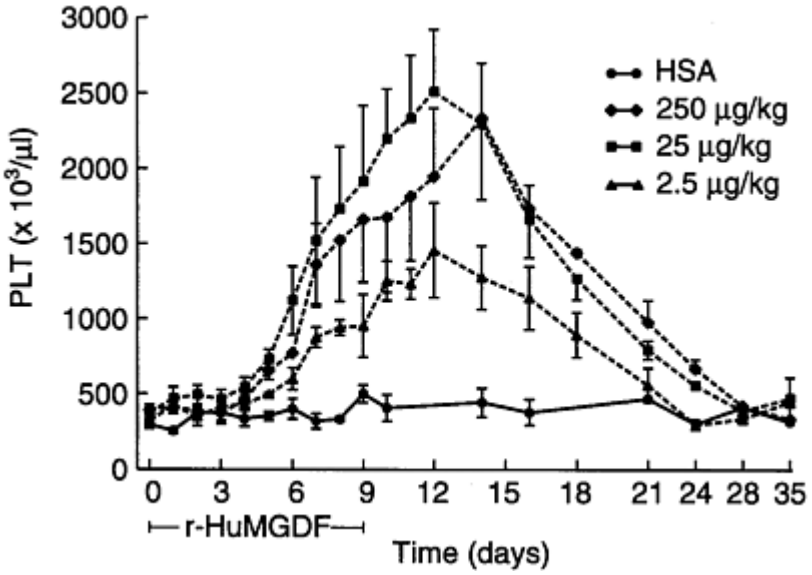

Figure 6.3 Effects of r-HuMGDF administration on platelet counts in normal primates. The platelet (PLT) counts observed in normal rhesus primates *(Macaca mulatta)* following r-HuMGDF which was administered subcutaneously (sc), once per day (qd) for 10 consecutive days at dosages of 2.5 (*n*=3), 25 (*n*=3) or 250 (*n*=2) μg/kg/d of body weight. Control animals (*n*=3) received 25 μg/kg/d of human serum albumin sc, QD for 10 days. Daily CBCs were drawn over the first week and at selected intervals thereafter. Data represent mean±SEM absolute platelet counts for the cytokine or control-treated animals

(Figure 6.3). The peak mean platelet count noted with the 25 μg/kg/d dose was approximately 2500×10^3 μl $^{-1}$. Neither the red nor white blood cell counts were modulated by the administration of MGDF. Bone-marrow-derived clonogenic activity showed increases in CFU-Meg and CFU-GEMM while that of GM-CFC and BFU-E remained unaffected during MGDF administration. Other studies have confirmed that while c-*mpl* ligand plays a central role in thrombopoiesis, other cytokines such as IL-3 alone or together with IL-6 or IL-11 can induce CFU-MK even in the presence of soluble c-*mpl* (Kaushansky *et al.,* 1994). Further evidence that a combination of growth factors other than c-*mpl* ligand can contribute to the regulation of megakaryocytes and platelets is provided by the observation that c-*mpl* deficient mice are not devoid of megakaryocytes and platelets (Gurney *et al.,* 1994).

Two recent studies by Jiang *et al.* (1994) and Wickenhauser *et al.* (1995) underscore the potential haemopoietic regulatory aspects of increased megakaryocytes in a marrow microenvironment recovering from radiation or drug-induced aplasia through the stimulatory action of MGDF/TPO. Human bone-marrow-derived megakaryocytes were found to constituitively express genes for IL-1 , IL-6 and GM-CSF but not TNF (Jiang *et al.,* 1994). Wickenhauser *et al.* (1995) also noted that IL-3 was constituitively secreted and, when used to stimulate megakaryocytes, significantly increased the release of IL-1 , IL-3, IL-6 and GM-CSF. Stimulation of megakaryocytes with either IL-1 , IL-6, IL-11 or GM-CSF also increased the secretion of IL-1 , IL-3, IL-6 and GM-CSF, although not to the same degree observed for the stimulatory effect of IL-3. These data collectively suggest that marrow megakaryocytes participate in autocrine and paracrine loops that not only influence megakaryocyte production and maturation but may also modulate the growth and maturation of other haemopoietic lineages through survival, proliferation and differentiation mechanisms.

6.3.3.2 Flt-3/flk-2 ligand

It has been recognised that cognate ligands for the tyrosine kinase receptors, c-*fms* (CSF-1) and c-*kit* (KL) play a role in regulatory aspects of haemopoiesis at both committed progenitor and stem cell levels. A search to find other tyrosine kinase receptors that may play a role in haemopoiesis led to the discovery of a novel receptor tyrosine kinase designated foetal liver kinase-2 (*flk*-2) (Matthews, 1991) and *flt*-3 (Rosnet *et al.,* 1993). Analysis of *flk*-2 message expression showed that it was largely confined to primitive haemopoietic cell populations, including those capable of long-term reconstitution of lethally irradiated mice (Matthews *et al.,* 1991). Recently the cognate ligand for *flt*-3/*flk*-2 receptor (FL) has been cloned (Lyman *et al.,* 1993; Hannum *et al.,* 1994; Lyman *et al.,* 1994). FL was shown to stimulate proliferation and modest colony formation of a subpopulation of human $CD34^+$ bone marrow cells. FL was also shown to synergise with IL-3, GM-CSF, IL-6 and PIXY321 for colony formation by purified progenitors (Hannum *et al.,* 1994). No synergy was observed with KL or EPO (Lyman *et al.,* 1994). A recent analysis of murine foetal liver and bone marrow stem cell populations that express *flt*-3/*flk*-2 receptor showed that expression is not uniform (Zeigler *et al.,* 1994). There are stem cell populations that do not express the receptor. The *flk*-2^- cell fractions from foetal liver and bone marrow had fewer cycling cells and displayed greater repopulating capacity than their *flk*-2^+ counterparts. Zeigler *et al.* (1994) suggests that the *flt*-3/*flk*-2 receptor is expressed on a subset of haemopoietic cells destined to differentiate to more committed progenitor cells. They further suggest that the murine bone marrow-derived stem cells express $Lin^{lo}Sca^+kit^+CD34^+flk\text{-}2^{\pm}$. Muench *et al.* (1995) recently evaluated FL on purified subpopulations of human foetal liver progenitors. FL alone induced modest proliferation and no colony formation of selected $CD34^{++}CD38^-Lin^-$ and $CD34^{++}CD38^+Lin^-$ progenitor cell populations, whereas synergy was observed between FL and IL-3, GM-CSF or KL in promoting growth of HPP-CFC and LPP-CFC. FL did not have any effect on the most mature subpopulation of foetal liver progenitors and a two-cytokine combination was required for growth of the most primitive subset. These cytokines, FL, IL-3, GM-CSF or KL, also promoted the survival of the early progenitor subset. Muench *et al.* (1995) suggest that FL is active on early haemopoietic progenitors and that it may be part of a redundant network of cytokines that maintains the viability and regulates the growth of stem and progenitor cells. FL does not potentiate the growth of erythropoiesis (Hannum *et al.,* 1994; Lyman *et al.,* 1994) nor affect the growth of mature CFCs.

6.3.3.3 Synthokines

Another approach to the development of new growth factors is the analysis of structure and activity relationships for known cytokines that have a narrow therapeutic index. IL-3 is a cytokine with limited therapeutic potential. Recent information on structure activity relationships of IL-3 have been explored in order to design clinically useful derivatives which would afford enhanced therapeutic efficacy and decreased toxicity (Kaushansky *et al.,* 1992; Lopez *et al.,* 1992; Klein *et al.,* 1994; Thomas *et al.,* 1995).

The synthetic cytokine (Synthokine) SC-55494 is a high affinity IL-3 receptor ligand which has demonstrated greater *in vitro* multilineage growth factor activity than native IL-3 while inducing no significant increase in inflammatory activity relative to native IL-3 (Klein *et al.,* 1994; McKearn *et al.,* 1994; Thomas *et al.,* 1995). We recently evaluated the therapeutic efficacy of this Synthokine on haemopoietic reconstitution in a non-human primate model of high dose (7 Gy) radiation-induced marrow aplasia (Farese *et al.,* 1995). Synthokine was evaluated relative to dose and treatment schedule. Synthokine administered (sc, bid) for 14 or 23 consecutive days beginning on day 1 after exposure to 7 Gy total body

irradiation significantly reduced the duration of thrombocytopenia relative to control animals regardless of dose (25 or 100 μg/kg/d) or protocol length. The most striking reduction was noted in the animals administered 100 μg/kg/d for 23 consecutive days (3.5 days *v.* 12.5 days in control, HSA-treated animals). However, the duration of neutropenia was not altered, although the depth of nadir was significantly lessened in all animal cohorts regardless of dose *v.* schedule length.

These data signal the potential for use of modified cytokines that possess narrow therapeutic indices due to associated systemic toxicities at clinically relevant doses and schedule. Furthermore, the data suggest that combined cytokine protocols utilising G-CSF or GM-CSF would provide the desired effect of enhancing production of both platelets and neutrophils. The demonstrated efficacy of concurrent IL-3 and GM-CSF (Farese *et al.,* 1993b) as well as the IL-3/GM-CSF fusion protein PIXY321 (Williams *et al.,* 1993a) and concurrent IL-6 plus G-CSF or GM-CSF (Farese *et al.,* 1994a) in primate models of radiation-induced marrow aplasia would forecast the comparable efficacy of Synthokine plus G-CSF or GM-CSF.

Despite effective monotherapy with the CSFs, IL-6 and IL-11, neutropenia and thrombocytopenia remain as severe consequences of acute, high dose radiation exposure. Further reduction of this obligate period of cytopenia will require combinations of existing and/or new generation cytokines (Tables 6.1 and 6.3). Combination cytokine protocols of concomitant IL-3/GM-CSF, concomitant IL-6/GM-CSF and IL-6/G-CSF in addition to the fusion protein PIXY321 have demonstrated increased efficacy in promoting increased production of both neutrophils and platelets. Whereas combinations of IL-3/IL-6 promoted increased production of platelets without affecting neutrophil production. Additional combination protocols are now being evaluated. These include the Synthokine plus G-CSF, PIXY321 plus G-CSF and c-*mpl* ligand (MGDF) plus G-CSF. We would also advocate more direct evaluation of combination protocols utilising the survival promoting and/or synergistic capability of cytokines such as IL-3, IL-6, IL-7, IL-11, IL-12, IL-13 or *flt*-3/*flk*-2 ligand in a protocol where early high dose, brief exposure for one to two days immediately after irradiation is followed by sequential or concomitant combination protocols noted above (Table 6.3). Lowry *et al.* (1992) proposed the concept of a cytokine having an 'anchoring activity' in which the candidate cytokine such as KL, IL-3 or FL could preserve the responsiveness of HPCs in the presence of reduced concentrations of other accessory factors. In this way the efficacy of a single factor, administered soon after irradiation, may be the consequence of the *in situ* microenvironmental availability of other interactive cytokines. Our ability to enhance recovery of HPCs further following high dose irradiation will depend upon the informed use of available current and new generation cytokines to regulate survival, proliferation and differentiation of short- and long-term reconstitutional cells.

6.4
Reconstitution of the Haemopoietic System after Radiation-induced Marrow Aplasia: Stem and Progenitor Cell Transplantation

6.4.1
Peripheral Blood-derived Stem Cells

The potential of transplantation using peripheral blood (PB)-derived haemopoietic stem cells was demonstrated in mice 33 years ago by Goodman and Hodgson (1962). This was followed by the demonstration of successful marrow reconstitution with peripheral blood progenitor cells (PBPC) in dogs (Debalik-Fehir *et al.,* 1975; Storb *et al.,* 1967) and non-human primates (Storb *et al.,* 1977). The concept of PB transplantation as a surrogate or adjunct for bone marrow transplantation remains a pleasing one from several standpoints. It would obviate the need for general anaesthesia and risk associated with bone marrow

aspiration, increase the potential for less tumour cell involvement and raise the possibility of autotransplant in patients with prior irradiation of substantial marrow space. The clinical potential of PB autografts has been demonstrated in patients with submyeloablative regimens of chemotherapy and irradiation (Juttner *et al.,* 1985; Kessinger *et al.,* 1988; Reiffers *et al.,* 1986; Korbling *et al.,* 1986; Gianni *et al.,* 1989b). The recent demonstrations that cytokines can efficiently mobilise haemopoietic progenitor cells (HPC) into the peripheral blood in concentrations that may equate or exceed those found in normal steady-state bone marrow has significantly enhanced the clinical potential of peripheral blood stem and/or progenitor cell transplantation (Sheridan *et al.,* 1992; Gianni *et al.,* 1989a, 1990; Elias *et al.,* 1992; Chao *et al.,* 1993; Bensinger *et al.,* 1993; Brugger *et al.,* 1992). Tjonnfjord *et al.* (1994) recently characterised the $CD34^+$ peripheral blood cells mobilised by G-CSF in healthy adults to that of normal bone marrow. PBHSC grafts are enriched for $CD34^+$ cells as a whole in addition to $CD34^+$, $CD33^+$ cells and colony-forming cells; all factors should correlate to accelerated haemopoietic reconstitution and reduction in supportive care with autologous transplantation. The question of whether long-term reconstitution in man will result from mobilised PB haemopoietic stem cell (HSC) transplantation must await allogeneic or genetically marked cell transplantation. The clinical and experimental data suggests that PB autografting of mobilised HPCs will provide for early and rapid engraftment with complete haemopoietic reconstitution (Bensinger *et al.,* 1993; Bolwell *et al.,* 1993; Hohaus *et al.,* 1993; To *et al.,* 1992). The major dilemma faced in the radiation accident scenario for treatment of severely irradiated personnel is the inability to utilise the autologous graft.

Peripheral blood mononuclear cell allografts performed in lethally irradiated dogs several years ago illustrate that PB derived HPCs can provide for long-term reconstitution (Fliedner *et al.,* 1976; Storb *et al.,* 1967). Studies in mice, dogs and non-human primates attest to the ability of mobilised PBHSCs to provide both early and late haemopoietic engraftment in lethally irradiated recipients (Molineux *et al.,* 1990; Bodine *et al.,* 1993; Fleming *et al.,* 1993; Andrews *et al.,* 1992a, 1995; de Revel *et al.,* 1994).

The strategems to mobilise HSC and CFCs in the accident setting are limited to the use of growth factors. Three growth factors or cytokines have been used predominantly to mobilise haemopoietic progenitor cells for transplantation; these are GM-CSF, G-CSF and SCF (*kit* ligand) (Andrews *et al.,* 1992a,b, 1994, 1995; Duhrsen *et al.,* 1988; Socinski *et al.,* 1998; Molineux *et al.,* 1990; Sheridan *et al.,* 1992; Siena *et al.,* 1989; Gianna *et al.,* 1989a, 1990; Haas *et al.,* 1990; Dreger *et al.,* 1993; Ho *et al.,* 1991; Matsunaga *et al.,* 1993). The potential usefulness of IL-3 is also being explored in this area. A preclinical study has demonstrated a modest expansion of circulating haemopoietic progenitors in primates administered IL-3 alone (Geissler *et al.,* 1990). Mobilisation was increased in primates treated with IL-3 followed by GM-CSF relative to IL-3 or GM-CSF alone. The combination of low dose SCF with G-CSF has also been evaluated in several animal models including mice (Briddell *et al.,* 1993; Van *et al.,* 1994), dogs (de Revel *et al.,* 1994) and non-human primates (Andrews *et al.,* 1995). Most recently, Andrews *et al.* (1995) showed that combined SCF and G-CSF effectively mobilised cells that will rapidly engraft lethally irradiated non-human primates (baboons). The efficiency of mobilisation allowed this group to perform a single, 2-hour leukapheresis comparable to that achieved in humans with G-CSF (Pettengell *et al.,* 1993). The kinetics of engraftment using the combined GFs was more rapid than with PBMNCs mobilised by G-CSF alone. Transfusion of non-mobilised PBMNCs in control animals was uniformly lethal. The duration of neutropenia in the combined GF treated group was also less than that noted for baboons transplanted with PBMNCs mobilised by SCF alone (Andrews *et al.,* 1995).

The increased engraftment potential noted for cells mobilised by combined SCF/ G-CSF was similar to that described in murine (Briddel *et al.,* 1993; Van *et al.,* 1994) and canine (de Revel *et al.,* 1994) models. In mice it was also noted that numerically, fewer PBPCs mobilised by combined SCF/G-CSF are required

to rescue lethally irradiated animals compared with G-CSF alone. Long-term reconstitution of engrafted cells was distinguished through use of genetically marked stem cells (Yan *et al.,* 1994). A direct comparison of low and high dose SCF, G-CSF and combined SCF/G-CSF was made in lethally irradiated dogs (de Revel *et al.,* 1994). Although numbers were low, the data suggest that dogs transplanted with the combined SCF/ G-CSF mobilised PBMNCs had reduced neutropenic durations relative to either the high dose SCF or G-CSF groups. The dogs treated with PBMNCs collected after low-dose SCF did not engraft. While neutrophil recovery was enhanced, platelet recovery was slow in each group with prolonged thrombocytopenia requiring 37 to 46 days to reach more than 20000 platelets/μl^{-1}. This differential, favouring neutrophil recovery, was not noted in the above-mentioned baboon model. Platelet recovery was more rapid with the combined treated PBMNCs than with the G-CSF alone mobilised cells. These results speak to the potential differential mechanism(s) of mobilising agents on quantity and quality of HPCs, and CFCs, not to mention the temporal aspect of the mobilisation process. G-CSF and GM-CSF appear to induce a transient increase in circulating CFCs whereas SCF-induced circulating CFCs persist throughout administration period (Andrews *et al.,* 1995). A recent report shows that an inhibitor, MIP-1 , used in combination with G-CSF is also useful in mobilising stem cells (Lord *et al.,* 1995). In particular, cells with marrow repopulating ability appear in large numbers and in a sharply defined time window.

These advantages should translate into an effective PBHSC allograft relative to conventional bone marrow allografts. The effective dose for mobilised PBHSC allografting is unknown but is likely to be larger than for autografts. The other unknown variable is the effect of larger numbers of T-lymphocytes in the PBHSC preparations (Weaver *et al.,* 1993). $CD34^+$ selection with its consequent T-cell depletion and/or additional selective T-cell depletion techniques may be required.

In an effort to explore the use of PBSC/CFC from mobilised PB for allo-transplantation, Lane *et al.* (1995) and Tjonnfjoid *et al.* (1994) administered G-CSF, GM-CSF, or G-CSF plus GM-CSF to normal donors and characterised the percentage of $CD34^+$ cells and subsets, content of clonogenic CFCs and T-cell depletion. Lane *et al.* (1995) demonstrated that G-CSF and combined G/GM-CSF mobilised equivalent numbers of $CD34^+$ and GM-CFU that were both greater than that from the group treated with GM-CSF. T-cells were also depleted by 3 log intervals after $CD34^+$ selection using paramagnetic microspheres (Baxter, Isolex, Baxter Healthcare Corp.). These investigators concluded that sufficient numbers of progenitor cells can be mobilised and collected from the PB of normal donors to perform or support allogeneic bone marrow transplantation by one to maximally two leukapheresis procedures. The Tjonnfjord *et al.* (1994) study emphasised that G-CSF-mobilised PB cells are enriched for $CD34^+$ cells including the $CD34^+$ subsets $CD33^+$, $CD38^-$ and HLA-DR^- as well as increased numbers of CFCs relative to normal BM. All of the above parameters correlate with enhanced auto-engraftment and led the authors to predict that allogeneic transplantation using G-CSF primed PBHSC/CFC grafts will result in more rapid haemopoietic reconstitution after myeloablative conditioning than BM grafting. The recently reported results of phase I/II trials (McNiece *et al.,* 1993; Glaspy *et al.,* 1994) using combined SCF/G-CSF demonstrated enhanced mobilisation of progenitor cells in blood compared with G-CSF alone. These results would support the potential efficacy of cytokine-mobilised PBHSC/CFCs for allogeneic engraftment and long-term reconstitution.

One of the dilemmas presented in the use of mobilised PBPCT for the radiation accident victim is the time required to mobilise and harvest PBPCs from the appropriate donor. It is of interest that two studies evaluated the transplantation potential of PBPCs mobilised after a single injection of a cytokine (Fibbe *et al.,* 1992; Gasparetto *et al.,* 1994). Fibbe *et al.* used a murine transplantation model of syngeneic sex mismatched recipients to demonstrate that a single injection of IL-1 mobilised sufficient PBPCs to provided for long-term engraftment. Gasparetto *et al.* (1994) demonstrated similar efficacy of PBPCs

collected between 1 and 4 days after mobilisation by a single injection of IL-1 . A limiting dose PBMCs was used to evaluate the engraftment potential of transplanted stem cells compared with the non-mobilised PBPCs. All four of the primates which received the IL-1 mobilised PBPCs survived more than 60 days relative to one of four control primates. Durable engraftment for greater than 2 years was noted in two of the four primates. These experiments suggest the possibility that a single injection of IL-1 may increase the concentration of PBPCs over a short period of time to levels capable of short-and long-term engraftment of the severely irradiated host.

6.4.2
Ex vivo Expansion of Haemopoietic Cells

Insights gained from the myriad number of studies investigating the *in vitro* regulation of haemopoietic stem and progenitor cell self-renewal, proliferation and differentiation have provided seminal information for the development of culture conditions providing for regulation of maintenance and expansion of these cells *in vitro*. The ability to manipulate stem and progenitor cells *in vitro* will significantly increase the therapeutic potential of marrow and/or peripheral blood cell transplantation. Translation of potential into reality will revolve around two key capabilities: (1) expansion of haemopoietic stem cells and/progenitor cells with maintenance of long-term pluripotent and short-term multipotent reconstitution, and (2) expansion of haemopoietic CFCs to provide post-progenitor neutrophil and/or platelet precursors. To date, *in vitro* and *in vivo* preclinical studies have provided encouraging results.

Most primitive cells require multiple growth factors for self-renewal and proliferation of both long- and short-term reconstitutional cells. Many experiments have provided clues to the combination of growth factors required for expansion of haemopoietic stem and progenitor cells. Moore and Warren (1987) showed that IL-1 in combination with G-CSF had synergistic activity for production of stem cells and high proliferative potential (HPP) and low proliferative potential (LPP) CFCs using 5-FU resistant bone marrow. Leary *et al.* (1988) showed efficacy of IL-3 and IL-6 in supporting proliferation of human blast cell colonies while Brandt *et al.* (1990) and Kobayashi *et al.* (1991) described production of human progenitor cells from purified bone marrow populations in response to IL-3 and IL-6 or IL-3 and IL-1. Further studies assessing development of HPP-CFU and blast-CFU in post 5-FU bone marrow have shown synergy between IL-1 and IL-6 and with IL-3, G-CSF, GM-CSF and M-CSF (Ikebuchi *et al.,* 1987, 1988). These studies were extended to show that SCF synergises with IL-1, IL-3 or IL-6 in generation of secondary HPP-CFU (Zsebo *et al.,* 1990b). The cloning of the ligand for the c-*kit* receptor, *kit* ligand (KL) by several groups in 1990 (Zsebo *et al.,* 1990a,b; Huang *et al.,* 1990; Anderson *et al.,* 1990; Williams, D.E. *et al.,* 1990) had a major impact on manipulation of stem cells for the purpose of expansion and enhanced mobilisation of stem and progenitor CFCs into the peripheral blood. Short-term, pre-CFC suspension cultures of murine bone marrow cells showed maximum output of both lineage restricted progenitors and HPP-CFC was enriched when four factor combinations of KL, IL-1 and IL-6 with IL-3 or GM-CSF were used (Moore, 1991). Cultures of human $CD34^+$ lin^- bone marrow cells with KL, IL-3 and G-CSF increased colony formation ten-fold (Bernstein *et al.,* 1991). Additional studies with purified human bone marrow cells showed a fifty-fold increase in $CD34^+$, $HLA\text{-}DR^-$ cells with a five-fold increase in HPP-CFC with combined KL and the IL-3/GM-CSF fusion protein, PIXY321 (Srour *et al.,* 1993). It is clear from these and many other studies (Muench *et al.,* 1992a) that *kit* ligand, IL-1, IL-6, G-CSF and IL-3 are important growth factors in stem cell survival and proliferation.

These *in vitro* studies led to the recent demonstration of *in vivo* stem cell fidelity being maintained during *ex vivo* short-term culture conditions (Muench *et al.,* 1992b, 1993). Muench *et al.* (1992b) initially

demonstrated that lethally irradiated mice receiving transplants of *ex vivo* expanded, post 5-FU bone marrow had an accelerated recovery of peripheral blood cells, required fewer transplanted cells for survival and had a comparable haemopoietic proliferative capacity up to 10 weeks after transplant relative to control marrow. Their subsequent study (Muench *et al.,* 1993) clearly documented the long-term reconstitutional efficacy of IL-1 and KL expanded stem cells. The short-term *ex vivo* growth of bone marrow pre-CFU did not appear to damage the lineage potential or proliferative capacity of the stem cell compartment.

A second capability of *ex vivo* expansion proposed by Haylock *et al.* (1992) is for purposes of short-term cellular therapy, that is, the production of post progenitor committed precursors. The obligate periods of cytopenia, both neutropenia and thrombocytopenia associated with transplantation of marrow or peripheral blood stem cells will require the use of more lineage committed precursor cells to accelerate the production of mature cells. Haylock *et al.* (1992) investigated the combination of growth factors required to generate nascent GM-CFC in a 7-day suspension culture of peripheral blood-derived $CD34^+$ cells. A combination of six growth factors including IL-1, IL-3, IL-6, G-CSF, GM-CSF and KL was identified as the most potent combination of those tested. This system generated a 66-fold increase in GM-CFC within 10 days and a 1300-fold increase if extended to 21 days of culture. Cells produced in culture were predominantly neutrophil precursors which developed normally as assessed by morphology, immunophenotype and superoxide production. In the 10-day culture, 40 per cent of the neutrophil precursors were identified as promyelocytes although this phase was enriched for blast-type cells, $CD34^+$ cells and GM-CFC. It was the second phase of culture from 14–21 days that resulted in the largest increase of mature myeloid cells. These investigators calculated that the mix of cells present in day 14–17 cultures of G-CSF-mobilised PB $CD34^+$ cells was capable of providing enough neutrophil precursors to prevent severe neutropenia for a 10-day period post transplant. Further examination of the cytokine cocktail revealed that *kit* ligand and G-CSF were the most important cytokines for generation of neutrophil precursors, while optimal production also required IL-3 and IL-6. IL-1 and GM-CSF could be eliminated without significant detriment to output. It was also noted that the day-14 cultures contained between 2 and 8 per cent megakaryocytic cells which suggested increased potential for the *in vivo* generation of platelets. The recent identification, cloning and production of thrombopoietin provides, to expansion cultures, the lineage specific cytokine critical for the production of megakaryocytes and precursors which may result in the *ex vivo* generation of adequate numbers of these cells required for the successful treatment of the thrombocytopenic patient with cellular infusion therapy.

The body of experiments that have focused on *in vitro* stem cell biology have led to the recent demonstration, that *in vitro,* stroma-free, cytokine-dependent cultures of selected pre-CFC marrow can generate expansion and maintenance of both long-and short-term haemopoietic reconstitutional cells capable of rescuing lethally irradiated animals. Also, mobilised peripheral blood stem cells can provide for the generation of both lineage-committed CFCs and post-progenitor precursors that can generate mature functional neutrophils within days of infusion.

These recent advances may well provide the clinician with an alternative as well as an adjunct to bone marrow or peripheral blood cell transplantation. Utility in the clinical setting, however, is different from that subsequent to a radiation accident. The variable of time is underscored in importance considering that 4–10 days are currently required to mobilise stem cells into the peripheral blood as well as the 7–10 days that are required for *ex vivo* expansion and maintenance of stem cells or CFCs and post-progenitor, lineage-specific precursors (7–17 days) derived from peripheral blood or bone marrow. MIP-1 may help to reduce that initial mobilisation phase. Nevertheless, these data demonstrate the feasibility of transplanting mobilised allogeneic peripheral blood stem cells with adjunctive cellular support for rapid production of mature neutrophils and platelets provided by *ex vivo* expanded, peripheral blood-derived lineage committed precursors. Further research defining the optimum starting cell populations, *ex vivo* culture conditions and

cytokine cocktails for specific target cell production will undoubtedly enhance the clinical potential of these procedures.

6.4.3
Cord Blood-derived Stem Cells

The use of cord blood stem and progenitor cells (CBSC, CBPC) as an alternative source of haemopoietic stem cells has stimulated significant interest in preclinical and clinical evaluation of transplantation potential, efficient separation and storage techniques, and *ex vivo* expansion. Indeed, the use of CBSCs for transplantation following myeloablative therapy for malignant and non-malignant disease, has proved successful for children and adults (Gluckman *et al.,* 1989; Vilmer *et al.,* 1992, 1994; Wagner *et al.,* 1992, 1994a, b; Broxmeyer *et al.,* 1991; Shen *et al.,* 1994). Recent analysis by Wagner *et al.* (1994b) of the International Cord Blood Registry, data on fifty patients aged from 1.3 to 47.8 years continued to support the contention that CBSCs are an acceptable source of transplantable stem cells with a low GVHD potential. It was also reported that neither engraftment of donor cells nor time to haemopoietic recovery correlated with numbers of transplanted nucleated cells or GM-CFC. The number of nucleated cells or other markers such as GM-CFC or CD34+cells required to transplant a child or adult is unknown. The data reported for the registry were 4.3×10^7/kg and 1.9×10^4/kg as median numbers of CB nucleated cells and GM-CFC per transplant respectively.

The potential advantages of using CB as a source of stem cells for transplantation are that:

1 It is abundantly available.
2 It can be harvested without risk to mother or infant and stored for future use.
3 It may have fewer infectious agents than adults.
4 It has a quality of CBSCs greater than those in marrow or peripheral blood.
5 It results in a potential decrease in GVHD after transplant.
6 It can provide a source of rare HLA-phenotypes.

The major disadvantages relative to the radiation accident scenario are the limited number of cells obtainable from a single donor, lack of second access in case of graft failure and the observed relatively slow engraftment (Gluckman *et al.,* 1989; Broxmeyer *et al.,* 1992; Wagner *et al.,* 1992).

The disadvantages may be overcome through preclinical analysis and evaluation of better selection and purification techniques that allow maintenance and expansion of stem cell numbers and quality. A recent conference on cord blood transplantation and biology focused on current knowledge and future directions in cord blood stem cell biology (Broxmeyer, 1994). Presentations at this conference emphasised the potential for *ex vivo* expansion of CB-derived stem and progenitor cells relative to similar phenotypically selected early haemopoietic cell subsets from adult bone marrow and mobilised peripheral blood cells. Lansdorp *et al.* (1993, 1994) emphasised the age-related differences in proliferative potential of purified stem cell subsets. Using $CD34^+$, CD45 Ra^{lo}, $CD71^{lo}$ purified cells from adult bone marrow, foetal liver and cord blood, evidence was presented to support the notion that haemopoietic cells from adult bone marrow have diminished proliferative potential compared with cells from foetal liver or cord blood as evidenced by an age-related decline in their cytokine-stimulated proliferative response. The increased proliferative potential, and hence quality, of cord blood-derived HPCs relative to adult bone marrow has been suggested by their ability to generate increases in $CD34^+$ cells (Xaio *et al.,* 1994; Traycoff *et al.,* 1994), total nucleated cells and progenitor cell populations in *ex vivo,* cytokine-stimulated culture systems (Broxmeyer *et al.,* 1992;

Migliaccio *et al.,* 1992; Mayani *et al.,* 1993; Mayani and Landsdorp, 1994; De Bruyn *et al.,* 1992; Ruggieri *et al.,* 1993; Moore, 1993; Moore and Hoskins, 1994; Landsdorp *et al.,* 1993, 1994) and in culture with irradiated stroma (Hows *et al.,* 1992). Moore and Hoskins (1994) further demonstrated that cells with stem cell features, the LTC-IC and $CD34^+$, $CD38^-$ phenotype, are present in CB with a frequency similar to that in adult bone marrow. However, in contrast to adult bone marrow, the LTC-IC population in CB-derived subsets can be expanded *in vitro* via cytokine cocktail stimulation by 20–100-fold.

The ability to expand CB-derived stem cells and/or progenitor cells while maintaining the quality of engraftable short- and long-term reconstituting cells in concert with the noted advantages of cord blood stem cells may provide an excellent alternative to use of bone marrow transplantation. Further insights into age-related differences in stem cell biology and the interactive relationship with the adult marrow microenvironment and cytokine modulation will help in defining the true potential of cord blood-derived stem cells relative to graft stability, level of GVHD and post graft manipulation by cytokine administration.

6.5 Conclusions

The *in vitro* and *in vivo* preclinical experience to date suggest a major role for the current and future generation cytokines in enhancing haemopoietic recovery from severe radiation exposure (Tables 6.1 and 6.3). However, efficacy in animal models must be viewed with regard to species, cytokine type, dose and protocol and pharmacokinetics as well as radiation dose and dose rate. It is worth noting that in spite of the demonstrated efficacy of cytokine therapy, the majority of experiments were probably not conducted with the optimally defined therapeutic dose or protocol in the myeloablated animal. In addition, the irradiated animals may be less sensitive to the inflammatory and 'other organ system' effects of cytokines at the dose and protocol utilised.

Use of cytokines in preclinical animal models has demonstrated remarkable efficacy in modulating the morbidity and mortality associated with severe radiation exposures. One of the fears associated with initial cytokine protocols was that the excessive pressure to differentiate and produce mature cells would exhaust the surviving numbers of stem cells below that required to reconstitute the haemopoietic system. This has proved not be the case, and in fact the use of cytokines G-CSF, PIXY321, and KL has induced long-term survival in otherwise lethally irradiated and clinically supported animals. In addition, use of cytokine therapy in high dose, sublethally irradiated animals ensured survival with significant enhancement of haemopoietic recovery relative to the non-treated controls.

The uncontrolled and ill-defined nature of radiation accidents forecasts a non-uniform exposure with variable dose distribution over the haemopoietic tissue. Such exposure geometry suggests a potential sparing of haemopoietic stem cells. The availability of surviving stem and progenitor cells allows for potential manipulation with selective combination cytokine therapy. Early but brief use of survival-promoting cytokines may prevent radiation-induced apoptosis and allow time for repair and stabilisation of a small surviving population of stem and progenitor cells. Subsequent use of combination cytokine protocols that promote self-renewal and proliferation of respective stem cell populations in sequential or concomitant fashion may allow maximum expansion and differentiation of cell clones responsible for short- and long-term reconstitution of the haemopoietic system.

Several areas remain to be evaluated with regard to radiation effects. These include the determination of cytokine efficacy in animals exposed to acute, high dose and high dose rate exposure as well as to fractionated and continuous irradiation scenarios. The question of high dose rate exposure is an interesting one because of the potential involvement of radiation effects on other organ systems such as lung and

cutaneous tissue that may impact on long-term morbidity of cytokine-treated and haemopoietically reconstituted hosts. Will the pleiotropic action of certain haemopoietic cytokines exacerbate the radiation effects on non-haemopoietic tissues? The questions pertaining to treatment during fractionated irradiation or continuous exposure are similar to the clinical situations of multiple-course chemotherapy and radiolabelled immunotherapy. These questions will focus research on the interaction among radiation biology, stem cell heterogeneity, radiosensitivity, and recovery and the ability of the selected cytokines to modulate the viability, proliferation and differentiation of surviving HSC. The information and insight gained relative to stem cell kinetics and therapeutic potential of cytokine manipulation will allow for more rational design of treatment protocols.

References

AGLIETTA, M., SANAVIO, F., STACCHINI, A. *et al.* (1993) Interleukin-3 *in vivo:* kinetic of response of target cells, *Blood,* **82**, 2054–61.

AINSWORTH, E.J. (1988) From endotoxins to newer immunomodulators: survival promoting effects of microbial polysaccharide complexes in irradiated mice, *Pharmacology Therapy,* **39**, 233–41.

ANDERSON, D.M., LYMAN, S.D., BAIRD, A. *et al.* (1990) Molecular cloning of mast cell growth factor: a hematopoietin that is active in both membrane bound and soluble forms, *Cell* **63**, 235–43.

ANDREWS, R.G., BENSINGER, W.I., KNITTER, G.H. *et al.* (1992a) The ligand for c-*kit,* stem cell factor, stimulates the circulation of cells that engraft lethally irradiated baboons, *Blood,* **80**, 2715–20.

ANDREWS, R.G., BARTELMEZ, S.H., KNITTER, G.H. *et al.* (1992b) A c-*kit* ligand, recombinant human stem cell factor, mediates reversible expansion of multiple $CD34^+$ colony-forming cell types in blood and marrow of baboons, *Blood,* **80**, 920–7.

ANDREWS, R.G., BRIDDELL, R.A., KNITTER, G.H. *et al.* (1994) *In vivo* synergy between recombinant human stem cell factor and recombinant human granulocyte colony-stimulating factor in baboons: enhanced circulation of progenitor cells, *Blood,* **84**, 800–10.

ANDREWS, R.G., BRIDDELL, R.A., KNITTER, G.H. *et al.* (1995) Rapid engraftment by peripheral blood progenitor cells mobilized by recombinant human stem cell factor and recombinant human granulocyte colony-stimulating factor in nonhuman primates, *Blood,* **85**, 15–20.

ARNAOUT, M.A., WANG, E.A., CLARK, S.C. & SIEFF, C.A. (1986) Human recombinant granulocyte-macrophage colony-stimulating factor increases cell-to-cell adhesion and surface expression of adhesion-promoting surface glycoproteins on mature granulocytes, *J. Clinical Investigation,* **78**, 597–601.

ASANO, S., OKANO, A, OZAWA, K. *et al.* (1990) *In vivo* effects of recombinant human interleukin-6 in primates: stimulated production of platelets, *Blood,* **75**, 1602–5.

BAGBY, G.J., PLESSALA, K.J., WILSON, L.A., THOMPSON, J.J. & NELSON, S. (1991) Divergent efficacy of antibody to tumor necrosis factor- in intravascular and peritonitis models of sepsis, *J. Infectious Disease,* **163**, 83–8.

BAGDASAROV, A.A., RAUSHENBAKK, M.O., ABDULLAEV, G.M. *et al.* (1959) The treatment of acute radiation sickness with packed platelets, *Problems in Hematology Blood Transfusion,* **4**, 1–5.

BARTLEY, T. BOGONBERGER, J. HUNT, P. *et al.* (1994) Identification and cloning of a megakaryocyte growth and development factor that is a ligand for the cytokine receptor *Mpl, Cell,* **77**, 1117–24.

BAZZONI, F., CASSATELLA, M.A., ROSSI, F., CESKA, M., DEWALD, B. & BAGGIOLINI, M. (1991) Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/interleukin-8, *J. Experimental Medicine,* **173**, 771–4.

BEGLEY, C.G., LOPEZ, A.F., NICOLA, N.A. *et al.* (1986) Purified colony-stimulating factor enhances the survival of human neutrophils and eosinophils *in vitro:* using a rapid and sensitive assay for colony-stimulating factor, *Blood,* **68**, 162–6.

BENJAMIN, W.R., TARE, N.S., HAYES, T.J. BECKER, J.M. & ANDERSON, T.D. (1989) Regulation of hemopoiesis in myelosuppressed mice by human recombinant IL-1 alpha, *J Immunology,* **142**, 792–9.

BESINGER, W., SINGER, J., APPELBAUM, F. *et al.* (1993) Autologous transplantation with peripheral blood mononuclear cells collected after administration of recombinant granulocyte stimulating factor, *Blood,* **81**, 3158–63.

BERG, R.D. (1981) Promotion of the translocation of enteric bacteria from the gastrointestinal tracts of mice by oral treatment with penicillin, clindamycin or metronidazole, *Infection and Immunity,* **33**, 854–61.

BERMUDEZ, L.E., MARTINELLI, J.C., GASCON, R., WU, M. & YOUNG, L.S. (1990) Protection against gram-negative bacteremia in neutropenic mice with recombinant granulocyte-macrophage colony-stimulating factor, *Cytokine,* **2**, 287–93.

BERNSTEIN, I.D., ANDREWS, R.G. & ZSEBO, K.M. (1991) Recombinant human stem cell factor enhances the formation of colonies by $CD34^+$ and $CD34^+lin^-$ cells and the generation of colony-forming cell progeny from $CD34^+lin^-$ cells cultured with interleukin-3, granulocyte colony-stimulating factor, or granulocyte-macrophage colony-stimulating factor, *Blood,* **77**, 2316–21.

BERTONCELLO, I., HODGSON, G.S. & BRADLEY, T.R. (1985) Multiparameter analysis of transplantable hemopoietic stem cells: the separation and enrichment of stem cells homing to marrow and spleen on the basis of rhodamine-123 fluorescence , *Experimental Hematology,* **13**, 999–1006.

BIESMA, B., WILLEMSE, P.H., MULDER, N.H. *et al.* (1992) Effects of interleukin-3 after chemotherapy for advanced ovarian cancer, *Blood,* **80**, 1141–8.

BLEIBERG, I. RIKLIS, I. & FABIAN, I. (1990) Enhanced resistance of bone marrow transplanted mice to bacterial infection induced by recombinant granulocyte-macrophage colony-stimulating factor, *Blood,* **75**, 1262–6.

BODEY, G.P. (1986) Infection in cancer patients: a continuing association, *Am. J. Medicine,* **81** (suppl. 1A), 11–26.

BODEY, G.P., BUCKLEY, M., SATHE, Y.S. & FREIREICH, E.J. (1966) Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia, *Annals of Internal Medicine,* **64**, 328–40.

BODINE, D.M., ORLIC, D., BIRKETT, N.C. & SEIDEL, N.E. (1992) Stem cell factor increases colony-forming unit-spleen number *in vitro* in synergy with interleukin-6, and *in vivo* in SI/SId mice as a single factor, *Blood,* **79**, 913–19.

BODINE, D.M., SEIDEL, N.E., ZSEBO, K.M. & ORLIC, D. (1993) *In vivo* administration of stem cell factor to mice increases the absolute number of pluripotent hematopoietic stem cells, *Blood,* **82**, 445–55.

BOLWELL, B.J., FISHLEDER, A., ANDRESEN, S.W. *et al.* (1993) G-CSF primed peripheral blood progenitor cells in autologous bone marrow transplantation: parameters affecting bone marrow engraftment, *Bone Marrow Transplantation,* **12**, 609–14.

BRACH, M.A., DEVOS, S., GRUSS, J.J. & HERRMANN, F. (1992) Prolongation of survival of human polymorphonuclear neutrophils by granulocyte-macrophage colony-stimulating factor is caused by inhibition of programmed cell death, *Blood,* **80**, 2920–4.

BRANDT, J., SROUR, E.F., VAN BESIAN, K., BRIDDELL, R.A. & HOFFMAN, R. (1990) Cytokine-dependent long-term culture of highly enriched precursors of hematopoietic progenitor cells form human bone marrow, *J. Clinical Investigation,* **86**, 932–41.

BRANDT, J.E., BHALLA, K. & HOFFMAN, R. (1994) Effects of interleukin-3 and c-*kit* ligand on the survival of various classes of human hematopoietic progenitor cells, *Blood,* **83**, 1507–14.

BREE, A., SCHLERMAN, F., TIMONY, G., MCCARTHY, K., STOUDEMIRE, J. & GARNICK, M. (1991) Pharmacokinetics and thrombopoietic effects of recombinant human interleukin 11 (rhIL-11) in nonhuman primates and rodents, *Blood,* **78**, 132a.

BRIDDELL, R.A., HARTLEY, C.A., SMITH, K.A. & MCNIECE, I.K. (1993) Recombinant rat stem cell factor synergizes with recombinant human granulocyte colony-stimulating factor *in vivo* in mice to mobilize peripheral blood progenitor cells that have enhanced repopulating potential, *Blood,* **82**, 1720–3.

BROOK, I., WALKER, R.I. & MACVITTIE, T.J. (1986) Effects of radiation dose on the recovery of aerobic and anaerobic bacteria from mice, *Canadian J. Microbiology,* **37**, 719–22.

(1988) Effect of antimicrobial therapy on the gut flora and bacterial infection in irradiated mice, *In. J. Radiation Biology,* **5**, 175–91.

BROXMEYER, HAL E. (ed.) (1994) International Conference of Cord Blood Transplantation and Biology/ Immunology. *Blood Cells,* **20** (2/3).

BROXYMEYER, H.E., KURTZBERG, J., GLUCKMAN, E. *et al.* (1991) Umbilical cord blood hematopoietic stem and repopulating cells in human clinical transplantation, *Blood Cells,* **17**, 313–29.

BROXMEYER, H.E., HANGOC, G., COOPER, S. *et al.* (1992) Growth characteristics and expansion of human umbilical cord blood and estimation of its potential for transplantation in adults, *Proceedings for the National Academy of Sciences, USA,* **89**, 4109–13.

BRUGGER, W., BROSS, K., FRISCH, J. *et al.* (1992) Mobilization of peripheral blood progenitor cells by sequential administration of interleukin-3 and granulocyte-macrophage colony stimulating factor following polychemotherapy with etoposide, ifosfamide and cisplatin, *Blood,* **79**, 1193–200.

BRUNO, E., MILLER, M.E. & HOFFMAN, R. (1989) Interacting cytokines regulate *in vitro* human megakaryocytopoiesis, *Blood,* **73**, 671–7.

BURSTEIN, S.A., DOWNS, T., FRIESE, P. *et al.* (1992) Thrombocytopoiesis in normal and sublethally irradiated dogs: response to human interleukin-6, *Blood,* **80**, 420–8.

BURSTEIN, S.A., MEI, R., HENTHRON, J., FRIESE, P. & TURNER, K. (1992b) Leukemia inhibitory factor and interleukin-11 promote the maturation of murine and human megakaryocytes *in vitro, Journal of Cellular Physiology,* **153**, 305–12.

CACERES-CORTES, J., RAJOTTE, D., DUMOUCHEL, J., HADDAD, P. & HOANG, T. (1994) Product of the steel locus suppresses apoptosis in hemopoietic cells. Comparison with pathways activated by granulocyte macrophage colony-stimulating factor, *J. Biological Chemistry,* **269**, 12084–91.

CARACCIOLO, D., CLARK, S.C. & ROVERA, G. (1989) Human interleukin-6 supports granulocyte differentiation of hematopoietic cells and acts synergistically with GM-CSF, *Blood,* **73**, 666–70.

CARRINGTON, P.A., HILL, R.J., STENBERG, P.E. *et al.* (1991) Multiple *in vivo* effects of interleukin-3 and interleukin-6 on murine megakaryocytopoiesis, *Blood,* 77, 34–41.

CARRINGTON, P.A., HILL, R.J., LEVIN, J. & VEROTTA, D. (1992) Effects of interleukin-3 and interleukin-6 on platelet recovery in mice treated with 5-fluorouracil, *Experimental Hematology,* **20**, 462–9.

CARTER, P.B. & COLLINS, F.M. (1974) The route of enteric infection in normal mice, *J. Experimental Medicine,* **139**, 1189–203.

CASSATELLA, M.A., BAZZONI, F., CESKA, M., FERRO, I., BAGGIOLINI, M. & BERTON, G. (1992) IL-8 production by human polymorphonuclear leukocytes, *J. Immu nology,* **148**, 3216–20.

CASTELLI, M.P., BLACK, P.L., SCHNEIDER, M., PENNINGTON, R. ABE., F. & TAL-MADGE, J.E. (1988) Protective, restorative and therapeutic properties of recombinant human IL-1 in rodent models. *J. Immunology,* **140**, 3830–7.

CHAO, N.J., SCHRIBER, J.R., GRIMES, K. *et al.* (1993) Granulocyte colony-stimulating factor 'mobilized' peripheral blood progenitor cells accelerate granulocyte and platelet recovery after high-dose chemotherapy, *Blood,* **81,** 2031–5.

CICCO, N.A., LINDEMANN, A., CONTENT, J. *et al.* (1990) Inducible production of interleukin-6 by human polymorphonuclear neutrophils: role of granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-alpha, *Blood,* **75**, 2049– 52.

COHEN, A.M., HINES, D.K., KORACH, E.S. & RATZKIN, B.J. (1988) *In vivo* activation of neutrophil function in hamsters by recombinant human granulocyte-macrophage colony-stimulating factor, *Infection and Immunity,* **56,** 2861–5.

COLLINS, F.M. (1956) Mucosal defenses against Salmonella infection in the mouse, *J. Infectious Diseases,* **129,** 503–10.

COLLINS, M.K., MARVEL, J., MALDE, P. & LOPEZ-RIVAS, A. (1992), Interleukin-3 protects murine bone marrow cells from apoptosis induced by DNA damaging agents, *J. Experimental Medicine,* **176,** 1043–51.

COLLOTTA, R., RE, F., POLENTARUTTI, N., SOZZANI, S. & MANTOVANI, A. (1992) Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products, *Blood,* **80**, 2012–20.

COX, G., GAULDIE, J. & JORDANA, M. (1992) Bronchial epithelial cell-derived cytokines (G-CSF and GM-CSF) promote the survival of peripheral blood neutrophils *in vitro, Am. J. Respiratory Cell Molecular Biology,* **7**, 507–13.

CRAIG, W.A. (1993) Postantibiotic effects in experimental infection models: relationship to *in vitro* phenomena and to treatment of infections in man. *J. Antimicrobial Chemotherapy,* **31**, 149–58.

CURTIS, B.M. WILLIAMS, D.E., BROXMEYER, H.E. *et al.* (1991) Enhanced activity of a human granulocyte macrophage colony stimulating factor-interleukin-3 fusion protein, *Proceedings of the National Academy of Sciences, USA,* **88**, 5809–13.

DAVIDSON, D.E., GRENAN, M.M. & SWEENEY, T.R. (1980) Biological characteristics of some improved radioprotectors. In L.W.Brady (ed.) *Radiosensitizers: Their Use in the Clinical Management of Cancer.* New York: Masson, pp. 309–20.

DE BRUYN, C., DELFORGE, A., BERNIER, M. *et al.* (1992) Expansion of purified $CD34^+$ cord blood cells in stromal cell-free long-term cultures in presence of recombinant growth factors, *Blood,* **82** (suppl. 1), 481a.

DEBELAK-FEHIR, K.M. & EPSTEIN, R.B. (1975) Restoration of hematopoiesis in dogs by infusion of cryopreserved autologous peripheral white cells following busulfancyclophosphamide treatment, *Transplantation,* **20**, 63–7.

DE REVEL, T., APPELBAUM, F.R., STORB, R. *et al.* (1994) Effects of granulocyte colony-stimulating factor and stem cell factor, alone and in combination, on the mobilization of peripheral blood cells that engraft lethally irradiated dogs, *Blood,* **83**, 3795–9.

DE SAUVAGE, F.J., HASS, P.E., SPENCER, S.D. *et al.* (1994) Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand, *Nature,* **369**, 533–8.

DJEU, J.Y., SERBOUSEK, D. & BLANCHARD, D.K. (1990) Release of tumor necrosis factor by human polymophonuclear leukocytes, *Blood,* **76**, 1405–9.

DONAHUE, R.E., SEEHRA, J., METZGER, M. *et al.* (1988) Human IL-3 and GM-CSF act synergistically in stimulating hematopoiesis in primates, *Science,* **241**, 1820–2.

DREGER, P., SUTTORP, M., HAFERLACH, T., LÖFFLER, H., SCHMITZ, N. & SCHROYENS, W. (1993) Allogeneic granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells for treatment of engraftment failure after bone marrow transplantation, *Blood,* **81**, 1404.

DU, X.X., NEBEN, T., GOLDMAN, S. & WILLIAMS, D.A. (1993) Effects of recombinant interleukin-11 on hematopoietic reconstitution in transplant mice: acceleration of recovery of peripheral blood neutrophils and platelets, *Blood,* **81**, 27–34.

DUHRSEN, U., VILLEVAL, J-L., BOYD, J., KANNOURAKIS, G., MORSTYN, G. & METCALF, D. (1988) Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients, *Blood,* **72**, 2074–81.

ELIAS, A.D. AYASH, L., ANDERSON, K.C. *et al.* (1992) Mobilization of peripheral blood progenitor cells by chemotherapy and granulocyte-macrophange colony-stimulating factor for hematologic support after high-dose intensification for breast cancer, *Blood,* **79**, 3036–44.

EMERSON, S.G., YANG, Y.C., CLARK, S.C. & LONG, M.W. (1988) Human recombinant granulocyte-macrophage colony stimulating factor and interleukin-3 have overlapping but distinct hematopoietic activities, *Journal of Clinical Investigation,* **82**, 1282–7.

FAIRBAIRN, L.J., COWLING, G.J., REIPERT, B.M. & DEXTER, T.M. (1993) Suppression of apoptosis allows differentiation and development of a multipotent hemopoietic cell line in the absence of added growth factors, *Cell,* **74**, 823–32.

FARESE, A.M. KIRSCHNER, K.F., PATCHEN, M.L., ZSEBO, K.M. & MACVITTIE, T.J. (1993a) The effect of recombinant canine stem cell factor and/or recombinant canine granulocyte colony stimulating factor on marrow aplasia recovery in lethally irradiated canines, *Experimental Hematology,* **21**, 1169.

FARESE, A.M., WILLIAMS, D.E., SEILER, F.R., MACVITTIE, T.J. (1993b) Combination protocols of cytokine therapy with interleukin-3 and granulocyte-macrophage, colony-stimulating factor in a primate model of radiation-induced marrow aplasia, *Blood,* **82**, 3012–18.

FARESE, A.M., MYERS, L.A. & MACVITTIE, T.J. (1994a) Therapeutic efficacy of the combined administration of either recombinant human interleukin-6 and rh-granulocyte colony stimulating factor or rh-granulocyte-macrophage colony stimulating factor in a primate model of radiation-induced marrow aplasia, *Experimental Hematology,* **22**, 684.

FARESE, A.M., MYERS, L.A. & MACVITTIE, T.J. (1994b) Therapeutic efficacy of recombinant human leukemia inhibitory factor in a primate model of radiation-induced marrow aplasia, *Blood,* **84**, 3675–8.

FARESE, A.M., HERODIN, F., GRAB, L.A. *et al.* (1995) Acceleration of hematopoietic reconstitution with a synthetic cytokine (SC-55494) following radiation induced bone marrow aplasia, *Blood,* in press.

FARESE, A.M., HUNT, P., BOONE, T.C. & MACVITTIE, T.J. (1995) Recombinant human megakaryocyte growth and development factor (r-HuMGDF) simulates megakaryocytopoiesis in normal primates, *Blood,* **26**, 54–9.

FASANO, M.B., COUSART, S., NEAL, S. & MCCALL, C.E. (1991) Increased expression of the interleukin-1 receptor on blood neutrophils of human with the sepsis syndrome, *J. Clinical Investigation,* **88**, 1452–9.

FIBBE, W.E., RAEMAEKERS, J., VERDONCK, L.F. *et al.* (1992) Human recombinant interleukin-3 after autologous bone marrow transplantation for malignant lymphoma, *Annals of Oncology,* **3** (suppl. 1), 163.

FLEISCHMANN, J., GOLDE, D.W., WEISBART, R.H. & GASSON, J.C. (1986) Granulocyte-macrophage colony-stimulating factor enhances phagocytosis of bacteria by human neutrophils, *Blood,* **68**, 708–11.

FLEMING, W.H., ALPERN, E.J., UCHIDA, N., IKUTA, K. & WEISSMAN, I.L. (1993) Steel factor influences the distribution and activity of murine hematopoietic stem cells *in vivo, Proceedings of the National Academy of Sciences, USA,* **90**, 3760–4.

FLIEDNER, T.M., FLAD, H.D., BRUCH, C.H., *et al.* (1976) Treatment of aplastic anemia by blood stem cell transfusion: a canine model, *Haematologica,* **61**, 141–56.

FRENCK, R.W., SARMAN, G., HARPER, T.E. & BUESCHER, E.S. (1990) The ability of recombinant murine granulocyte-macrophage colony-stimulating factor to protect neonatal rats from septic death due to Staphylococcus aureus, *J. Infectious Diseases,* **162**, 109–14.

FURTH, F.W., COULTER, M.P. MILLER, R.W., HOWLAND, J.W. & SWISHER, S. N. (1953) The treatment of acute radiation syndrome in dogs with auromycin and whole blood, *J. Laboratory Clinical Medicine,* **41**, 918–28.

FUSHIKI, M., ONO, K., SASAI, K. *et al.* (1990) Effect of recombinant human granulocyte colony stimulating factor on granulocytopenia in mice induced by irradiation. *In. J. Radiation Oncology, Biology and Physics,* **18**, 353–7.

GANSER, A., SEIPELT, G., LINDEMANN, A. *et al.* (1990) Effects of recombinant human interleukin-3 in patients with myelodysplastic syndromes, *Blood,* **76**, 455–62.

GASPARETTO, C., LAVER, J., ABBOUD, M. *et al.* (1989) Effects of IL-1 on hematopoietic progenitors: evidence of stimulatory and inhibitory activities in a primate model, *Blood,* **74**, 547–50.

GASPARETTO, C., SMITH, C., GILLIO, A., STOPPA, A.M., MOORE, M.A.S. & O'REILLY, R.J. (1994) Enrichment of peripheral blood stem cells in a primate model following administration of a single dose of rh-IL-1 , *Bone Marrow Transplantation,* **14**, 717–23.

GASSON, J.C., WEISBART, R.H., KAUFMAN, S.E. *et al.* (1984) Purified human granulocyte-macrophage colony-stimulating factor: direct action on neutrophils, *Science,* **226**, 1339–42.

GEISSLER, K., VALENT, P., MAYER, P. *et al.* (1990) Recombinant human IL-3 expands the pool of circulating hematopoietic progenitor cells in primates. Synergism with recombinant human granulocyte-macrophage colony stimulating factor, *Blood,* **75**, 2305–10.

GEISSLER, K., VALENT, P., BETTELHEIM, P. *et al.* (1992) *In vivo* synergism of recombinant human interleukin-3 and recombinant human interleukin-6 on thrombopoieses in primates, *Blood,* **79**, 1155–60.

GERHARTZ, H.H., WALTHER, J., BUNICA, O. *et al.* (1992) Clinical-hematological-and cytokine-response to interleukin-3 (IL-3) supported chemotherapy in resistant lymphomas: a phase II study, *Proceedings of the American Society of Clinical Oncology,* **11**, 329.

GIANNI, A.M., SIENA, S, BREGNI M. *et al.* (1989a) Granulocyte-macrophage colony-stimulating factor to harvest circulating haemotopoietic stem cells for autotransplantation. *Lancet,* **2**, 580–5.

GIANNI, A.M., BREGNI, M., SIENA, S. *et al.* (1989b) Rapid and complete hemopoietic reconstitution following combined transplantation of autologous blood and bone marrow cells. A changing role for high dose chemo-radiotherapy? *Hematology Oncology,* **7**, 139–48.

GIANNI, A.M., TARELLA, C., SIENA, S. *et al.* (1990) Durable and complete hematopoietic reconstitution after autografting of rhGM-CSF exposed peripheral blood progenitor cells, *Bone Marrow Transplant,* **6**, 143–5.

GILLIO, A.P., GASPARETTO, C., LAYER, J. *et al.* (1990), Effects of interleukin-3 on hematopoietic recovery after 5-fluorouracil or cyclophosphamide treatment of cynomolgus primates, *J. Clinical Investigation,* **85**, 1560–5.

GLASPY, J., MCNIECE, I., LEMAISTRE, F. *et al.* (1994) Effects of stem cell factor (rhSCF) and Filgrastim (rhG-CSF) on mobilization of peripheral blood progenitor cells (PBPC) and on hematological recovery posttransplant: early results from a phase I/II study, *Proceedings of American Society of Clinical Oncology,* **13**, 76a.

GLUCKMAN, E., BROXYMEYER, H.E., AUERBACH, A.D. *et al.* (1989) Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling, *New England Journal of Medicine,* **321**, 1174–8.

GONTER, P.W. HILLYER, C.D., STROBERT, E.A. *et al.* (1993a) The effect of varying ratios of administered rhIL-3 and rhGM-CSF on post-chemotherapy marrow regeneration in a nonhuman primate model, *Blood,* **82** (suppl. 1), 365a.

(1993b) Enhanced post-chemotherapy platelet and neutrophils recovery using combination rhIL-6 and rhGM-CSF in a nonhuman primate model, *Blood,* **82**, (suppl. 1), 365a.

GOODMAN, J.W. & HODGSON, G.S. (1962) Evidence for stem cells in the peripheral blood of mice, *Blood,* **19**, 702–14.

GORDON, M.S. & HOFFMAN, R. (1992) Growth factors affecting human thrombocytopoiesis: potential agents for treatment of thrombocytopenia, *Blood,* **80**, 302–7.

GÖRGEN, I., HARTUNG, T., LEIST, M. *et al.* (1992) Granulocyte colony-stimulating factor treatment protects rodents against lipopolysaccharide-induced toxicity via suppression of systematic tumor necrosis factor- , *J. Immunology,* **149**, 918–24.

GURNEY, A.L., CARVER-MOORE, K., DE SAUVAGE, F.J. & MOORE, M.W. (1994) Thrombocytopenia in c-*mpl*-deficient mice, *Science,* **265**, 1445–7.

HAAS, R., HO, A.D., BREDTHAUER, U., CAYEUX, S., EGERER, G., KNAUF, W. & HUNSTEIN, W. (1990) Successful autologous transplantation of blood stem cells mobilized with recombinant human granulocyte-macrophage colony-stimulating factor, *Experimental Hematology,* **18**, 94–8.

HANNUM, C. CULPEPPER, J., CAMPBELL, D. *et al.* (1994) Ligand for FLT3/FLK2 receptor tyrosine kinase regulates growth of haematopoietic stem cells and is encoded by variant mRNAs, *Nature,* **368**, 643–8.

HARRISON, D.E. & LERNER, C.P. (1991) Most primitive hemopoietic stem cells are stimulated to cycle rapidly after treatment with 5-fluorouracil, *Blood,* **78**, 1237–40.

HATHORN, J.W., RUBIN, M. & PIZZO, P.A. (1987) Empiric antibiotic therapy in febrile neutropenic cancer patients: clinical efficacy and impact of monotherapy, *Antimicrobial Agents and Chemotherapy,* **31**, 971–7.

HAYLOCK, D.N., TO, L.B., DOWSE, T.L., JUTTNER, C.S. & SIMMONS, P.J. (1992) *Ex vivo* expansion and maturation of peripheral blood $CD34^+$ cells into the myeloid lineage, *Blood,* **80**, 1405–12.

HENDRY, J.H. & LORD, B.I. (1983) The analysis of the early and late response to cytotoxic injury in the haematopoietic cell hierarchy. In C.S.Potten & J.H.Hendry (eds), *Cytotoxic Insult to Tissues.* Edinburgh: Churchill-Livingstone. pp. 1–66.

HERODIN, F., MESTRIES, J.C., JANODET, D. *et al.* (1992) Recombinant glycosylated human interleukin-6 accelerates peripheral blood platelet count recovery in radiation-induced bone marrow depression in baboons, *Blood,* **80**, 688–95.

HILL, R.J., WARREN, M.K., STENBERG, P. *et al.* (1991) Stimulation megakaryocytopoiesis in mice by human recombinant interleukin-6, *Blood,* **77**, 42–8.

HIRANO, T., AKIRA, S., TAGA, T. & KISHIMOTO, T. (1990) Biological and clinical aspects of interleukin-6, *Immunology Today,* **11**, 443–9.

HO, A.D., HAAS, R., KORBLING, M., DIETA, M. & HUNSTEIN, W. (1991) Utilization of recombinant human GM-CSF to enhance peripheral progenitor cell yield for autologous transplantation, *Bone Marrow Transplant,* **7** (suppl. 1), 13–17.

HODGSON, G.S. & BRADLEY, T.R. (1979) Properties of haematopoietic stem cells surviving treatment: evidence for a pre-CFU-S cell, *Nature,* **281**, 381–2.

HOFFMAN, R. (1989) Regulation of megakaryocytopoiesis, *Blood,* **74**, 1196–212.

HOHAUS, S., GOLDSCHMIDT, H., EHRHARDT, R. & HAAS, R. (1993) Successful autografting following myeloablative conditioning therapy with blood stem cells mobilized by chemotherapy plus rhG-CSF, *Experimental Hematology,* **21**, 508–14.

HOWS, J.M., BRADLEY, B.A., MARSH, J.C.W. *et al.* Growth of human umbilical-cord blood in long-term haemopoietic cultures, *Lancet,* **340**, 73–6.

HUANG, E., NOCKA, K., BEIER, D.R. *et al.* (1990) The hematopoietic growth factor KL is encoded at the SI locus and is the ligand of c-*kit* receptor, the gene product of the *W* locus, *Cell,* **63**, 225–33.

IKEBUCHI, K., WONG, G.G., CLARK, S.C., IHLE, J.N., HIRAI, T. & OGAWA, M. (1987) Interleukin-6 enhancement of interleukin-3-dependent proliferation of multipotential hemopoietic progenitors, *Proceedings of the National Academy of Science, USA,* **84**, 9035–9.

IKEBUCHI, K., IHLE, J.N., HIRAI, Y., WONG, G.G., CLARK, S.C. & OGAWA, M. (1988) Synergistic factors for stem cell proliferation: further studies of the target stem cells and the mechanism of stimulation by interleukin-1, interleukin-6 and granulocyte colony-stimulating factor, *Blood,* **72**, 2007–14.

IMAI, T., KOIKE, K., KUBO, T. *et al.* (1991) Interleukin-6 supports human megakaryocytic proliferation and differentiation *in vitro, Blood,* **78**, 1969–74.

INOUE, H., KADOYA, T., KABAYA, K. *et al.* (1994) A highly enhanced thrombopoietic activity by monmethoxy polyethylene glycol-modified recombinant human interleukin-6 , *J. Laboratory Clinical Medicine,* **124,** 529–36.

ISHIBASHI, T., KIMURA, H., SHIKAMA, Y. *et al.* (1989a) Interleukin-6 is a potent thrombopoietic factor *in vivo* in mice, *Blood,* **74**, 1241–4.

ISHIBASHI, T., KIMURA, H., UCHIDA, T. KARIYONE, S., FRIESE, P. & BURSTEIN, S. A. (1989b) Human interleukin-6 is a direct promoter of maturation of megakaryocytes *in vitro, Proceedings of the National Academy of Science, USA,* **86**, 5953–7.

JACKSON, D.P., SORENSEN, D.K., CRONKITE, E.P. BOND, V.P. & FLIEDNER, T. M. (1959) Effectiveness of transfusions of fresh and lyophiloized platelets in controlling bleeding due to thrombocytopenia, *J. Clinical Investigation,* **38**, 1689–97.

JACOBSEN, F.W., VEIBY, O.P., SKJØNSBERG, C, & JACOBSEN, S.E.W. (1993) Novel role of interleukin-7 in myelopoiesis: stimulation of primitive murine hematopoietic progenitor cells. *J. Experimental Medicine,* **178**, 1777–82.

JACOBSEN, S.E.W., VEIBY, O.P. & SMELAND, E.B. (1993) Cytotoxic lymphocyte maturation factor (interleukin-12) is a synergistic growth factor for hematopoietic stem cells , *Journal of Experimental Medicine,* **178**, 413–18.

JACOBSEN, S.E.W., OKKENHAUG, C., VEIBLY, O.P., CAPUT, D., FERRARA, P. & MINTY, A. (1994) Interleukin-13: novel role in direct regulation of proliferation and differentiation of primitive hematopoietic progenitor cells, *J. Experimental Medicine,* **180**, 75–82.

JIANG, S., LEVINE, J.D., FU, Y. *et al.* (1994) Cytokine production by primary bone marrow megakaryocytes, *Blood,* **84**, 4151–6.

JONES, R.J. CELANO, P., SHARKIS, S.J. & SENSENBRENNER, L.L. (1989) Two phases of engraftment established by serial bone marrow transplantation in mice, *Blood,* **73**, 397–401.

JONES, R.J., WAGNER, J.E., CELANO, P., ZICHA, M.S. & SHARKIS, S.J. (1990) Separation of pluripotent haematopoietic stem cells from spleen colony-forming cells, *Nature,* **347**, 188–9.

JORDAN, C.T. & LEMISCHKA, I.R. (1990) Clonal and systematic analysis of long-term hematopoiesis in the mouse, *Gene Development,* **4**, 220–32.

JUTTNER, C.A. TO, L.B., HAYLOCK, D.N., BRANDFORD, A. & KIMBER, R.J. (1985) Circulating autologous stem cells collected in very early remission from acute non-lymphoblastic leukaemia produce a prompt but incomplete haemopoietic reconstitution after high dose melphalan or supralethal chemoradiotherapy, *Br. J. Haematology,* **61**, 739–45.

KATAYAMA, N, CLARK, S.C. & OGAWA, M. (1993) Growth factor requirement for survival in cell cycle dormancy of primitive murine lymphohematopoietic progenitors, *Blood,* **81**, 610–16.

KAUSHANSKY, K. SHOEMAKER, S.G., BROUDY, V.C. *et al.* (1992) Structure-function relationships of interleukin-3. An analysis based on the function and binding characteristics of a series of interspecies chimera of gibbon and murine interleukin-3, *J. Clinical Investigation,* **90,** 1879–88.

KAUSHANSKY, K., LOK, S., HOLLY, R.D. *et al.* (1994a) Promotion of megakaryocyte progenitor expansion and differentiation by the c-Mpl ligand thrombopoietin, *Nature,* **369**, 568–71.

KAUSHANSKY, K, LIN, N. FOX, N. *et al.* (1994b) Thrombopoietin (Tpo) the MPL-ligand, is the primary regulator of megakaryocyte (Mk) development and maturation, *Blood,* **84** (suppl. 1), 241a.

KAVNOUDIAS, H., JACKSON, H., ETTLINGER, K., BERTONCELLO, I., MCNIECE, I. & WILLIAMS, N. (1992) Interleukin-3 directly stimulates both megakaryocyte progenitor cells and immature megakaryocytes, *Experimental Hematology,* **20**, 43–6.

KESSINGER, A., ARMITAGE, J.O., LANDMARK, J.D., SMITH, D.M. & WEISENBURGER, D.D. (1988) Autologous peripheral hematopoietic stem cell transplantation restores hematopoietic function following marrow ablative therapy, *Blood,* **71**, 223–7.

KHWAJA, A., MILLS, W., LEVERIDGE, K., GOLDSTONE, A.H. & LINCH, D.C. (1992) Efficacy of delayed granulocyte colony-stimulating factor after autologous BMT, *Bone Marrow Transplantation,* **11**, 479–82.

KISHIMOTO, T. (1989) The biology of interleukin-6, *Blood,* **74**, 1–10.

KITAGAWA, S., YUO, A., SUOZA, L.M., SAITO, M., MIURA, Y. & TAKAKU, F. (1987) Recombinant human granulocyte-macrophage colony-stimulating factor enhances superoxide release in human granulocytes stimulated by chemotactic peptide, *Biochemical Biophysic Research Communication,* **144**, 1143–6.

KLEIN, B., OLINS, P., BAUER, C. *et al.* (1994) Structure activity relationships for IL-3 and related synthokines, *Blood,* **84**, 369a.

KOBAYASHI, Y., OKABE, T., URABE, T., URABE, A., SUZUKCI, N. & TAKAKU, F. (1987) Human granulocyte colony stimulating factor produced by *Escherichia coli* shortens the period of granulocytopenia induced by irradiation in mice, *Japan. J. Cancer Research,* **78**, 763–8.

KOBAYASHI, M., IMAMURA, M., GOTOHDA, Y. *et al.* (1991) Synergistic effects of interleukin-1 and interleukin-3 on the expansion of human hematopoietic progenitor cells in liquid cultures, *Blood,* **78**, 1947–53.

KOIKE, K., STANLEY, E.R., IHLE, J.N. & OGAWA, M. (1986a) Macrophage colony formation supported by purified CSF-1 and/or interleukin-3 in serum-free culture: evidence for hierarchical difference in macrophage colony-forming cells, *Blood,* **67**, 859–64.

KOIKE, K., IHLE, J.N. & OGAWA, M. (1986b) Declining sensitivity to interleukin-3 of murine multipotential hemopoietic progenitors during their development. Application to a culture system that favors blast cell colony formation, *J. Clinical Investigation,* **77**, 894–9.

KORBLING, M., DORKEN, B., HO, A.D., PEZZUTTO, A., HUNSTEIN, W. & FLIEDNER, T.M. (1986) Autologous transplantation of blood-derived hemopoietic stem cells after myeloablative therapy in a patient with Burkitt's lymphoma, *Blood,* **67**, 529–32.

KORSMEYER, S.J. (1992) Bcl-2 initiates a new category of oncogenes: regulators of cell death, *Blood,* **80**, 879–86.

KOURY, M.J. & BONDURANT, M.C. (1990) Erythropoietin retards DNA breakdown and prevents programmed death in erythroid progenitor cells, *Science,* **248**, 378–81.

KRUMWIEH, D., WEINMANN, E. & SEILER, F.R. (1990) Different effects of interleukin-3 (IL3) on the hematopoiesis of subhuman primates due to various combinations with granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF), *Int. J. Cell Cloning,* **8**, 229–48.

KURZROCK, R., TALPAZ, M., ESTROV, Z., ROSENBLUM, M.G. & GUTTERMAN, J. U. (1991) Phase I study of recombinant human interleukin-3 in patients with bone marrow failure, *J. Clinical Oncology,* **9**, 1241–50.

LANE, T.A. LAW, P., & MARUYAMA, M. *et al.* (1995) Harvesting and enrichment of hematopoietic progenitor cells mobilized into the peripheral blood of normal donors by granulocyte-macrophage colony-stimulating factor (GM-CSF) or G-CSF: potential role in allogeneic marrow transplantation, *Blood* **85**, 275–82.

LANSDORP, P.M., DRAGOWSKA, W. & MAYANI, H. (1993) Ontogeny-related changes in proliferative potential of human hematopoietic cells, *J. Experimental Medicine,* **178**, 787–91.

LANSDORP, P.M., DRAGOWSKA, W., THOMAS, T.E., LITTLE, M.-T. & MAYANI, H. (1994) Age-related decline in proliferative potential of purified stem cell candidates, *Blood Cells,* **20,** 376–81.

LEARY, A.G., IKEBUCHI, K., HIRAI, Y., WONG, G.G., YANG, Y.C. & CLARK, S. C. (1988) Synergism between the interleukin-6 and interleukin-3 in supporting proliferation of human hematopoietic stem cells: comparison with interleukin-1- , *Blood,* **71,** 1759–63.

LEARY, A.G., WONG, G.G., CLARK, S.C., SMITH, A.G. & OGAWA, M. (1990) Leukemia inhibitory factor differentiation-inhibiting activity/human interleukin for DA cells augments proliferation of human hematopoietic stem cells, *Blood,* **75**, 1960–4.

LEONARD, J.P., QUINTO, C.M., GOLDMAN, S.J., KOZITZA, M.K. NEBEN, T.Y. (1994) Recombinant human interleukin-11 (rhIL-11) multilineage hematopoietic recovery in mice after a myelosuppressive regimen of sublethal irradiation and carboplatin, *Blood,* **83**, 1499–506.

LERNER, C. & HARRISON, D.E. (1990) 5-Fluorouracil spares hemopoietic stem cells responsible for long-term repopulation, *Experimental Hematology,* **18,** 114–18.

LI, C.L & JOHNSON, G.R. (1992) Rhodamine 123 reveals heterogeneity within murine Lin^- $Sca1^+$ hemopoietic stem cells. *J. Experimental Medicine,* **175**, 1443–7.

LINDEMANN, A., RIEDEL, D., OSTER, W. *et al.* (1988) Granulocyte/macrophage colony-stimulating factor induces interleukin-1 production by human polymophonuclear neutrophils, *J. Immunology,* **140**, 837–9.

LINDEMANN, A., RIEDEL, D., OSTER, W. & ZIEGLER-HEITBROCK, H.W. (1989) Granulocyte-macrophage colony-stimulating factor induces cytokine secretion by human polymorphonuclear leukocytes. *J. Clinical Investigation,* **83**, 1308–12.

LLOYD, A.R. & OPPENHEIM, J.J. (1992) Poly's lament: the neglected role of the polymorphonuclear neutrophil in the afferent limb of the immune response, *Immunology Today,* **13**, 169–72.

LOK, S. KAUSHANSKY, K., HOLLY, R.D. *et al.* (1994) Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production *in vivo, Nature* **369,** 565–8.

LOPEZ, A.F., NICOLA, N.A., BURGESS, A.W. *et al.* (1983) Activation of granulocyte cytotoxic function by purified mouse colony-stimulating factors, *J. Immunology,* **131**, 2983–8.

LOPEZ, A.F., WILLIAMSON, D.J., GAMBLE *et al.* (1986) Recombinant human granulocyte-macrophage colony-stimulating factor stimulates *in vitro* mature human neutrophil and eosinophil function, surface receptor expression, and survival, *J. Clinical Investigation,* **78**, 1220–8.

LOPEZ, A.F., SHANNON, M.F., BARRY, S. *et al.* (1992a) A human interleukin-3 analog with increased biological and binding activities, *Proceedings of the National Academy of Sciences, USA,* **89**, 11842–6.

LOPEZ, A.F., ELLIOTT, M.J., WOODCOCK, J. & VADAS, M.A. (1992b) GM-CSF, IL-3, and IL-5: cross-competition on human haemopoietic cells, *Immunology Today,* **13**, 495–500.

LORD, B.I., GURNEY, H., CHANG, J., THATCHER, N. CROWTHER, D. & DEXTER, J. M. (1992) Haemopoietic cell kinetics in humans treated with GM-CSF, *Int. J. Cancer,* **50,** 26–31.

LORD, B.I., TESTA, N.G., BRETTI, S. *et al.* (1994) Haemopoietic progenitor and myeloid cell kinetics in humans treated with interleukin-3 and granulocyte/macrophage colony stimulating factor in combination, *Int. J. Cancer,* **59,** 483–90.

LORD, B.I., WOOLFORD, L.B., WOOD, L.M. *et al.* (1995) Mobilization of early hemopoietic progenitor cells with BB.100101A genetically engineered variant and human macrophage inflammatory protein-1 , *Blood,* **85**, 3412–15.

LOTEM, J., SHABO, Y. & SACHS, L. (1989) Regulation of megakaryocyte development by interleukin-6, *Blood,* **74**, 1545–51.

LOWRY, P.A., DEACON, D., WHITEFIELD, P., MCGRATH, H.E. & QUESENBERRY, P.J. (1992) Stem cell factor induction of *in vitro* murine hematopoietic colony formation by 'subliminal' cytokine combinations: the role of 'anchor factors', *Blood,* **80**, 663–9.

LYMAN, S.D. JAMES, L., VANDEN, BOS, T. *et al.* (1993) Molecular cloning of a ligand for the *flt3*/*flk*-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells, *Cell,* **75**, 1157–67.

LYMAN, S.D., JAMES, L., JOHNSON, L. *et al.* (1994) Cloning of the human homologue of the murine flt3 ligand: a growth factor for early hematopoietic progenitor cells, *Blood,* **83**, 2795–801.

MACVITTIE, T.J. *et al.* (1991) Casualty Management Research Group. Reconstitution of hemopoiesis and resistance to sepsis and septic shock in preclinical models of radiation-induced marrow aplasia and gram-negative infection: the efficacy of cytokine therapy and blockade of the inflammatory cascade. Annual report on AFRRI Research.

MACVITTIE, T.J., MONROY, R.L., PATCHEN, M.L. & SOUZA, L.M. (1990) Therapeutic use of recombinant human G-CSF in a canine model of sublethal and lethal whole-body irradiation, *Int. J. Radiation Biology,* **57**, 723–36.

MACVITTIE, T.J., MONROY, R., VIGNEULLE, R.M. ZEMAN, G.H. & JACKSON, W. E. (1991) The relative biological effectiveness of mixed fission-neutron- radiation on the hematopoietic syndrome in the canine: effect of therapy on survival, *Radiation Research,* **128**, S29–36.

MACVITTIE, T.J., FARESE, A.M., PATCHEN, M.L. & MYERS, L.A. (1994) Therapeutic efficacy of recombinant interleukin-6 (IL-6) alone and combined with recombinant human IL-3 in a nonhuman primate model of high-dose, sublethal radiation-induced marrow aplasia, *Blood,* **84**, 2515–22.

MADONNA, G.S., LEDNEY, G.D., ELLIOTT, T.B. *et al.* (1989) Trehalose dimycolate enhances resistance to infection in neutropenic animals, *Infection and Immunity,* **57**, 2495–501.

MAGLI, M.C., ISCOVE, N.N. & ODARTCHENKO, N. (1982) Transient nature of early hematopoietic spleen colonies, *Nature,* **295**, 527–9.

MARUCHA, P.T., ZEFF, R.A. & KREUTZER, D.L. (1990) Cytokine regulation of IL- gene expression in the human polymorphonuclear leukocyte, *J. Immunology,* **145**, 2932–7.

MATSUMOTO, M., MATSUBARA, S., MATSUNO, T. *et al.* (1987) Protective effect of human granulocyte colony-stimulating factor on microbial infection in neutropenic mice, *Infection and Immunity,* **55**, 2715–20.

MATSUMOTO, M., MATSUBARA, S. & YOKOTA, T. (1991) Effect of combination therapy with recombinant granulocyte colony-stimulating factor (rG-CSF) and antibiotics in neutropenic mice unresponsive to antibiotics alone, *J. Antimicrobial Chemotherapy,* **28**, 447–53.

MATSUNAGA, T., SAKAMAKI, S., KOHGO, Y., OHI, S., HIRAYAMA, Y. & NIITSU, Y. (1993) Recombinant human granulocyte colony stimulating factor can mobilize sufficient amounts of peripheral blood stem cells in healthy volunteers for allogeneic transplantation, *Bone Marrow Transplant,* **11**, 103–8.

MATTHEWS, W., JORDAN, C.T., WIEGAND, G.W., PARDOLL, D. & LEMISCHKA, I. R. (1991) A receptor tyrosine kinase specific to hematopoietic stem and progenitor cell-enriched populations, *Cell,* **65**, 1143–52.

MAYANI, H., DRAGOWSKA, W. & LANSDORP, P.M. (1993) Cytokine-induced selective expansion and maturation of erythroid versus myeloid progenitors from purified cord blood precursor cells, *Blood,* **81**, 3252–8.

MAYANI, H. & LANSDORP, P.M. (1994) Thy-1 expression is linked to functional properties of primitive hematopoietic progenitor cells from human umbilical cord blood, *Blood,* **83**, 2410–17.

MAYER, P. LAM, C., OBENAUS, H., LIEHL, E. & BESEMER, J. (1987) Recombinant human GM-CSF induces leukocytosis and activates peripheral blood polymorphonuclear neutrophils in nonhuman primates, *Blood,* **70**, 206–13.

MAYER, P., VALENT, P., SCHMIDT, G., LIEHL, E. & BETTELHEIM, P. (1989) The *in vivo* effects of recombinant human interleukin-3: demonstration of basophil differentiation factor, histamine-producing activity, and priming of GM-CSF responsive progenitors in nonhuman primates, *Blood,* **74**, 613–21.

MAYER, P., GEISSLER, K., VALENT, P., CESKA, M., BETTELHEIM, P. & LIEHL, E. (1991a) Recombinant human interleukin-6 is a potent inducer of the acute phase response and elevates the blood platelets in nonhuman primates, *Experimental Hematology,* **19,** 688–96.

MAYER, P., SCHÜTZE, E., LAM, C., KRICEK, F., & LIEHL, E. (1991b) Recombinant murine granulcotye-macrophage colony-stimulating factor augments recovery and enhances resistance to infections in myelosuppressed mice. *J. Infectious Diseases,* **163,** 584–90.

MAYER, P., GEISSLER, K., WARD, M. & METCALF, D. (1993) Recombinant human leukemia inhibitory factor induces acute phase proteins and raises the blood platelet counts in nonhuman primates. *Blood,* **81,** 3226–33.

MCCOLL, S.R., PAQUIN, R., MÉNARD, C. & BEAULIEU, A.D. (1992) Human neutrophils produce high levels of the interleukin-1 receptor antagonist in response to granulocyte/macrophage colony-stimulating factor and tumor necrosis factor , *J. Experimental Medicine ,* **176**, 593–8.

MCKEARN, J.P. BAUER, C., KLEIN, B. *et al.* (1994) Evaluation of synthetic cytokine agonist of the human IL-3 receptor with significantly improved activity relative to native IL-3, *Blood,* **84** (suppl. 1) 422a.

MCNIECE, I., GLASPHY, J. LEMAISTRE, F. BRIDDELL, R., MENCHACA, D. & SHPALL, E.J. (1993) Effects of recombinant methionyl human stem cell factor (rhSCF) and filgrastim (rhG-CSF) on mobilization of peripheral blood progenitor cells: preliminary laboratory results from a phase I/II study, *Blood,* **82,** 84a.

MEINJE, E.I.M., VAN DER WINDEN-VAN-GROENEWEGEN, R.J., PLOEMACHER, R. E., VOS, O., DAVIDS, J.A.G. & HUISKAMP, R. (1991) The effect of X-irradiation on hematopoietic stem cell compartments in the mouse, *Experimental Hematology,* **19,** 617–23.

MEISENBERG, B.R., DAVIS, T.A., MELARAGNO, A.J., STEAD, R. & MONROY, R.L. (1992) A comparison to therapeutic schedules for administering granulocyte colony-stimulating factor to nonhuman primates after high-dose chemotherapy, *Blood,* **79**, 2267– 72.

METCALF, D., NICOLA, N.A. & GEARING, D.P. (1990) Effects of injected leukemia inhibitory factor (LIF) on hemopoietic and other tissues in mice, *Blood,* **76**, 50–6.

METCALF D. & NICOLA, N.A. (1991) Direct proliferative actions of stem cell factor on murine bone marrow cells *in vitro:* effects of combination with colony-stimulating factors, *Proceedings of the National Academy of Sciences, USA,* **88,** 6239–43.

METCALF, D., HILTON, D. & NICOLA, N.A. (1991) Leukemia inhibitory factor can potentiate murine megakaryocytopoiesis *in vitro, Blood,* **77**, 2150–3.

METHIA, N. LOUACHE, F., VAINCHENKER, W. & WENDLING, F. (1994)Oligodeoxynucleotides antisense to the proto-oncogene c-mpl specifically inhibit *in vitro* megakaryocytopoiesis, *Blood,* **82**, 1395–1401.

MIGLIACCIO, G., MIGLIACCIO, A.R. & VISSER, J.W.M. (1988) Synergism between erythropoietin and interleukin-3 in the induction of hematopoietic stem cell proliferation and erythroid burst colony formation, *Blood,* **72**, 944–51.

MIGLIACCIO, G., MIGLIACCIO, A.R., DRUZIN, M.L., GIARDINA, P.J.V., ZSEBO, K.M. & ADAMSON, J.W. (1992) Long-term generation of colony-forming cells in liquid culture of $CD34^+$ cord blood cells in the presence of recombinant human stem cell factor , *Blood,* **79**, 2620–7.

MILLER, C.P. (1956) The effect of irradiation on natural resistance to infection, *Annals of New York Academy of Sciences,* **66**, 250–61.

MOLINEUX, G., SCHOFIELD, R. & TESTA, N.G. (1986) Development of spleen CFU-s colonies from day 8 to 11: relationship to self-renewal capacity, *Experimental Hematology,* **14**, 710–13.

MOLINEUX, G., POJDA, A., HAMPSON, I.N., LORD, B.I. & DEXTER, T.M. (1990) Transplantation potential of peripheral blood stem cells induced by granulocyte colony-stimulating factor, *Blood,* **76**, 2153–8.

MOLINEUX, G., MIGDALSKA, A., SZMITKOWSKI, M., ZSEBO, K., & DEXTER, T.M. (1991), The effects on hematopoiesis of recombinant stem cell factor (ligand for c-*kit*) administered *in vivo* to mice either alone or in combination with granulocyte colony-stimulating factor, *Blood,* **78**, 961–6.

MONROY, R.L., SKELLY, R.R., MACVITTIE, T.J. *et al.* (1987) The effect of recombinant GM-CSF on the recovery of monkeys transplanted with autologous bone marrow, *Blood,* **70**, 1696–9.

MONROY, R.L., SKELLY, R.R., TAYLOR, P., DUBOIS, A., DONAHUE, R.E. & MACVITTIE, T.J. (1988) Recovery from severe hemopoietic suppression using recombinant human granulocyte-macrophage colony stimulating factor, *Experimental Hematology,* **16**, 334–8.

MONROY, R.L., DAVIS, T.A., DONAHUE, R.E. & MACVITTIE, T.J. (1991) *In vivo* stimulation of platelet production in a primate model using IL-1 and IL-3, *Experimental Hematology,* **19**, 629–35.

MOORE, M.A.S. (1989) Role of interleukin-1 in hematopoiesis, *Immunologic Research,* **8**, 165–75.

MOORE, M.A.S. (1991) Clinical implications of positive and negative hematopoietic stem cell regulators. *Blood,* **78**, 1–19.

MOORE, M.A.S (1993) *Ex vivo* expansion and gene therapy using cord blood $CD34^+$ cells. *J. Hematotherapy,* **2**, 221–4.

MOORE, M.A.S. & HOSKINS, I. (1994) *Ex vivo* expansion of cord blood-derived stem cells and progenitors, *Blood Cells,* **20**, 468–81.

MOORE, M.A.S. & WARREN, D.J. (1987) Interleukin-1 and G-CSF synergism: *in vivo* stimulation of stem cell recovery and hematopoietic regeneration following 5-fluorouracil treatment in mice. *Proceedings of the National Academy of Science, USA,* **84**, 7134–8.

MORRISSEY, P., CHARRIER, K. BRESSLER, L. & ALPERT, A. (1988) The influence of IL-1 treatment on the reconstitution of the hemopoietic and immune systems after sublethal irradiation, *J. Immunology,* **140**, 4204–10.

MUENCH, M.O. & MOORE, M.A.S. (1992) Accelerated recovery of peripheral blood cells counts in mice transplanted with *in vitro* cytokine-expanded hematopoietic progenitors, *Experimental Hematology,* **20**, 611–18.

MUENCH, M.O., SCHNEIDER, J.G. & MOORE, M.A.S. (1992) Interactions among colony-stimulating factors, IL-1 , IL-6 and *kit*-ligand in the regulation of primitive murine hematopoietic cells, *Experimental Hematology,* **20**, 339–49.

MUENCH, M.O., FIRPO, M.T. & MOORE, M.A.S. (1993) Bone marrow transplantation with interleukin-1 plus *kit*-ligand *ex vivo* expanded bone marrow accelerates hematopoietic reconstitution in mice without the loss of stem cell lineage and proliferative potential, *Blood,* **81**, 3463–73.

MUENCH, M.O. RONCAROLO, M.G., MENON, S. *et al.* (1995) FLK-2/FLT-3 ligand regulates the growth of early myeloid progenitors isolated from human fetal liver, *Blood,* **85**, 963–72.

MUSASHI, M., YANG, Y-C., PAUL, S.R., CLARK, S.C. SUDO, T. & OGAWA, M. (1991) Direct and synergistic effects of interleukin-11 on murine hemopoiesis in culture, *Proceedings of the National Academy of Sciences, USA,* **88**, 765–9.

NEBEN, T.Y., LOEBELENZ, J., HAYES, L. *et al.* (1993) Recombinant human interleukin-11 stimulates megakayocytopoiesis and increases peripheral platelets in normal and splenectomized mice, *Blood,* **81**, 901–8.

NEMUNAITIS, J., BUCKNER, C.D., APPLEBAUM, F.R. *et al.* (1992) Phase I trial with recombinant human interleukin-3 (rhIL-3) in patients with lymphoid cancer undergoing autologous bone marrow transplantation (ABMT), *Blood,* **80**, (suppl. 1) 85a.

NOTHDURFT, W., SELIG, C., FLIEDNER, T.M. *et al.* (1992) Hematological effects of rhGM-CSF in dogs exposed to total body irradiation with a dose of 2.4 Gy, *In. J. Radiation Biology,* **61**, 519–31.

O'REILLY, M., SILVER, G.M., GREENHALGH, D.G., GAMELLI, R.L., DAVIS, J.H. & HEBERT, J.C. (1992) Treatment of intra-abdominal infection with granulocyte colony-stimulating factor, *J. Trauma,* **33**, 679–82.

ONO, M., MATSUMOTO, M. MATSUBARA, S., TOMIOKA, S. & ASANO, S. (1988) Protective effect of human granulocyte colony-stimulating factor on bacterial and fungal infections in neutropenic mice, *Behring Institute Mitt.* **83**, 216–21.

ORLIC, D., FISCHER, R., NISHIKAWA, S.I., NIENHUIS, A.W. & BODINE, D.M. (1993) Purification and characterization of heterogeneous pluripotent hematopoietic stem cell populations expressing high levels of *c-kit* receptor, *Blood,* **82**, 762–70.

PATCHEN, M.L. (1995) Immunomodulators and cytokines: their use in the mitigation of radiation-induced hemopoietic injury. In. E.A.Bump & K.Malaker (eds), *Radio-protectors: Chemical, Biological and Clinical Perspectives.* Boca Raton: CRC Press (in press).

PATCHEN, M.L., MACVITTIE, T.J., SOLBERG, B.D. & SOUZA, L.M. (1990) Therapeutic administration of recombinant human granulocyte colony stimulating factor accelerated hemopoietic regeneration and enhances survival in a murine model of radiation-induced myelosuppression, *Int. J. Cell Cloning,* **8**, 107–22.

PATCHEN, M.L., MACVITTIE, T.J., WILLIAMS, J.L., SCHWARTZ, G.N. & SOUZA, L.M. (1991) Administration of interleukin-6 stimulates multilineage hematopoiesis and accelerates recovery from radiation-induced hematopoietic depression, *Blood,* **77**, 472–80.

PATCHEN, M.L., FISCHER, R. & MACVITTIE, T.J. (1993) Effects of combined administration of IL-6 and G-CSF on recovery from radiation-induced hemopoietic aplasia, *Experimental Hematology,* **21**, 338–44.

PATCHEN, M.L., FISCHER, R., MACVITTIE, T.J., SEILER, F.R. & WILLIAMS, D.E. (1994a) Mast cell growth factor (c-kit ligand) in combination with granulocyte-macrophage colony-stimulating factor and interleukin-3: *in vivo* hemopoietic effects in irradiated mice compared to *in vitro* effects. *Biotherapy,* **7**, 13–26.

PATCHEN, M.L., FISCHER, R., SCHMAUDER-CHOCK, E.A. & WILLIAMS, D.E. (1994b) Mast cell growth factor enhances multilineage hematopoietic recovery *in vivo* following radiation-induced aplasia, *Experimental Hematology,* **22**, 31–9.

PAUL, S.R., BENNETT, F., CALVETTI, J.A. *et al.* (1990) Molecular cloning of a cDNA encoding interleukin-11, a stromal cell-derived lymphopoietic and hematopoietic cytokine, *Proceedings of the National Academy of Science, USA,* **87**, 7512–16.

PERMAN, V., CRONKITE, E.P., BOND, V.P. & SORENSEN, D.K. (1962), The regenerative ability of hemopoietic tissue following lethal X-irradiation in dogs, *Blood,* **19**, 724– 37.

PETTENGELL, R., MORGERNSTERN, G.R., WOLL, P.J. *et al.* (1993) Peripheral blood progenitor cell transplantation in lymphoma and leukaemia using a single apheresis, *Blood,* **82**, 3770.

PIZZO, P.A. (1984) Granulocytopenia and cancer therapy. *Cancer,* **54**, (suppl.) 2649–61.

(1987) After empiric therapy: what to do until the granulocyte comes back, *Review of Infectious Diseases,* **9**, 214–19.

(1993) Management of fever in patients with cancer and treatment-induced neutropenia. *N. Engl. J. Medicine,* **328**, 1323–32.

PLOEMACHER, R.E. & BRONS, N.H.C. (1988) Cells with marrow and spleen repopulation ability and forming spleen colonies on day 16, 12 and 8 are sequentially ordered on the basis of rhodamine-123 retention, *Journal of Cellular Physiology,* **136**, 531–6. (1989) Separation of CFU-S from primitive cells responsible for reconstitution of the bone marrow hemopoietic stem cell compartment following irradiation: evidence for a pre-CFU-S cell, *Experimental Hematology,* **17**, 263–6.

PLOEMACHER, R.R., VAN OS, R., VAN BEURDEN, A.J. & DOWN, J.D. (1992)Murine haemopoietic stem cells with long-term engraftment and marrow repopulating ability are more resistant to gamma-radiation than are spleen colony forming cells, *Int. J. Radiation Biology,* **61**, 489–99.

PORTEU, F. & NATHAN, C. (1990) Shedding of tumor necrosis factor receptors by activated human neutrophils, *J. Experimental Medicine,* **172**, 599–607.

QUESENBERRY, P., IHLE, J.N. & MCGRATH, E. (1985) The effect of interleukin-3 and GM-CSA-2 on megakaryocyte and myeloid clonal colony formation, *Blood,* **65,** 214.

QUESENBERRY, P.J., MCGRATH, H.E., WILLIAMS, M.E. *et al.* (1991) Multifactor stimulation of megakaryocytopoiesis: effects of interleukin-6, *Experimental Hematology,* **19**, 35–41.

RE, F., MEGOZZI, M., MUZIO, M., DINARELLO, C.A., MANTOVANI, A. & COLOTTA, F. (1993) Expression of interleukin-1 receptor anatogonist (IL-1ra) by human circulating polymorphonuclear cells, *Eur. J. Immunology,* **23**, 570–3.

REIFFERS, J., BERNARD, P., DAVID, B. *et al.* (1986) Successful autologous transplantation with peripheral blood hemopoietic cells in a patient with acute leukemia , *Experimental Hematology,* **14**, 312–15.

RENNICK, D., JACKSON, J., YANG, G., WIDEMAN, J., LEE, F. & HUDAK, S. (1989) Interleukin-6 interacts with interleukin-4 and other hematopoietic growth factors to selectively enhance the growth of megakaryocytic, erythoid, myeloid, and multipotential progenitor cells. *Blood,* **73**, 1828–35.

ROLLIDES, E., WALSH, T.J., PIZZO, P.A. & RUBIN, M. (1991) Granulocyte colony-stimulating factor enhances the phagocytic and bactericidal activity of normal and defective human neutrophils, *J. Infectious Disease,* **163**, 579–83.

ROSE, T.M. & BRUCE, A.G. (1991) Oncostatin M is a member of a cytokine family that includes leukemia-inhibitory factor, granulocyte colony-stimulating factor, and interleukin-6, *Proceedings of the National Academy of Sciences, USA,* **88**, 8641–5.

ROSNET, O., SCHIFF, C., PEBUSQUE, M-J. *et al.* (1993) Human *FLT-3/FLK2* gene: cDNA cloning and expression in hematopoietic cells, *Blood,* **82**, 1110–19.

RUGGIERI, L., HEIMFELD, S. & BROXMEYER, H.E. (1993), Cytokine-dependent *ex vivo* expansion of early subserts of CD34+ cord blood progenitors is greatly enhanced by cord blood plasma, but expansion of the more mature subsets of progenitors is favored, *Blood,* **82** (suppl. 1), 16a.

SCHLERMAN, F., BREE, A. & SCHAUB, R. (1992) Effects of subcutaneous administration of recombinant human interleukin-11 (rhIL-11) or recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF) alone, or rhIL-11 in combination with recombinant human interleukin-3 (rhIL-3) or rhGM-CSF in nonhuman primates, *Blood,* **80**, 64a.

SCHUENING, F.G., STORB, R., GOEHLE, S. *et al.* (1989) Effects of recombinant human granulocyte colony-stimulating factor on hematopoiesis of normal dogs and on hematopoietic recovery after otherwise lethal total body irradiation, *Blood,* **74**, 1308–13.

SCHUENING, F.G., APPLEBAUM, F.R., DEEG, H.J. *et al.* (1993) Effects of recombinant canine stem cell factor, a c-*kit* ligand and recombinant granulocyte colony stimulating factor on hematopoietic recovery after otherwise lethal total body irradiation, *Blood,* **81**, 20–6.

SELIG, C., KREJA, L., MÜLLER, H., SEIFRIED, E., & NOTHDURF, W. (1994)Hematologic effects of recombinant human interleukin-6 in dogs exposed to a total-body radiation dose of 2.4 Gy, *Experimental Hematology,* **22**, 551–8.

SHEN, B.J., HOU, H.S., ZHANG, H.Q. & SUI, X.W. (1990) Unrelated, HLA-mismatched multiple human umbilical cord blood transfusion in four cases with advanced solid tumors: initial studies, *Blood Cells,* **20,** 285–92.

SHERIDAN, W.P., BEGLEY, G., JUTTNER, C.A. *et al.* (1992) Effect of peripheral blood progenitor cells mobilized by filgrastim (G-CSF) on platelet recovery after high dose chemotherapy, *Lancet,* **339,** 640–4.

SHIMAMURA, M., KOBAYASHI, T., YUO A. *et al.* (1987) Effect of human recombinant granulocyte-colony-stimulating factor on hemopoietic injury in mice induced by 5-fluorouracil, *Blood,* **69,** 353–5.

SHINOMIYA, N., TSURA, S., KATSURA, Y., KAYASHIMA, S. & NOMOTO, K. (1991) Enhanced resistance against *Listeria monocytogenes* achieved by pretreatment with granulocyte colony-stimulating factor, *Infection and Immunity,* **59,** 4740–3.

SHIRAFUJI, N., MATSUDA, S. OGURA, H. *et al.* (1990) Granulocyte colony stimulating factor stimulates human mature neutrophilic granules to produce interferon- , *Blood,* **75**, 17–19.

SIENA, S., BREGNI, M., BRANDO, B., RAVAGNANI, F., BONADONNA, G. & GIANNI, A.M. (1989) Circulation of CD34-positive hematopoietic stem cells in peripheral blood of high-dose cyclophosphamide treated patients: enhancement by intravenous recombinant human GM-CSF, *Blood,* **74**, 1905–14.

SOCINSKI, M.A., ELIAS, A., SCHNIPPER, L., CANNISTRA, S.A., ANTMAN, K.H. & GRIFFIN, J.D. (1988) Granulocyte-macrophage colony stimulating factor expands the circulating haemophoietic progenitor cell compartment in man, *Lancet,* **1**, 1194–8.

SORENSEN, D.K., BOND, V.P., CRONKITE, E.P. & PERMAN, V. (1960) An effective therapeutic regimen for the hemopoietic phase of the acute radiation syndrome in dogs, *Radiation Research,* **13,** 669–85.

SOUYRI, M., VIGON, I., PENCIOIELLI, J-F., HEARD, J-M., TAMBOURIN, P. & WENDLING, F. (1990) A putative truncated cytokine receptor gene transduced by the myeloproliferative leukemia virus immortalizes hematopoietic progenitors, *Cell,* **63,** 1137–47.

SROUR, E.F., BRANDT, J.E., BRIDDELL, R.A., GRIGSBY, S., LEEMHUIS, T. & HOFFMAN, R. (1993) Long-term generation and expansion of human primitive hematopoietic progenitor cells *in vitro, Blood,* **81**, 661–9.

STAHL, C.P., WINTON, E.F., MONROE, M.C. *et al.* (1991a) Recombinant human granulocyte-macrophage colony stimulating factor promotes megakaryocyte maturation in nonhuman primates , *Experimental Hematology,* **19**, 810–16.

STAHL, C.P., ZUCKER-FRANKLIN, D., EVATT, B.L. & WINTON, E.F. (1991b) Effects of human interleukin-6 on megakaryocyte development and thrombocytopoiesis in primates, *Blood,* **78**, 1467–75.

STAHL, C.P., WINTON, E.F., MONROE, M.C. *et al.* (1992) Differential effects of sequential, simultaneous, and single agent interleukin-3 and granulocyte macrophage colony-stimulating factor on megakaryocyte maturation and platelet response in primates, *Blood,* **80**, 2479–85.

STANLEY, E.R., MARTOCCI, A., PATINKIN, D., ROSENDAAL, M. & BRADLEY, T.R. (1986) Regulation of very primitive, multipotent, hemopoietic cells by hemopoietin-1, *Cell,* **45**, 667–74.

STORB, R., EPSTEIN, R.B., RAGDE, H., BRYANT, J. & THOMAS, E.D. (1967) Marrow engraftment by allogeneic leukocytes in lethally irradiated dogs, *Blood,* **30,** 805–11.

STORB, R., GRAHAM, T.C., EPSTEIN, R.B., SALE, G.E. & THOMAS, E.D. (1977) Demonstration of hemopoietic stem cells in the peripheral blood of baboons by cross circulation, *Blood,* **50,** 537–42.

SUDA, T., YAMAGUCHI, Y., SUDA, J., MIURA, Y., OKANO, A. & AKIYAMA, Y. (1988) Effect of interleukin-6 (IL-6) on the differentiation and proliferatition of murine and human hemopoietic progenitors. *Experimental Hematology,* **16**, 891–5.

SUREDA, A., VALLS, A., KADAR, E. *et al.* (1993) A single dose of granulocyte colony stimulating factor modifies radiation-induced death in B6D2F1 mice, *Experimental Hematology,* **21**, 1605–7.

TAGA, T. & KISHIMOTO, T. (1992) Cytokine receptors and signal transduction. *Federation of the American Society for Experimental Biology Journal,* **6**, 3387–96.

TAKATSUKI, F., OKANO, A., SUZUKI, C. *et al.* (1990) Interleukin-6 perfusion stimulates reconsitution of the immune and hematopoietic systems after 5-fluorouracil treatment, *Cancer Research,* **50**, 2885–90.

TANAKA, T., OKAMURA, S., OKADA, K. *et al.* (1989) Protective effect of recombinant murine granulocyte-macrophage colony-stimulating factor against *Pseudomonas aeruginosa* infection in leukocytopenic mice, *Infection and Immunity,* **57**, 1792–9.

TANIKAWA, S., NAKAO, I., TSUNESKA, K. & NOBIO, N. (1989) Effects of recombinant granuloctye colony-stimulating factor (rG-CSF) and recombinant granulocyte-macrophage colony-stimulating factor (rGM-CSF) on acute radiation hematopoietic injury in mice, *Experimental Hematology,* **17**, 883–8.

TANIKAWA, S., NOSE, M. YOSHIRO, A., TSUNEOKA, K., SHIKITA, M. & NARA, N. (1990) Effects of recombinant human granulocyte colony-stimulating factor on the hematologic recovery and survival of irradiated mice, *Blood,* **76**, 445–9.

TERAMURA, M. KOBAYASHI, S., HOSHINO, S., OSHIMI, K. & MIZOGUCHI, H. (1992) Interleukin-11 enhances human megakaryocytopoiesis *in vitro, Blood,* **79**, 327–31.

THÈZE, J. (1994) Cytokine receptors: a combinative family of molecules. *European Cytokine Network,* **5**, 353–68.

THOMAS, I.W., BAUM, C.M., HOOD, W.F. *et al.* (1995) Potent interleukin-3 receptor agonist with selectively enhanced hematopoietic activity relative to recombinant interleukin-3, *Proceedings of the National Academy of Sciences, USA,* **92,** 3779–83.

TILL, J.E. & MCCULLOCH, E.A. (1961) A direct measurement of the radiation sensitivity of normal bone marrow cells, *Radiation Research,* **14**, 213–22.

TJØNNFJORD, G.E., STEEN, R., EVENSEN, S.A., THORSBY, E. & EGELAND, T. (1994) Characterization of $CD34^+$ peripheral blood cells from healthy adults mobilized by recombinant human granulocyte colony-stimulating factor, *Blood,* **84,** 2795–801.

TO, L.B., ROBERTS, M.M., HAYLOCK, D.N. *et al.* (1992) Comparison of haematological recovery times and supportive care requirements of autologous recovery phase peripheral blood stem cell transplants, autologous bone marrow transplants and allogeneic bone marrow transplants, *Bone Marrow Transplant,* **9**, 277–84.

TODA, H., MURATA, A., MATSUURA, N. *et al.* (1993) Therapeutic efficacy of granulocyte colony-stimulating factor against rat cecal ligation and puncture model, *Stem Cells,* **11**, 228–34.

TODA, H., MURATA, A., UDA K-I. *et al.* (1994) Effects of granulocyte macrophage colony stimulating factor on sepsis-induced organ injury in rats, *Blood,* **83,** 2893–8.

TRAYCOFF, C.M., ABBOUD, M.R., LAYER, J. *et al.* (1994) Human umbilical cord blood hematopoietic progenitor cells: are they the same as their adult bone marrow counterparts? *Blood Cells,* **20,** 382–91.

TROWBRIDGE, A.A. (1977) Neutropenia: when and when not to treat, *Postgraduate Medicine,* **61**, 208–15.

VADHAN-RAJ, S., PAPADOPOULOS, N.E., BURGESS, M.A. *et al.* (1994) Effects of PIXY321, a granulocyte-macrophage colony-stimulating factor/interleukin-3 fusion protein, on chemotherapy-induced multilineage myelosuppression in patients with sarcoma, *J. Clinical Oncology,* **12**, 715–24.

VAN ZANT, G. (1984) Studies of hematopoietic stem cells spared by 5-fluorouracil. *J. Experimental Medicine,* **159**, 679–90.

VAN ZANT, G., CHEN, J-J. & SCOTT-MICUS, K. (1991) Developmental potential of hematopoietic stem cells determined using retrovirally marked allophenic marrow, *Blood,* **77**, 756–63.

VAN DER WAAIJ, D., HOFSTRA, I.T. & WIEGERSMA, N. (1982) Effects of beta-lactam antibiotics on the resistance of the digestive tract of mice to colonization, *J. Infectious Diseases,* **146**, 417–22.

VILMER, E., STERKERS, G., RAHIMY, C. *et al.* (1992) HLA-mismatched cord blood transplantation in a patient with advanced leukemia, *Transplantation,* **53**, 1155–7.

VILMER, E., QUELVENNEC, E., PLOUVIER, E. *et al.* (1994) HLA-mismatched cord blood transplantation: immunological studies, *Blood Cells,* **20**, 235–41.

WAGEMAKER, G., VAN GILS, F.C.J.M., BURGER, H. *et al.* (1990) Highly increased production of bone marrow derived blood cells by administration of homologous interleukin-3 to rhesus monkeys. *Blood,* **76**, 2235–41.

WAGNER, J.E. (1994) Umbilical cord blood transplantation: overview of the clinical experience, *Blood Cells,* **20**, 227–34.

WAGNER, J.E., BROXMEYER, E., BYRD, R.L. *et al.* (1992) Transplantation of umbilical cord blood after myeloablative therapy: analysis of engraftment, *Blood,* **79**, 1874–81.

WAGNER, J.E., KORPAN, N.A., BROXMEYER, H.E. & GLUCKMAN, E. (1994) Transplantation of umbilical cord blood in 50 patients: analysis of the registry data, *Blood,* **84**, (Suppl. 1), 395a.

WAKIYAMA, H., TSURU, S., HATA, N. *et al.* (1993) Therapeutic effect of granulocyte colony-stimulating factor and cephem antibiotics against experimental infections in neutropenic mice induced by cyclophosphamide. *Clinical Experimental Immunology,* **92**, 218–24.

WALLACE, P.M., MACMASTER, J.F., RILLEMA, J.R. *et al.* (1995) Thrombocytopoietic properties of oncostatin M, *Blood,* **86**, 1310–15.

WEAVER, C.H., BUCKNER, C.D., LONGIN, K. *et al.* (1993) Syngeneic transplantation with peripheral blood mononuclear cells collected after the administration of recombinant human granulocyte and colony-stimulating factor, *Blood,* **82**, 1981–4.

WEIGH, N.S., TULLAI, J., GUIDO, E. *et al.* (1993) Interleukin-3/erythropoietin fusion proteins: *in vitro* effects on hematopoietic cells, *Experimental Hematology,* **21**, 647–55.

WEINTHAL, J., GARRISON, L., KRAILO, M. *et al.* (1994) Accelerated multi-lineage hematopoietic recovery during a phase I trial of PIXY321 following ICE chemotherapy in children with recurrent solid tumors, *Blood,* **84**, 27a.

WEISBART, R.H., GOLDE, D.W., CLARK, S.C., WONG, G.G. & GASSON, J.C. (1985) Human granulocyte-macrophage colony-stimulating factor in a neutrophil activator, *Nature,* **314**, 361–3.

WEISBART, R.H., GASSON, J.C. & GOLDE, D.W. (1989) Colony-stimulating factors and neutrophils: colony-stimulating factors and host defense. *Annals of Internal Medicine,* **110**, 297–303.

WELTE, K., BONILLA, M.A., GILLIO, A.P. *et al.* (1987) Recombinant human granulocyte colony-stimulating factor. Effects on hematopoiesis in normal and cyclophosphamide-treated primates. *J. Experimental Medicine,* **165**, 941–8.

WENDING, F., MARASKOVSKY, E., DEBILL, N. *et al.* (1994) c-Mpl ligand is a humoral regulator of megakaryocytopoiesis, *Nature,* **369**, 571–4.

WICKENHAUSER, C., LORENZEN, J., THIELE, J. *et al.* (1995) Secretion of cytokines (Interleukins-1 , -3, and -6 and granulocyte macrophage colony-stimulating factor) by hormal human bone marrow megakaryocytes, *Blood,* **85**, 685–91.

WILLIAMS, D.E., EISENMAN, J., BAIRD, A. *et al.* (1990) Identification of a ligand for the *c*-kit proto-oncogene, *Cell,* **63**, 167–74.

WILLIAMS, D.E., DUNN, J.T., PARK, L.S. *et al.* (1993a) A GM-CSF/IL-3 fusion protein promotes neutrophil and platelet recovery in sublethally irradiated rhesus monkeys , *Biotechnology Therapeutics,* **4**, 17–29.

WILLIAMS, D.E., FARESE, A. & MACVITTIE, T.J. (1993b) PIXY321, but not GM-CSF plus IL-3, promotes hematopoietic reconstitution following lethal irradiation, *Blood,* **82**, 366a.

WILLIAMS, G.T., SMITH, C.A., SPOONCER, E., DEXTER, T.M. & TAYLOR, D.R. (1990) Haemopoietic colony stimulating factors promote cell survival by suppressing apoptosis, *Nature,* **343,** 76–9.

WILLIAMS, N., BERTONCELLO, I., KAVNOUDIAS, H., ZSEBO, K. & MCNIECE, I. (1992) Recombinant rat stem cell factor stimulates the amplification and differentiation of fractionated mouse stem cell populations. *Blood,* **79,** 58–64.

WINTON, E.F., SRINIVASIAH, J., KIM, B.K. *et al.* (1994) Effect of recombinant human interleukin-6 (rhIL-6) and rhIL-3 on hematopoietic regeneration as demonstrated in a nonhuman primate chemotherapy model, *Blood,* **84**, 65–73.

WONG, G.G., WITEK-GIANNOTTI, J.S., TEMPLE, P.A. *et al.* (1988) Stimulation of murine hemopoietic colony formation by human IL-6 *J. Immunology,* **140**, 3040–4.

XIAO, M., BROXMEYER, H.E., HORIE, M., GRIGSBY, S. & LU, L. (1994) Extensive proliferative capacity of single isolated $CD34^{+++}$ human cord blood cells in suspension culture, *Blood Cells,* **20**, 455–67.

YAN, X-Q, BRIDDELL, R., HARTLEY, C., STONEY, G., SAMAL, B. & MCNIECE, I. (1994) Mobilization of long-term hematopoietic reconstituting cells in mice by the combination of stem cells factor plus granulcoyte colony-stimulating factor, *Blood,* **84**, 795–9.

YANG, Y.C. & YIN, T. (1992) Interleukin-11 and its receptor, *Biofactors,* **4**, 15–21.

YEE, N.S., PAEK, I. & BESMER, P. (1994) Role of kit-ligand in proliferation and suppression of apoptosis in mast cells: basis for radiosensitivity of white spotting and steel mutant mice, *J. Experimental Medicine,* **179**, 1777–87.

YONEMURA, Y., KAWAKITA, M., MASUDA, T., FUJIMOTO, K., KATO, K. & TAKA-SUKI, K. (1992) Synergistic effects of interleukin-3 and interleukin-11 on murine megakaryopoiesis in serum-free culture, *Experimental Hematology,* **20**, 1011–16.

ZEIDLER, C., KANZ, L., HURKUCK, F. *et al.* (1992) *In vivo* effects of interleukin-6 on thrombopoiesis in healthy and irradiated primates, *Blood,* **80**, 2740–5.

ZEIGLER, F.C., BENNETT, B.D., JORDAN, C.T. *et al.* (1994) Cellular and molecular characterization of the role of the FLK-2/FLT-3 receptor tyrosine kinase in hematopoietic stem cells, *Blood,* **84**, 2422–30.

ZSEBO, K.M., WILLIAMS, D.A., GEISSLER, E.N *et al.* (1990a) Stem cell factor is encoded at the S1 locus of the mouse and is the ligand for the *c-kit* tyrosine kinase receptor, *Cell,* **63**, 213–24.

ZSEBO, K.M., WYPYCH, J., MCNIECE, I.K. *et al.* (1990b) Identification, purification, and biological characterization of hematopoietic stem cell factor from buffalo rat liver-conditioned medium, *Cell,* **63**, 195–201.

ZSEBO, K.M., SMITH, K.A., HARTLEY, C.A. *et al.* (1992) Radioprotection of mice by recombinant rat stem cell factor, *Proceedings of the National Academy of Sciences, USA,* **89**, 9464–8.

7
Clinical Approaches to Treatment of Radiation-induced Haemopoietic Injury

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7
Clinical Approaches to Treatment of Radiation-induced Haemopoietic Injury

7.1
General Background

7.1.1
Introduction

Since the 1950s there has been a continuous medical and biological interest in the effects of whole-body radiation exposure of the haemopoietic system mainly because of the use of total-body irradiation (TBI) in the treatment of disseminated malignancies and of the persistent possibility of accidental exposures. Sources of information on the clinical management of patients suffering from haemopoietic injury originate from records of TBI therapy of patients for cancer and other diseases, case studies of radiological accident victims and composite analyses based on data from various sources (UNSCEAR, 1988; Anno *et al.,* 1989). Although the information from patients medically treated with TBI is well documented, its usefulness is limited to its application in the treatment of radiation-induced haemopoietic injury: these patients are already suffering from a severe disease. Additionally, they are exposed to high doses of radiation as part of the treatment of their disease and the side-effects of their exposure may be mitigated by corrective therapeutic agents. In addition, when TBI is performed as preparation for bone marrow transplantation (BMT), the combined effects of the two components of the treatment may introduce some confusion in understanding the observed phenomena. Consequently it is difficult from this category of patients to derive any generic rules for the medical management of patients with severe haemopoietic injury. On the other hand, victims of accidental high-dose exposures represent a small group of individuals, each case being specific and related to various parameters such as the circumstances of the exposure, the quality of the radiation, etc. In spite of this scarcity (worldwide, the number of deaths due to accidental radiation exposure since the 1940s is about one hundred), these accidental cases represent the best basis for evaluating the medical management of acute or protracted whole body irradiation.

In cases of radiological accidents, a clear distinction must be made between two particular categories, because of fundamental differences in their management:

1 Accidents which result in a few victims. The medical response will be comparable to that of any medical casualty, with the current rules of medical ethics, where the participants are limited to the victim and the responsible physician. Most such accidents have been related to industrial and medical sources of radiation, each involving only one or very few individuals.

2 Accidents concerning large groups of people, either workers or individuals in the general population. The management of such cases is performed according to the same principles as those prevailing in any major catastrophic event. This category requires medical triage, distinguishing overexposed individuals with other types of injury, and individuals likely to have received doses below the thresholds for deterministic effects. The general management of this latter category relies first on the responsible national authorities. Such events are illustrated by the Chernobyl and Goiânia accidents. In the Chernobyl accident, about 135000 people were evacuated and 500 casualties were hospitalized. Of these, 237 were diagnosed with an acute radiation syndrome. In the Goiânia accident, 10 victims were transferred to Rio de Janeiro. Two hundred and forty-nine individuals were contaminated, of which 129 were kept under dosimetric surveillance. Both accidents resulted in environmental contamination of large areas of land.

In addition, the radiation source may be external or internal or both, with regards to the body. Internal exposure may result from inhalation, ingestion, direct absorption through the skin or penetration of radioactive materials through open wounds. Accidental exposures involving internal contamination of medically significant importance are rare events; some of them have resulted in high enough doses to induce bone marrow depression which one normally only associates with whole-body external exposure. In the case of association of external and internal exposures, the latter may complicate the bone marrow depression and compromise its recovery, as the internal exposure, although delivered at relatively low dose rates, may be prolonged and result in continuous injury to haemopoietic tissues.

This chapter provides practical bases, information and proposals for the medical management of individuals exposed to high doses of ionising radiation.

7.1.2
High-dose Total-body Medical Irradiation

High-dose TBI in combination with BMT was originally applied in the management of haematological diseases such as acute leukaemia, and it has found increasing use since the late 1970s in the systemic treatment of a variety of other malignancies including multiple myeloma, neuroblastoma and oat cell carcinoma of the lung. In addition, TBI is widely used prior to organ transplantation, the first experience being kidney transplantation (Ferrebee and Thomas, 1958; Mathé *et al.* 1964; Gale and Champlin, 1986; Thomas, 1986a; Barret, 1987). Total-body irradiation is currently given in combination with cytotoxic agents, or multidrug treatment.

The TBI objectives as a preparation for transplantation are:

1 achievement of immune suppression—the first applications were performed prior to kidney transplantation in the late 1950s (Tubiana and Lalanne, 1963).
2 Elimination of malignant stem cells, resulting in eradication of leukaemia or other haematological disease, using doses as high as permitted by the early and late tolerance of other healthy tissues.
3 Bone marrow ablation, providing ‘space’ which is needed by the grafted cells to become established (Nothdurft, 1991). Techniques for delivering these doses are now established and currently high energy X-rays, as produced by linear accelerators, are used to minimise dose variations related to the different depths of various anatomical volumes of interest within the body. The distance between the source and the patient is large enough to include the whole body in the field of radiation; generally the patient is in a supine position, in such a way that longitudinal variations in the midline dose are acceptable.

In the 1960s the reference protocol for TBI was 10 Gy, given as a single dose at 0.05–0.1 Gy min^{-1}. A better result was expected from dose fractionation, based on the low fractionation (or dose rate) sensitivity of bone marrow progenitors and leukaemic cells as opposed to the high fractionation (or dose rate) sensitivity of most healthy organs at risk (Cosset *et al.,* 1992). Thus, since the 1960s, most TBIs have been delivered following different regimens, involving different dose rates or number of fractions. For several years, many TBIs were delivered following an identical fractionation scheme, i.e. five daily fractions of 2 Gy. Since then, various protocols, adapted to the patients, have been used. The general conclusion was that a 'generic schedule' did not exist. It was concluded in the early 1990s that the past experience with TBI 'fails to disclose any regimen which is clearly superior' and that 'there is little evidence that methods for delivering TBI have been improved since the initial trials' (Thomas, 1990; Appelbaum *et al.* 1992). The extensive use of TBI over several decades, however, allows us to make a reasonable assessment of its role and the various impacts of TBI fractionation.

In summary, the main characteristics of TBI are:

1 Application to patients suffering from severe diseases.
2 Very frequent combination with BMT.
3 Variable protocols.
4 Various fractionation schemes, combining different doses rates.

It might seem surprising that intensive follow-up of these patients, to whom the most modern medical care is provided, is insufficient to give appropriate information applicable to the general medical management of patients who received life-endangering doses of radiation. In fact, the interdependency of some of these parameters results in difficulties in comprehending the effectiveness of the medical management for most of the cases studied. For these reasons, it is necessary to rely on the information obtained from accidentally irradiated individuals.

7.1.3
Total-body Accidental Irradiation

Since the discovery of penetrating ionising radiations, X-rays, by Roentgen in 1895, and -rays by Becquerel in 1896, some accidents have occurred, a few resulting in whole-body irradiation. A few tens of the victims have died because of the acute radiation syndrome that they developed: severe aplasia associated with its classical complications. The comparison of severe whole-body irradiation cases is often difficult for several reasons such as: (1) lack or insufficiency of information (cases of historical accidents, especially from countries where politics prevented them coming to light); (2) significant improvement in the medical management of prolonged aplasia; and (3) difficulties in the assessment of the homogeneity of bone marrow exposure in most cases. The most reliable cases are represented by patients who received acute doses from penetrating radiations such as those produced by ^{60}Co (1.17 and 1.33 MeV) or ^{137}Cs (0.66 MeV) sources. Fewer examples are provided by ^{192}Ir (0.2–0.6 MeV), which needs much longer exposures than does Co or Cs to result in aplasia because of its much lower penetration. Accidents happen in the course of both medical and industrial uses of radiation (Nénot, 1990). In addition, in the 1940s and 1950s, criticality in nuclear reactors was responsible for the highest death rate among radiation accidents (eight deaths). Since then there has been only one criticality accident (in 1983) in a zero power research reactor. There is not much to be learnt from these historical accidents, as the dose levels did not allow long enough survival time to assess any therapeutic efficiency of medications.

Among the accidents which are reasonably documented from this point of view, the most useful are those which fulfil the following conditions:

1 Doses in the lethal range, preferably acute.
2 Relevant information on the circumstances, in order to reconstruct the patterns of exposure.
3 Whole-body exposure (it is sometimes difficult in accidental situations to define a clear distinction between whole-body and partial-body exposure).
4 In case of death of the victim, sufficient survival time to allow pertinent and comprehensive observation.

In this restricted registry, good examples are provided by the accidents which occurred in Vinca, Yugoslavia (1958), Mol, Belgium (1965), Pittsburgh, USA (1967), Setif, Algeria (1978), Shanghai, China (1980), Casablanca, Morocco (1984), Chernobyl, Ukraine (1986), Goiânia, Brazil (1987), San Salvador, El Salvador (1989), Soreq, Israel (1990) and Nesvizh, Belarus (1991). A review of these accidents provides good information on the progress in the medical approach for treating such cases.

7.1.3.1
Bone marrow transplantation for treatment of accidental radiation-induced aplasia

Vinca (Yugoslavia, 1958) Five scientists were irradiated by neutrons and -rays from a nuclear reactor. The estimated doses ranged approximately from 7 to 10 Gy. Blood cytopenia was well tolerated until day 25 following irradiation. One patient (estimated dose 8 Gy) had a foetal liver and spleen transplantation (4. 2×10^9 cells of foetal age 5 months) two weeks after the accident. No clinical or haematological improvement was observed from this treatment. The five victims received an allogeneic bone marrow transplantation one month after the accident. Bone marrow samples were collected on the basis of one donor per recipient of the same sex and ABO group. The patients received from 8.5×10^9 to 14×10^9 nucleated bone marrow cells. The patient who received the foetal cell transplantation died four days after the subsequent bone marrow cell transplantation (from a haemorrhage of the respiratory tract), although the transfusion of the cells was followed by a rapid improvement in the blood counts. The haematological and clinical signs of the other four patients improved following the bone marrow transplantation. The studies of the differences in the minor blood group phenotypes of the erythrocytes from the donors and recipients suggest that the presence of cells of donor origin lasted only one month and was followed by autologous reconstitution in the survivors. These transplantations were among the first ever performed and notably the first following an accidental irradiation exposure. Their efficiency is difficult to evaluate (Mathé *et al.,* 1959; Jammet *et al.,* 1959; Jammet, 1984).

Pittsburg (USA, 1967) A physicist was accidentally X-irradiated when working on a Van de Graff generator (estimated dose 6 Gy). He was grafted with the bone marrow of his identical twin. On day 20, leukocytes were less than 0.9×10^9 1^{-1}, platelets less than 24×10^9 1^{-1} and the bone marrow was acellular. The patient recovered and was discharged from isolation on day 42. Whether haemopoiesis was from donor or recipient origin could not be determined. Haemopoiesis started later than expected by the medical team, suggesting a possible autologous recovery (Gilberti, 1980).

Shanghai (People's Republic of China, 1980) Following an accidental irradiation exposure to a ^{60}Co source (estimated dose between 4.7 and 6.4 Gy), a patient showed the classical signs of a severe radiation-induced aplasia. The number of circulating lymphocytes dropped to 0.25×10^9 1^{-1} two days after the accident, and the nadirs for platelets (10^{10} 1^{-1}) and leukocytes were reached on days 24 and 25 respectively.

Blood cell counts then progressed steadily. Within three hours of the accident, the patient received an 'antiradiation' treatment (in order to promote a return to normal haemopoiesis) composed of oestrol and herbs from the traditional Chinese medicine pharmacopeia, without adverse effects for the patient. A foetal liver transplantation was performed six days after the accident. The engraftment was not clearly in evidence. The reticulocyte count drop stopped after the transplantation and the mitotic index was enhanced (there were erythroblasts in the bone marrow) two days after the transplant. The level of the circulating T-lymphocytes stayed high from day 11 to day 47, which could be related to a graft rejection process. Blood lymphocytes dropped to their nadir of $0.05\times10^9\ 1^{-1}$ on day 20. From day 12, fractionated or unfractionated blood from familial identical or semi-identical donors was transfused when the blood leukocytes dropped below $10^9\ 1^{-1}$, granulocytes below $0.5\times10^9\ 1^{-1}$ or platelets below $20\times10^9\ 1^{-1}$. The platelet and granulocyte counts returned to normal values on day 38 and day 49 respectively. The patient was discharged from hospital five months after the accident and had no further haematological problem. The bone marrow chimerism was not studied (Ye *et al.,* 1990).

Chernobyl (Ukraine, 1986) There was a follow-up concerning 117 patients after the nuclear plant accident. Fever and infections were correlated with an agranulocytosis ($0.05\times10^9\ 1^{-1}$ cells or less) related to the received dose (for patients classified in 2 Gy incremental groups). They were specially severe for exposures over 5 Gy. Thrombocytopenia was managed by fresh platelet transfusions of 10^{11} per day when platelets were lower than $20\times10^9\ 1^{-1}$. No death related to haemorrhage was reported. Exposures were essentially of the – type. The internal exposures were less than 3 per cent of the total exposure. Six patients with estimated exposure over 9 Gy received a foetal liver transplant but died rapidly before the impact of the transplant could be evaluated. Some patients received a GM-CSF infusion. The dose, schedules and effects of the infusions are, however, unknown.

Thirteen patients who had received doses estimated to be over 6 Gy had a bone marrow transplantation 4–16 days after irradiation. HLA typing and tissue compatibility assays were difficult to realise due to the leukopenia of the patients. Five patients received HLA identical transplants, three patients had haploidentical donors, four patients had haploidentical donors with one more identical locus. One patient was either HLA identical or haploidentical with one more locus. In an attempt to limit the risk of graft-versus-host disease (GVHD) the patients with haploidentical donors received T-lymphocyte depleted cells. There were three cases of major ABO incompatibility and the red cells were removed. Seven patients died from gastrointestinal lesions or radiation-induced burns during the first month following exposure.

Six patients with less severe lesions survived more than one month. A partial engraftment (10 per cent of the marrow cells tested) and an elevation of the granulocyte count in the blood was observed. From these patients, two survived and four died within three months. Both survivors (5.6 and 8.7 Gy estimated doses) had received a T-lymphocyte depleted haploidentical graft (0.23 and 0.02×10^5 cells kg^{-1}, respectively). Immunosuppressive treatment included methotrexate, anti-lymphocytic serum and cyclosporin A. The graft had been quickly rejected and the patients had autologous reconstitution.

The death of four patients can be related to the transplantation:

- One patient with GVHD and pulmonary emboli died 34 days after the accident (estimated dose 4.4 Gy, HLA haploidentical transplant with one more identical locus, no T-lymphocyte depletion, 1.77×10^5 injected cells kg^{-1}).
- Two patients had GVHD, viral and bacterial infection. They died 48 and 86 days after the accident with estimated doses of 6.4 and 5.2 Gy (HLA identical transplant, 3.24 and 0.92×10^5 injected cells kg^{-1}, respectively).

- One patient had early myeloid rejection, a suspected GVHD (on the basis of histological post-mortem findings) and *cytomegalovirus* infection. He died on day 91 (estimated dose 10.2 Gy, haploidentical T-lymphocyte depleted transplantation, 0.92×10^5 cells kg^{-1}) (Baranov *et al.,* 1989, 1990).

7.1.3.2
Growth factor therapy for treatment of accidental radiation-induced aplasia

Goiânia (Brazil, 1987) Eight victims had bone marrow failure with granulocytes less than 10^9 l^{-1} (and seven had thrombocytopenia and anaemia) after prolonged external and internal exposure (up to 14 days) to a source of ^{137}Cs (estimated whole-body doses ranging from 2.5 to 7 Gy). They were treated with GM-CSF (500 μg m^{-2} per day intravenously) until granulocytes were higher than 2×10^9 l^{-1} for three consecutive days. The dose of GM-CSF was then halved and halved again after three additional days. Treatment was discontinued when neutrophil levels were sustained (8–14 days duration for the four survivors). Treatment was initiated 1–11 days after the onset of neutropenia. Four patients died of gram-negative sepsis. Because of early death after the beginning of treatment, one patient's treatment could not be evaluated. For the seven remaining patients, improvement in neutrophil counts appeared after 1–5 days of GM-CSF administration. Platelet and red cell recovery seemed unaffected by the treatment. Side-effects included fever, shock and respiratory failure which could be related to infection. After treatment was discontinued the survivors' neutrophil levels returned to normal within 3–5 days. During 18 months follow-up, neutrophils remained normal in three patients. One patient developed a mild neutropenia 1 month after discontinuation of GM-CSF.

It was concluded that, by initiating GM-CSF use early after exposure in the lethal range, the period of granulocytopenia may be shortened and survival improved with minimal adverse effect (IAEA, 1988; Butturini and Gale; 1990; Butturini *et al.,* 1988; Valverde *et al.,* 1990).

San Salvador (El Salvador, 1989) Similar improvements to those seen at Goiânia were observed during the El Salvador accident. Three victims had bone marrow failure after external exposure to a source of ^{60}Co (mean dose estimates: 8.1, 3.7 and 2.9 Gy); they were treated with GM-CSF (240 μg m^{-2} per day i.v.) until neutrophil counts had increased to at least 1.5×10^9 l^{-1} after 20, 10 and 9 days of GM-CSF treatment starting 24, 26 and 32 days after the accident, respectively. It was considered that the increase in total neutrophil counts was due to the administration of GM-CSF, although the evidence was ambiguous (it had been administered when spontaneous recovery might have been expected). The spontaneous recovery of haemoglobin and platelets was greater than that of neutrophils, which bears out the fact that GM-CSF stimulates granulocyte precursors. Only two patients (doses of 8.1 and 3.7 Gy) had increased eosinophil counts. Mild side-effects included tremor and weakness for one patient who died on day 197 after the accident following surgery required for lung damage (IAEA, 1990).

Soreq (Israel, 1990) One victim received an accidental whole-body acute dose estimated to be higher than 10 Gy from a ^{60}Co source. GM-CSF (250 μg m^{-2} per day for 4 days) was administered starting about 9 hours after exposure, followed by a haploidentical (three loci were different) bone marrow transplantation. Daily GM-CSF treatment was continued and, from day 5, it was combined with IL-3 (125 μg m^{-2} per day continuous infusion). Administration of growth factors was discontinued on day 18 because of the normalisation of the white blood cell counts. Chimerism studies confirmed the rapid donor marrow engraftment. Side-effects included nausea and vomiting. The patient died on day 36. Some of the postmortem findings were compatible with acute GVHD. However, the severity of GVHD and its specific role in the death of the patient could not be fully assessed. It was concluded that although the role of growth factor alone in enhancing residual host cells cannot be judged on present findings, the data clearly indicated

that a combination of GM-CSF and IL-3 may lead to early and effective engraftment and maturation of donor marrow cells (IAEA, 1993).

Nesvizh (Belarus, 1991) One victim suffered accidental whole-body exposure to an acute dose around 10 Gy from a ^{60}Co source. The patient was treated with GM-CSF (days 2–6 and 16–41, 250 μg m^{-2} per day) and IL-3 (days 6–41, 250 μg m^{-2} per day). Both factors were infused intravenously as a daily 6 hour infusion. Neutrophil recovery started on day 21 reaching 5×10^{9} l^{-1} on day 40. Lymphocyte recovery also began and reticulocytes appeared 10–12 days later. No platelet recovery was observed in that period. The patient died on day 113 from pneumonia and acute respiratory failure. Haemopoietic recovery was incomplete but the results suggested a real improvement from the growth factor therapy (Baranov, 1993; Baranov *et al.,* 1994; IAEA, 1995).

7.1.3.3
Transfusion therapy for treatment of accidental radiation-induced aplasia

Mol (Belgium, 1965) A worker in a research nuclear reactor, which underwent a criticality excursion, received a mean estimated dose of 5 Gy (gamma)+0.5 Gy (neutron) with very heterogeneous values from 50 Gy to the left foot to about 2.5 Gy to the head and neck. Neutrophils were lower than 1×10^{9} l^{-1} from day 3 to day 31 with a nadir on day 21; platelets were lower than 50×10^{9} l^{-1} from day 6 to day 31 with a nadir on day 13. A bone marrow transplantation was suggested but was postponed as a sampling from the sixth cervical vertebra was normal in spite of all other samples taken from lower bones (sacrum, sternum, legs) being totally deprived of medullary cells. Dosimetry studies suggested a possible autologous reconstitution for this patient from a small but rather healthy bone marrow area. The patient received three transfusions of red cell concentrate on days 28–31. Haematological restoration was considered complete three months after the accident. Radiodermatitis lesions of the left leg were severe and required its amputation 25 weeks after the accident. The accident is an archetype of several accidents with very heterogeneous exposure leading to autologous haematological reconstitution, although other syndromes may be severe or lethal (burns and gastrointestinal syndrome as in the Nesvizh accident) (Jammet *et al.,* 1968).

Setif (Algeria, 1978) Four women received protracted irradiation from a gammagraphy ^{192}Ir source of about 1 TBq. Estimated accumulated doses to the marrow (more than 5 weeks of exposure with a daily exposure time of about 6–8 hours a day) ranged from 10 to 14 Gy. Initial bone marrow examination from various sites showed a total lack of cellularity. Blood lymphocytes were $15–20\times10^{7}$ l^{-1}, granulocytes $0–10^{8}$ l^{-1}, reticulocytes $0–20\times10^{7}$ l^{-1}, platelets $7–30\times10^{9}$ l^{-1}. Intensive care, anti-infectious treatment and daily balance of the haematological deficiency were applied. The patients were isolated for seven weeks in plastic, air-conditioned sterile chambers which were provided with sterile air. Asepsis was controlled daily and dust quantity and granulometry were monitored continuously. The four patients developed local infection or septicemia which necessitated major antibiotic and antimycotic treatment. The patients were fed intravenously. They also received hormonal therapy to compensate the adrenal deficiency induced by blocking the ovarian functions in order to inhibit menstrual flux which was likely to trigger a haemorrhage. A patient who was pregnant was given progesterone and chlormadinone acetate in an unsuccessful attempt to avoid spontaneous abortion. The three others were given ethinyl-estradiol, lynestrenol and norethisterone acetate; all received corticoids (Jammet, 1979; Jammet, *et al.,* 1980).

A very severe period, with infectious and haemorrhagic manifestations, lasted 8 weeks. One patient had retinal haemorrhage, another presented a buccal bleeding due to a vessel ulceration. The blood cell counts remained at a very low level for 3 weeks (granulocytes 0–4 per cent, reticulocytes 0–0.1 per cent, thrombocytes 8–15 per cent, lymphocytes 10–15 per cent of normal values). Despite daily transfusion

of erythrocytes, leukocytes and platelets, the blood count level remained at the limit of the infectious and haemorrhagic risks for a critical period which lasted between 30 and 60 days after the end of the exposure. One of the patients for example received 12 packed red blood cell transfusions (4125×10^{10} cells) and 10 transfusions of leukocytes (42×10^{10} cells). Maintenance of the hydroelectrolytic balance for the same patient took 222 litres of different sera.

The restoration period started late (myelocytes and metamyelocytes appearing in the peripheral blood) and progressed slowly before stabilising at levels below physiological values (granulocytes were between 35 and 60 per cent of normal values for two patients, lymphocytes between 35 and 60 per cent and thrombocytes 55–60 per cent of normal values for all patients) indicating the persistence of a long-term defect in haemopoiesis. On the other hand, reticulocyte levels showed a peak, reaching 140–280 per cent of control for the four patients.

The long-term follow-up showed a total recovery for all patients.

Casablanca (Morocco, 1984) Eight individuals were accidentally exposed to an ^{192}Ir source; the exposures were prolonged and fractionated. Six victims (parents and four children) died in 4–6 weeks. Among the other individuals exposed at lower levels of dose, two had a mean whole body dose estimated to be about 3 Gy (male, 32 years of age) protracted over 17 days, and about 7 Gy (female, 66 years of age), protracted over 82 days and more intensively over 17 days, respectively (Parmentier *et al.,* 1990). At the time of hospitalisation (day 43 and day 8 after the end of exposure, respectively) the first patient had deep and persistent asthenia and scars from nasal herpes. Haematological findings suggested a moderated hypoplasia, while the second one had biological signs and clinical symptoms of severe aplasia, paleness and haematoma and presented a total alopecia.

For the first patient, peripheral reticulocyte and neutrophil counts improved a few days after hospitalisation. An increase in eosinophil count was then observed. Three weeks later, however, haematological repair was still incomplete. Treatment of aplasia required two red cell concentrates on day 63 and one on day 68.

Platelet transfusion (10 units from day 12 to day 17) was required for the second patient. She received also four units of red blood cell concentrates on day 12 and two on day 14. During a phase of bacteremia, intensive antibiotic therapy had to be administered. Signs of bone marrow repair appeared when fever decreased. Haemopoiesis was stabilised (although at a level lower than normal average) on day 50. Ferrokinetic studies, scintigraphies and bone marrow cell cultures showed evidence of heterogeneous irradiation for both patients with higher doses to the upper part of the body. Both patients recovered within two months.

Table 7.1 provides a list of the accidents described above and summarises their main characteristics.

7.1.4
Comparison of Medically Irradiated Patients and Accidentally Overexposed Individuals

The two populations of interest for a rational approach in the treatment of haemopoietic injury, i.e. patients who received TBI for various medical reasons and victims of radiation accidents, are difficult to compare. The mixture of the experience

Table 7.1 Classification of radiation accidents distinguishing the type of treatment

	Place	Date	Source	No. patients	Dose[a] (Gy)	No. Deaths[b]
Bone marrow transplantation (and foetal liver)	Vinca	1958	Reactor	5	7–10	1

	Place	Date	Source	No. patients	Dose[a] (Gy)	No. Deaths[b]
Pittsburg	1967	Acceler.	1	6	–	
Shanghai	1980	^{60}Co	1 (FLT)	5	–	
Chernobyl	1986	Reactor	13	>6	11	
				6 (FLT)	>9	6
Growth factor therapy	Goiânia	1987	^{137}Cs	8	2.5–7	4
San Salvador	1989	^{60}Co	3	3–8	1	
Soreq	1990	^{60}Co	1	>10	1	
Nesvizh	1991	^{60}Co	1	10	1	
Transfusion therapy	Mol	1965	Reactor	1	>5	–
Setif	1978	^{192}Ir	5	10–14	1	
Casablanca	1984	^{192}Ir	9	3–7	6	

[a] Either dose range when several victims, or mean dose when only one victim.
[b] The total number of deaths may be higher; this table considers only the hospitalised victims.
[c] Only the four surviving victims are described in the text.

obtained from these two categories of irradiated individuals may result in incorrect interpretation of biological responses and a wrong choice when treating the victims. The main reasons for which any amalgamation may result in erroneous judgements are several:

1 In medical irradiation the exposure parameters, such as the type of radiation, the total dose, the dose rate, the dose delivered to single organs of particular interest, etc. are totally under control. In accidents, these parameters are generally completely or partially unknown and require prolonged efforts by experts to deduce the physical conditions of the accidental exposure.
2 In case of accidental exposure, the victims are generally in a healthy condition, or, at least, are not suffering from severe diseases. This is contrary to the case of patients receiving TBI who, in addition, have very often received previous cytotoxic agents, such as those commonly used in classical chemotherapy protocols.
3 TBI patients are medically prepared for their irradiation, both from the psychological and physical points of view. Stress is always an important component for accidentally irradiated individuals, especially in the prodromal period. In addition, in accidental circumstances, it can be expected that the medical team will also be subject to stress, as it has to face an unusual and unpredictable situation, the main parameters for decision-making being lacking or inconsistent. TBI patients are under high surveillance with regard to infection; preventive procedures as well as therapeutic actions are well defined. By contrast, the victims of an accident should be suspected to carry potential foci of infections, representing hazardous sources of complications for the futher development of aplasia.
4 Accidentally irradiated victims will rarely receive completely uniform radiation exposure, as occurs therapeutically. During the accident, shielding or partial shielding of a relatively small portion of the body will give a significant degree of protection. Experimental data suggest that the shielding of about 10 per cent of the active marrow while the remainder receives a dose close to the $LD_{50/60}$ may reduce the lethality to zero (UNSCEAR, 1988). This would also apply to some degree in the case of non-uniform exposure. As the distribution of the dose in the bone marrow will determine spontaneous regeneration capabilities (even a few, small isolated patches of bone marrow which have received relatively low doses will be enough for the repopulation of heavily irradiated areas), this specific

characteristic of accidental conditions has two issues: it may moderate the typical clinical course and will be of great importance when deciding whether a BMT is indicated or not.

5 Patients receiving TBI have their lungs shielded, in order to maintain their lung dose below about 8 Gy. Bone marrow doses around or in excess of 8 Gy are by themselves highly life-threatening; in addition, accident victims who receive whole-body doses of this order of magnitude may exhibit pulmonary effects, which will complicate the clinical course.

6 Radiation accident victims may be exposed to internal irradiation, the pattern of which will complicate the clinical picture and the prognosis. In addition, depending upon the circumstances of the accident, victims may exhibit classical injuries, such as wounds, thermal burns, etc. In general, the degree of emergency is related more to these injuries than to the radiation damage, which is not a real medical urgency; consequently sorting the victims may raise further problems, such as the adequacy of the adapted medical management.

Table 7.2 summarises the main differences between patients who receive TBIs for medical reasons and patients who have been accidentally whole-body irradiated.

7.1.5 Main Medical Issues

In the dose range where survival is possible with intensive medical care, the critical organ on which prognosis depends is the bone marrow. Nevertheless, the lethality after higher doses will reflect failure of particular organs which, in relation to the underlying cell kinetics, will fail after different periods of time. There is a latency period before the development of injury, and following the expression of injury there may be a dose-dependent recovery phase. The acute radiation syndrome is therefore the combination of various signs and symptoms, described by their temporal distribution, with a certain number of sequences of well documented events (ICRP, 1984; UNSCEAR, 1988).

Table 7.2 Main differences between patients receiving TBI for medical reasons and victims irradiated in an accident

	TBI therapy	Whole-body accidental irradiation
Exposure parameters	Chosen, known, under control	Unknown, difficult to assess
Exposure conditions	Homogeneous	Almost never homogeneous
Health state	Bad,+cytotoxic drugs	Normal
Medical preparation	Mandatory	None
Lung dose	<8Gy	?
Possible associated injury	None	Internal exposure, radiation burns, trauma, etc.

Because of the rapid renewal of many important types of blood cell, bone marrow is one of the best examples which can be quoted for organ radiation injury. Consequently, it will represent the greatest medical concern, at least during the first weeks of the acute manifestations of the radiation-induced illness. In summary, radiation leads to mitotic delay and a loss of the reproductive capability at all levels in the hierarchy of proliferating cells, including the stem cell compartment, in which the proliferation rate is rather low under normal conditions (Fliedner and Nothdurft, 1986). Acute doses of a few tenths of gray have been shown to cause injury to the haemopoietic cells in a given marrow site, presumably through induction of programmed

cell death (apoptosis). By contrast, most mature blood cells (except circulating lymphocytes) are quite resistant to radiation: after radiation injury, they maintain their cytological integrity and main specific functional properties. One important parameter for the medical management is the time-related pattern of development of the radiation injury, in particular in the form of critical blood cell concentrations (Nothdurft, 1991). This temporal pattern will depend upon the time which is needed by the various cell lines for their proliferation in the bone marrow and the residence time of non-dividing maturing cells, as well as the lifespan of the circulating blood cells which can be short (see Chapter 2). It can be concluded that radiation lethality is primarily, and in principle, a consequence of disturbed cellular kinetics in the renewal system critical for survival (Bond and Sugahara, 1969).

Consequently, the doses received by the bone marrow constitute a relevant index of severity, provided the exposure concerns the whole body and is not significantly non-uniform. In the case of accidental exposure, the physician will rely on the doses for planning purposes, as well as for a guide to the need for supportive or aggressive treatment. One major concept of interest in this case is the estimated median radiation dose leading to death in sixty days, i.e. the $LD_{50/60}$; the time period of two months is related to the length of the critical phase after the prodromal phase in the midlethal range of doses. Although the definition of $LD_{50/60}$ implies that no treatment has been given, there are, for obvious reasons, no accurate human data on the tolerance of the haemopoietic system after whole-body irradiation without the assistance of any kind of medical treatment. This fact, in addition with many other considerations, such as variation with age, body weight, health conditions, nature and energy of radiation, relative homogeneity of exposure, etc., explains why there have been in the literature so many estimates of the $LD_{50/60}$ over three decades (UNSCEAR, 1988). The midlethal range lies between 2.5 and 5 Gy to the bone marrow (mean dose in an annulus between 0 and 6–7 cm below the body surface) (ICRP, 1984). The bone marrow dose is higher than the free-in-air tissue kerma by a factor of 25–30 per cent and lower than the midline dose by about ten per cent for ^{60}Co - radiation. It has been concluded that the $LD_{50/60}$ for acute radiation is likely to be around 3 Gy marrow dose, provided the patients receive no or little medical care (UNSCEAR, 1988; US Nuclear Regulatory Commission, 1990).

Of more medical interest is the form of the dose-mortality relationship of the $LD_{50/60}$ in humans; it is expected to follow approximately a Gaussian distribution. The relationship is sigmoid on a linear plot of percentage mortality against dose. The points of interest are the dose values corresponding to:

1 The threshold, i.e. in the range $LD_{1–10}$, estimated to be around 1.5 Gy.
2 The dose values corresponding to mortality, i.e. in the range $LD_{90–99}$, estimated to be around 5 Gy.
3 The slope of the sharp increase in mortality.

The fact that the ratio between LD_{90} and LD_{10} (acute dose) is small shows that, for medical reasons, all efforts should be made to assess as accurately as possible the dose accidentally received.

Dose protraction or fractionation is very effective in increasing the $LD_{50/60}$. Depending upon the overall time and the irradiation schema, the $LD_{50/60}$ may be increased up to ten times for exposure periods of the order of several weeks.

Intensive medical treatment will increase the probability of survival for patients suffering from a bone marrow, radiation-induced depression. Experiences from radiotherapy patients and victims of accidents demonstrate that the LD_{50} after appropriate treatment may be increased by a factor of about 2–3, regarding lethality related to bone marrow. As for the other organs involved in a whole-body irradiation, some problems may arise after the specific bone marrow problems have been totally or partially solved.

From a medical point of view, especially when referring to therapy, it would be completely unrealistic to consider the bone marrow depression as an isolated syndrome. Because of their widely differing specialised functions and cell renewal capacities, all other organs and tissues, such as gut and central nervous system will produce complex but definable time and dose-dependent overlapping patterns of clinical symptoms. Whole-body irradiation will therefore include a sequence of combined interacting effects which will complicate the medical management (Dixon, 1985). Depending upon the organs and tissues concerned, the time pattern for each clinical and/or biological manifestation will differ. Some of these manifestations may appear late after the exposure and may last for long periods of time; they include effects on the lungs, liver, kidneys and endocrine systems, for example. All these effects may well be life-threatening. As for the more urgent medical management, it is possible to classify the early combined effects by taking into account the cell kinetics of the organ or tissue and their sensitivity to radiation, i.e. with reference to both overall exposure time and dose.

For doses in the sub- and infralethal range, prodromal effects on the gut may increase in severity and persistence, and then decrease. For doses slightly above the midlethal range, because of the clinical consequences of damage to the small intestine which are more acute than for bone marrow, death may result more quickly. Even so, latent damage to the bone marrow will be a contributory parameter at these dose levels. In addition, bleeding likely to occur as a result of thrombocytopenia may worsen the intestinal conditions. Above 15–20 Gy whole body (acute dose), injuries to both the bone marrow and the gut will be pre-empted by the overwhelming injury to the central nervous system and some other important tissues such as the vascular system.

7.2
Assessment of Severity

7.2.1
Clinical and Biological Features

Schematically, the higher the dose and the dose rate, the greater the effect and the more rapid the onset of symptoms. Guidance for classification of the acute radiation syndrome may help for the treatment of haemopoietic injury after whole body irradiation. Such guidance is provided on the basis of dose categories, corresponding to the severity of effects increasing with the exposure levels (Guskova *et al.,* 1987; Saenger, 1990; Baranov and Guskova, 1990). These categories include:

1 A mild degree of clinical damage (1–2 Gy).
2 Average severity representing a mild form of the acute radiation syndrome (2–4 Gy).
3 A severe clinical and biological course of events with haematological complications (4–6 Gy).
4 An accelerated version of the syndrome with gastroenteric signs complicating severely the haemopoietic injury (higher than 6 Gy).
5 Not amenable to medical treatment with a fulminating course including vascular and/or central nervous system impairment (a few tens of gray).

The clinical progression can be divided into four successive periods. The shorter the duration of each phase, the greater is the degree of injury to the patient. The prodromal period of initial shock in the first day after irradiation is characterised by several effects: there is marked prostration from the first day onwards, profuse sweating, nausea, vomiting and variable diarrhoea. The most frequent and reliable initial findings

are nausea and vomiting. An immediate erythema—or delayed up to a few hours—suggests high levels of exposure (above 3–5 Gy). The mechanism of the prodromal syndrome is not well understood, but it appears that abnormalities in the autonomic nervous system are responsible for the early digestive symptoms, even if they are partly psychogenic (Harding and Davis, 1986).

The latent period may last from a few hours for very high whole-body doses (more than 15 Gy) to a few weeks. In the range of the $LD_{50/60}$ it usually lasts less than one week. During this phase, only fatigue and headaches persist. During this period, subsequent signs and symptoms related to damage to progenitor cells in the bone marrow and lymphatic organs as well as a decreased immune response will be found. There is a rapid reduction in the lymphocyte count, usually within the first two days for doses higher than 2 Gy. This lymphocyte depletion is due mainly to the killing of circulating lymphocytes, which undergo interphase death within hours. The absolute lymphocyte count is of great help in evaluating the severity of injury within the first three days after exposure.

The critical phase is characterised by severe clinical findings such as anorexia, abdominal pain, signs of haemorrhage (epistaxis, gingival bleeding, haematemesis, melaena, haemoptysis and purpura), hyperthermia, prostration. For doses higher than 3–5 Gy to the skin, erythema may occur and may provide reliable information for dose assessment, as may epilation. The distribution of epilation may be useful in determining the direction of the radiation beam and distribution of the dose, and consequently may give reliable information for the treatment. The time course of changes in the blood cell lineages during this period is well documented. Among the white cell population, only neutrophils need to be considered. The changes are important over the entire clinical course. For doses in the $LD_{50/60}$ range, the typical evolution is characterised by:

1 An early peak, which does not last more than a few hours, and cannot be directly related to the dose.
2 A rapid dose-related fall.
3 An abortive rise, between days 10 and 20 after exposure, and probably due to mobilisation of cells from the bone marrow and extramedullary sites which may be mediated by haemopoietic growth factor release. The presence of the abortive rise has some value for the prognosis, as it is not seen for doses in excess of 5 Gy.
4 A second decline, which may result in very low absolute counts, from 100 to $10000\times10^9\ 1^{-1}$. For doses well in excess of 5 Gy, no white cells can be observed in the peripheral blood smears. In fact, doses up to 15 Gy do not completely ablate haemopoiesis; and a small number of lymphoid cells and haemopoietic progenitors persist (Butturini *et al.,* 1986). The nadir for granulocytes is reached after 20–30 days. A more rapid fall in granulocytes augurs a very poor or even desperate outlook, requiring very intensive therapy. Anyhow, this development indicates that the time period starting about 2 weeks after exposure is crucial with regards to fever and infection.
5 Bone marrow recovery, announced by the appearance in blood of monocytes and moderate myelemia (myelocytes and metamyelocytes). The presence in blood of very few precursors can be considered as a good prognosis.

The time course of thrombocytopenia is broadly similar to that for granulocytes, but there is no abortive rise. For doses around the $LD_{50/60}$, a decrease in platelets to $10–20\times10^9\ 1^{-1}$ is observed around day 14. Haemorrhage is likely to occur at thrombocyte levels below $20\times10^9\ 1^{-1}$, resulting in anaemia and possibly requiring transfusion. Recovery starts at about the same time as for the white cell series, but the platelets recover more slowly.

The erythrocyte counts fall much more slowly in the absence of haemorrhage. This can be related to the relatively long lifespan of red cells (100–130 days). The reticulocyte count is a good index to evaluate the future dev elopment of aplasia: a reticulocyte rise during the critical period can be considered as a good indicator of recovery from aplasia.

7.2.2
Parameters of Importance for Diagnosis and Prognosis

It is customary to classify severity of overexposures according to the estimated absorbed dose. Such a classification is to some extent arbitrary because many other variables influence both the symptomatology and the therapeutic choices.

7.2.2.1
Nature and energy of the radiation

It should be remembered in this respect that there are fundamental differences in the resulting effects of the irradiation between highly penetrating radiation and weakly penetrating radiation (see Chapters 4 and 5). Examples of highly penetrating radiation are given by photons of high energy (X and), neutrons, protons and mesons of high energy (as may be released by accelerators), which can penetrate the body to great depth. On the contrary, -rays cross distances of only 30–40μm and -rays (electrons) usually penetrate only several millimetres beneath the body surface. In general, only photons and neutrons will be considered with regard to bone marrow injury. The higher their initial energy, the deeper will be their level of absorption and the higher the dose to organs such as the bone marrow.

7.2.2.2
Nature, type and size of the source

In this regard, the main parameter is the geometry of the radiation source with respect to the individual. The relative position of the source to that of the victim, and its changes during the course of the exposure, are important determinants of the dose. For example, provided there is a sufficient distance between the source and the individual, and time of exposure is short enough not to allow significant variation in geometry, the inverse-square law (intensity of exposure decreases as the square of the distance) can be applied. It is relatively easy to estimate roughly the absorbed dose in a given volume. Great help can be provided by depth dose tables or curves, as exist in radiation therapy or radiological protection manuals.

Owing to the medical importance of the physical parameters, it is necessary to document in detail the point of radiation emission, the quality and quantification of scattered radiation from surrounding materials (shielding, collimators, ground, walls), the direction and spread of the beam, the position of the individual and his/her distance and orientation in relation to the source. The variations with time in these parameters should be identified although, in retrospect, precise determination has proved to be very difficult. The most difficult parameter to assess after an accident is time, in which errors of a factor two or more are not uncommon, depending on individual estimates.

7.2.2.3
Dose rate

After an accidental exposure it is difficult to be quantitative about the radiation effects because of variations of time, such as dose protraction or fractionation. Any exposure which occurs in seconds to hours can be considered as acute.

7.2.2.4
Spatial distribution of the exposure

The experience from radiation accidents shows that an accidentally exposed individual rarely receives completely uniform exposure, as is managed therapeutically with TBI. The exposure heterogeneity will determine, in part, the reversibility of the induced damage to the stem cell system since the surviving fraction of haemopoietic stem cells must be capable of both spontaneous regeneration in and repopulation of highly depleted bone marrow areas. The identification and relative evaluation of heterogeneity of accidental exposure will therefore be a determinant of the therapeutic choice.

7.2.3
Methods for Dose Assessment

The quantitative evaluation of exposure and of its main parameters as mentioned above rest mainly on physical and biological dosimetry. It should be stressed that most of the methods used for dose assessment need a minimum assay of time, depending upon the techniques. For instance, the full size reconstruction of the accident requires many technical and human efforts, and chromosome aberration scoring cannot be reduced below three days, when using current methodology. This implies that, at the early stage, the dose will be assessed on clinical findings and simple blood cells counts. In fact, these delays in obtaining important data will not hinder the medical management of the victim, since the duration of the latent period allows some flexibility in decision-making. On the other hand, all necessary dosimetric explorations should be considered and initiated as soon as the severity of the exposure is suspected.

7.2.3.1
Physical dosimetry

Under ideal circumstances the overexposed victim would be wearing a dosemeter. This is rarely the case in accidental situations, especially when members of the public are irradiated following the loss of a source. Anyhow, more information is needed than the measurement of a dose received at a particular point of the body.

The reconstruction of the accident, whether carried out by experimentation or by calculation according to the case, enables a realistic evaluation of the dose distribution within the body. An experimental reconstruction at the accident site, or simulated conditions of the accident, can be carried out with the help of phantoms made of a tissue-equivalent material for the various types of radiation. The appropriate detectors (thermoluminescent dosemeters for -rays, activation or junction detectors for neutrons) are placed in the phantom's slices and allow the measurement of the absorbed dose in the various regions of the bone marrow and other organs and tissues, after the phantom has been placed in such a way as to reproduce the position of the victim at the time of the accident (Parmentier *et al.,* 1968, 1980). The distribution of active bone marrow residing in the different bones of man is given by various authors and is currently presented in

comprehensive tables expressed in percentages. This enables an assessment to be made of the fractions of bone marrow which have received sufficiently low dose to allow spontaneous repair. When reconstruction is not feasible, the reconstruction by calculation can be performed, although its results are not generally as reliable as in a case of a realistic reconstruction.

Figure 7.1 shows the distribution of exposure in two cases of acute irradiation after the physical reconstruction of the accidents: in the Mol case (right), it was calculated that 16 per cent of the bone marrow received less than 3 Gy, while in the other case (Brescia, Italy, 1975) 13 per cent received between 4 and 8 Gy and 87 per cent more than 8 Gy (with death of the victim after 13 days).

Exposure to neutron or mixed -neutron radiation fields can result in activation of elements. Phosphorus-32 activation in the hair and nails and the activity of sodium-24 in the blood may be very helpful in estimating absorbed doses to particular areas of the body, and the mean absorbed dose, respectively.

The dose from accidental exposure may also be evaluated by using radiolyoluminescence, i.e. the luminescence which occurs during the dissolution of a sample of biological tissue (hair, nail, tooth, bone) and clothing. Radiolyoluminescence is caused by free radicals which are detected by the method of electron paramagnetic resonance. The main advantage of this method is that it allows measurements to be made at various points of the body and, consequently, the variations of the spatial distribution of exposure may be evaluated.

7.2.3.2
Biological dosimetry

Haematology Dose-response curves for the change in concentration of various blood cells of healthy humans after whole-body exposure are fairly reproducible, especially regarding the lymphocytes. As the reduction in lymphocyte numbers is very rapid and sensitive, this makes the best early biological indicator of severity, performed as soon as possible after the exposure and repeated once or twice at about six-hour intervals. This simple test is the best quick guide, within the first two to three days, of the degree of severity with an acceptable degree of uncertainty. Figure 7.2 shows the reduction in lymphocyte count after three radiation accidents, with doses ranging from around 2 Gy to 12 Gy. Later on, the nadir values for the various blood cells and the delay after which they are reached contributes to a confirmatory evaluation of the severity. Additional information on the distribution of the exposure within the body can be provided by bone marrow punctures in various sites which are chosen with regard to the exposure conditions. Various techniques for exploring bone marrow are available, each of them having specific advantages:

1 Myelograms—though it must be underlined that an empty bone marrow should not be considered necessarily as a poor prognostic indicator.
2 Progenitor cell culture which gives information on regeneration capabilities: from the bone marrow they will indicate the degree of radiation injury in each site, while from blood the presence of haemopoietic progenitors will suggest the survival of healthy haemopoietic stem cells in a viable, untested area.
3 Bone marrow mapping through scintigraphy using ^{59}Fe, allowing visualisation of possible extramedullary sites.

The two latter techniques, i.e. cell culture and bone marrow mapping, are time-consuming; consequently their results cannot serve as a basis for early diagnosis and prognosis. They will be useful at a later stage,

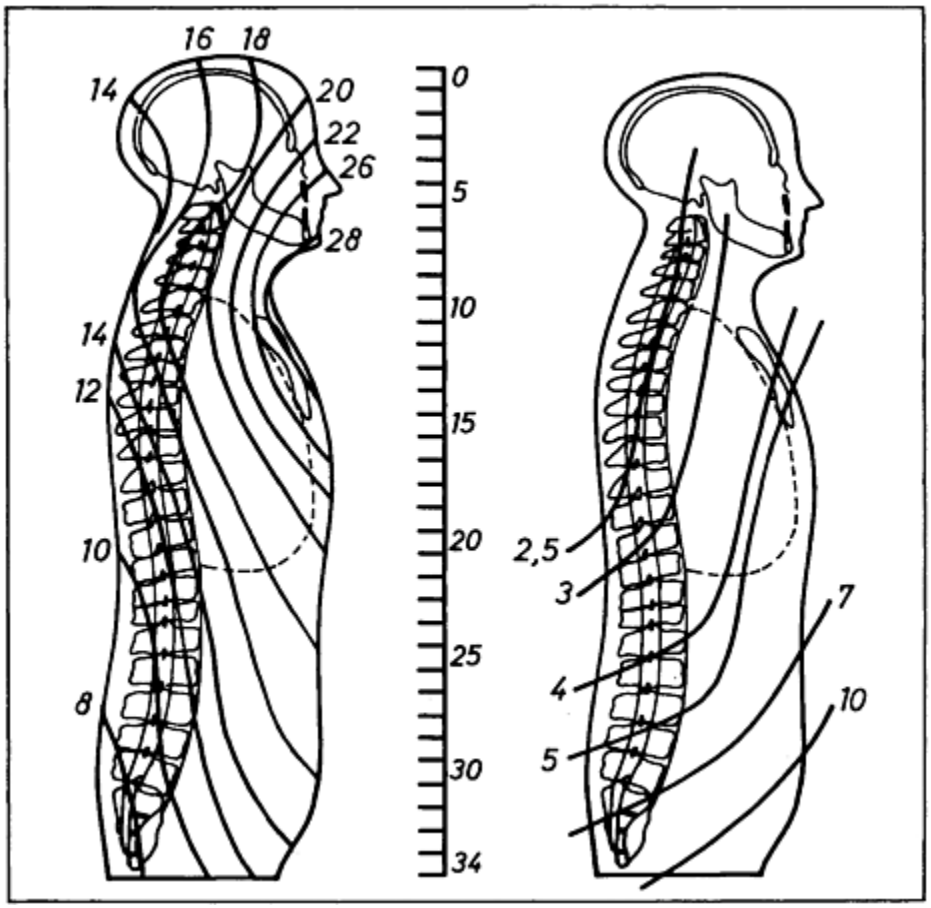

Figure 7.1 Isodoses curves after physical reconstruction of two radiation accidents: Mol, Belgium (1965) and Brescia, Italy (1975). Doses in Gy. (*Source*: Parmentier *et al.,* 1980)

especially in severe cases where the medical management of the critical phase needs as many data as possible.

Cytogenetic tests Many chromosomal aberrations may appear in irradiated lymphocytes. The aberrations currently taken as providing the most valuable information are the dicentrics, rings and fragments. The blood may be used as an absolute dosimeter in the weeks following the exposure, since human T-lymphocytes have a relatively long life and the reduction in the number of aberrations is not significant. In the circulating blood, lymphocytes are the only mature cells which have the capacity to divide. It is possible, after culture and during the first metaphase, to observe normally latent chromosome abnormalities. As the lymphocytes are from the circulation, their modifications can be a good indicator of the whole-body dose, even if the exposure was a relatively short duration, provided that the radiation energy was sufficiently high. Counting the number of abnormalities in circulating blood lymphocytes and comparing the number with reference values gives a reasonably accurate estimate of the mean dose to the whole body. This approach has its limitation in the case of heterogeneous acute exposures during which only some of the

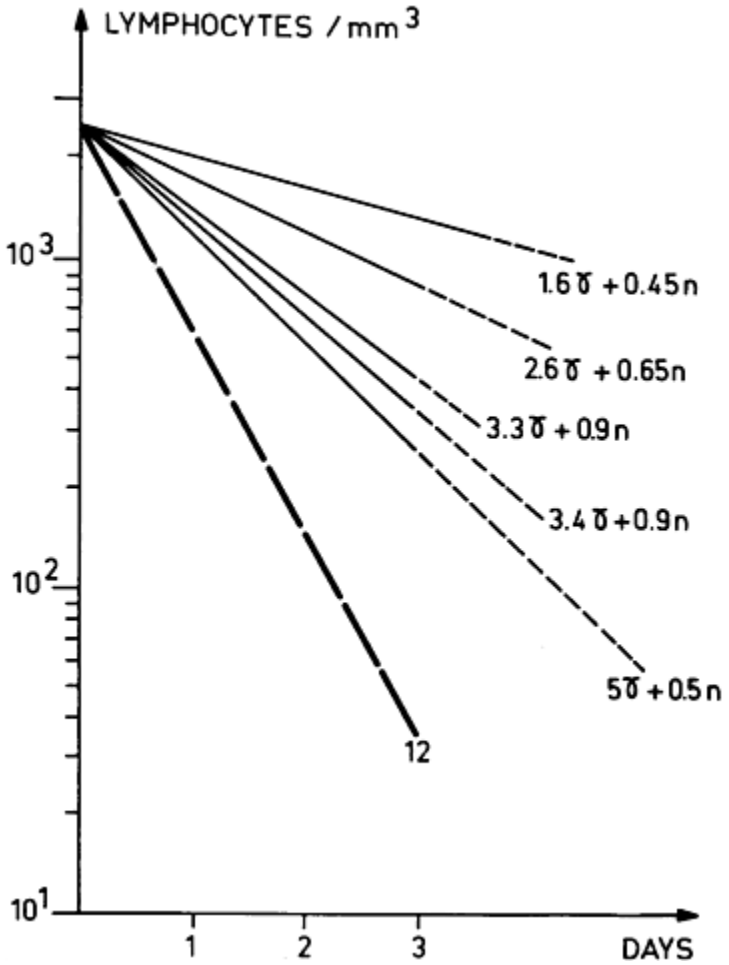

Figure 7.2 Initial reduction in lymphocyte count after three radiation accidents: Vinca, Mol and Brescia. Doses from -rays and neutrons are presented separately. (Source: Jammet, 1984)

lymphocytes are exposed and for which the dilution factor is not known, as well as in the case of prolonged exposure.

A dicentric aberration requires the interaction of two breaks, each induced in separate G_0 or G_1 chromosomes. For low-LET radiations, the yield of aberrations conforms well to a quadratic relationship:

$$Y=c+\alpha D+\beta D^2$$

where Y is the yield of aberrations, c is the background (about one aberration in one thousand cells or more), D is the dose, and are fitted parameters; the term D expresses the effect of a single ionising track causing two breaks, and the term D^2 represents those abnormalities produced when two breaks are caused by separate ionising tracks. The effect of the last term increases with increasing dose. Radiations which can be involved in accidental situations are various: most frequent are X- and -rays, sometimes together with neutrons. There are some differences in the dose-response relationships for these various

radiations, depending upon their energy. Several of the reference curves expressing these differences can be found in the literature (UNSCEAR, 1988; IAEA, 1979; Lloyd and Edwards, 1983; Littlefield *et al.,* 1980; Doloy *et al.,* 1991). For high-LET radiation, such as fission spectrum neutrons and -particles, the relationship is generally linear; the events are so densely distributed along the tracks that there is a very high probability that one track will deposit energy in both chromosomes. The relative biological effectiveness (RBE) decreases rapidly with increasing doses.

For assessing the accuracy of dose-response relationships, it is necessary to know the distribution of the aberrations. As the events following energy deposition seem to appear in a random fashion in the exposed cells, the distribution of the abnormalities can be described using a Poisson distribution in which the mean equals the variance. In non-uniform exposure, there is a larger dispersion of dicentrics, although the Poisson distribution is still representative. Anyhow, although the knowledge of the degree of exposure homogeneity is fundamental for medical decisions, the estimation of the blood volume irradiated using this method gives only a very rough evaluation of the percentage of the body (and consequently of the bone marrow) which has actually been irradiated.

Owing to the relative scarcity of radiation-induced chromosome aberrations, hundreds of metaphases, depending upon the dose level and the expected degree of precision in dose evaluation, have to be examined. Generally, evaluation of between 100 and 500 metaphases is sufficient to estimate the dose at levels which are of medical significance, i.e. of the order or higher than 1 Gy. For lower doses, a much higher number of metaphases is needed, and about 1000 are required for doses around or lower than 0.5 Gy. Uncertainties in the results are linked to the variations in the distribution and to the accuracy of the reference curve. For example, for a confidence interval of 95 per cent, a dose of 1 Gy will be estimated between 0.70 and 1.35 Gy when 100 cells are examined, and between 0.85 and 1.15 Gy on the basis of 1000 cells. For 0.25 Gy, precision falls between 0.03 and 0.60 Gy for 200 cells examined, and between 0.35 and 0.65 Gy for 1000 (IAEA, 1979). Schematically, for doses below 1 Gy, the accuracy is of the order of 0.10–0.25 Gy and depends on the number of cells observed. Sensitivity of the chromosomal method has undoubtedly been improved by the introduction of sister-chromatid differentiation which distinguished the first- and second-division metaphases and enables a selection of only first metaphases for chromosomal aberration analysis. The use of dicentrics for dose assessment is limited since the frequency of cells with such aberrations decreases with time after exposure. Scoring chromosome translocations avoids this limitation as they are stable with time after exposure. One reliable method for detecting and scoring reciprocal translocations is fluorescence *in situ* hybridisation. This is a promising method for biological dosimetry as it may be relatively rapid and can be automated (Lucas *et al.,* 1989).

An important feature of the dose-response curves is the relative importance of the dose rate. In the case of exposures extending over more than one day and up to around one week, there is some disparity between doses evaluated by chromosome aberration counting and their evaluation by clinical observation and physical evaluation or measurements. In general, cytogenetic analysis underestimates the haematological injury, especially as lymphocytes have already undergone one or several mitoses. In addition, reference curves, when existing, are never representative of the exact conditions prevailing at the time of exposure.

A simple method for scoring damage to chromosomal material is to count micronuclei in cells which have undergone one cell division after the clastogenic insult (Huber *et al.,* 1983; Fenech and Morley, 1985). Micronuclei are round bodies found in the cytoplasm outside the main nucleus and arise from acentric fragments which fail to incorporate into the daughter nuclei during cell division. There is a higher background incidence of micronuclei than of dicentrics, which results in a higher limit of detection. In principle, the counting of micronuclei appears faster and cheaper than the scoring of chromosome aberrations. In addition, it does not require such highly trained personnel as for chromosome aberration

identification. On the other hand, variability in cell cycle kinetics is likely to impose great uncertainties in the quantification of the dose-response. Several scoring methods have been proposed, and many laboratories are developing this technique for assessing individual doses in the case of accidents. The rationale for this relies on the acceptability of some uncertainties, especially in the low dose range, and on the greater availability of automated methods of analysis.

Sperm analysis The changes in sperm counts are extremely sensitive indicators at relatively low doses, because the early differentiating forms of spermatogonia are extremely sensitive (UNSCEAR, 1988; ICRP, 1984; Ladner, 1991). Assuming that the testes were exposed, the results of sperm counts and their variation with time can serve as a good indicator of the severity of exposure; in addition, they will be helpful for the assessment of the degree of uniformity of the exposure when compared with other biological parameters such as blood counts and chromosome analysis. Based on the time course of spermatogenesis (spermatogonial cell necrosis is detectable about five hours after testicular overexposure, while the more mature cells are much less affected and maintain normality for about six weeks) sperm counts should be obtained within the first month and subsequent ones obtained between the second and third month. Oligospermia is induced by doses as low as 0.15 Gy, and remains moderate; exposure of the testes may result in temporary sterility above 0.50 Gy, and in prolonged sterility above 1–2 Gy (Heller, 1967).

Neurophysiological disturbances Whole-body overexposure results rapidly in functional modifications in the central nervous system. Early reactions are intra- and extracellular oedema, inflammatory infiltration and metabolic disturbances. It acts as a stimulant of the brain, in particular of the structures in the bulbar protuberance and the hypothalamus, as well as of the synapses within the organ. The stimulation has also an indirect component, by the convergence of impulses to the brain from the spinal cord and the bulbus. These direct and indirect stimuli result in a defence response which is a part of the functional metabolic encephalopathy (UNSCEAR, 1988). Disturbances in the neurophysiological equilibrium are indicated by changes in excitability, paroxysmal abnormalities such as bursts of slow activity, to deformed spike-waves and grouped bursts of spike-wakes. After high doses around the $LD_{50/60}$, some structures, such as the hippocampus are unable to maintain the basic rhythms. These abnormalities can be detected by electroencephalography, with a threshold around a few tenths of gray.

7.2.4
Practical Consideration for Triage

Planning for the details of the medical management must be performed in a very practical way, as the clinical course is discrete in its early phase. Sorting victims into classes of severity will dictate the degree and kind of emergency. It allows medical requirements to be anticipated, most of which are necessary as they occur. One of the most important medical actions in the case of an overexposure accident is HLA typing; a severe whole-body overexposure knocks out peripheral lymphocytes so quickly that none can be found to serve as a basis for donor identification. In the same sense, heparinised blood samples will be taken as soon as possible for cytogenetic analysis. Since physical dosimetry may be lacking in accidental situations, the physician can reply only on the grouping of early signs and symptoms substantiated by biological parameters. The physician should give great importance to the reassurance of the patient, who needs warm, personalised support.

During the prodromal period, anti-emetics and sedatives will be given, depending on the level of severity. Cultures for micro-organisms should be performed, and foci of current infection, such as teeth, sinuses, throat, skin, colon, urogenital system, should be eradicated. Special attention should be paid to latent or documented viral infections, which may become extremely dangerous during the critical period.

Therapeutic needs become important when doses exceed about 2 Gy, and the patient should be held for observation and evaluation of severity. The extension of bone marrow aplasia will be judged by all available means and every effort will be made to support the patient, by treating infection and preventing bleeding. However, precipitous medical decisions will not be appropriate and may be misleading. Around the $LD_{50/60}$ for man, there is a absolute need for major therapeutic commitment. The patient must be prepared for barrier nursing care because of the expected rapid loss of granulocytes and related immune deficiency. Accompanying signs of the acute radiation syndrome will require specific treatments, as well as opportunistic infections which remain one of the main risks for the patient.

7.3
Conventional Treatment of Bone Marrow Depression

A clear distinction is needed between medical actions which aim to prevent complications related to bone marrow injury and those which try to restore the bone marrow functions. The first ones belong to the conventional management of patients immunodepressed with some other additional problems due to their global injury, while the latter correspond to specific haematological treatments. The latter are discussed in detail in the section 7.4.

7.3.1
Prevention and Treatment of Infection

Whatever the type of curative treatment chosen, the worst risk is the development of local or general superinfection, mainly as a result of immune deficiency. Patients with a severe degree of granulocytopenia (less than $0.1\times10^{9}\ 1^{-1}$) lasting for prolonged periods are at great risk of bacteremia, especially gram-negative rod bacteremia. These patients require aggressive approaches to prevent infection. Granulocytopenia alone is usually not sufficient to lead to infection. In concert with iatrogenic factors, cellular and humoral immune deficiencies, shifts of microbial flora and damage to normal anatomical barriers to organism penetration, it predisposes the irradiated patient to infection (Schimpff, 1990). Medical management of such patients is well defined and specialised intensive care units have means nowadays to keep alive immunocompromised patients, such as those suffering from AIDS or other diseases.

The first step is the prevention of exogenous infection. Whatever the degree to which prophylactic therapy should be pursued, isolation of the patient is mandatory. It should be underlined that using efficient and refined means of isolation is useless if the personnel are not correctly trained and aware of all the procedures used to control potential infection pathways. The use of laminar air flow rooms in which all air presented to the patient is previously filtered and maintained at a positive air pressure in relation to the remainder of the building is a current means for keeping patients correctly isolated. Bacteriological regular surveillance of the patient (at least skin and faeces) is mandatory. In addition, all monitoring, life support systems and consumable supplies should be sterile. The use of a suite with a separate work and change room is of great help for the medical personnel. These facilities should be periodically disinfected. In addition, when normal feeding is possible, all oral foods and fluids should be sterile. As these patients require frequent sampling and administration of parenteral drugs and fluids, the prior placement of a double or triple lumen central venous access line will greatly simplify the medical care and reduce the risks of infection. This intravenous access line should be inserted with great care, since it may be one of the major

iatrogenic factors increasing risks of infection, resulting in tunnel infection often due to *Staphylococcus aureus*.

The risk of endogenous contamination must be assessed as soon as the patient is admitted. It is standard practice to carry out antibacterial and antiviral therapy associated with digestive decontamination (antibacterial, antifungal) (Drum and Rappeport, 1990; Oliveira, 1990). Some authors recommend the use of associated antibiotics in order to obtain a broad-spectrum coverage and adequate serum bactericidal activity, for both gram-negative and gram-positive pathogens. As viral infections, such as herpetic infection, may cause a severe problem by its dissemination in other organs, prophylactic antiviral treatment should be carried out as soon as a radiation exposure at high dose is suspected. This treatment has proved efficient in the most recent accidents and to have positive results on herpetic lesions observed in immunodepressed patients. Available drugs which are successful against *herpes simplex* are not effective against *cytomegalovirus,* often encountered in patients in severe aplasia. Antiviral drug administration will be guided when possible by positive anti HSV *(herpes simplex virus)* antibody; otherwise, it will be performed empirically.

It seems that there are more advantages than risks to the institution of prophylactic oral antibiotherapy to eliminate pathogens usually present in the gut. It prevents subsequent systemic bacterial invasion, which may have disastrous effects on highly immunodepressed patients. In addition, maintenance of gastric acidity may prevent bacteria from colonising and invading the gastric mucosa and, therefore, reduce the probability of the development of nosocomial pneumonia. Antifungal therapy should be started very early, especially as damage to flora from antibiotic therapy encourages the settlement of yeasts which colonise the upper respiratory tract, and from there become disseminated.

With the onset of fever and the increase in the erythrocyte sedimentation rate, the source of infection must be sought as a matter of urgency. The immediate administration of several broad-spectrum antibiotics is required, and then adapted in accordance with the disc sensitivity test.

7.3.2
Nutrition and Fluid Balance

Whole-body irradiation in the midlethal range may lead to a syndrome of serious malnutrition. The nutritional and calorific equilibrium must therefore be maintained, without waiting until the syndrome develops. Oral feeding is preferable to parenteral feeding especially as it is psychologically reassuring. For patients in bad conditions total parenteral nutrition may be necessary, through the central venous line, especially for patients with severe mucositis of the oropharynx. Basal fluid intakes should be administered in accordance with the losses from diarrhoea, stomal output, nasogastric suction and possible drainage. When intravenous fluid replacement is absent, electrolyte imbalance can be life-threatening as a result of fluid loss due to severe vomiting (with or without diarrhoea) or sweating from exertion.

7.3.3
Blood Products

The use of blood products requires careful evaluation so that these materials are not given indiscriminately. Specific indications concern red blood cells and platelets. The frequency of transfusions varies with the daily reading of blood counts, the purpose being to maintain a level above that at which anaemia and haemorrhages can occur. All blood products should be irradiated at about 50 Gy to inactivate the viable immunocompetent lymphocytes present in the products and consequently to prevent GVHD. This

irradiation does not adversely affect the red blood cells or the platelets but may minimise the risk of viral diseases such as *cytomegalovirus*. If a bone marrow transplantation is envisaged for the future, the use of products from related donors should be avoided. A complete check-up should be made prior to the transfusions in order to reduce the risk of immunological complications; this includes determining or confirming ABO groups, the Rhesus factor together with the complete phenotype in other blood group systems and tests for irregular agglutinins and the HLA complex.

Erythrocytes should be provided in order to correct the tissue anoxaemia by maintaining haemoglobin at a sufficient level. Healthy individuals may tolerate rather low haematocrits, provided that their activities are circumscribed and appropriate care is given to other injuries. Transfusion of 200–250 ml packed red cells results in a 2–2.5 per cent increase in the haematocrit, provided there is no accompanying haemorrhage.

As for erythrocytes, the requirement for platelet support depends on the patient's condition. In the absence of other medical problems, the platelets should be maintained at least at $20\times10^9\ 1^{-1}$, although many victims have sustained lower counts without bleeding. The increment per transfusion is about 10×10^9 per body square metre.

Granulocyte transfusions should be reserved for patients presenting deep granulopenia, persistent signs of infection (especially gram-negative bacterial infection), and after antibiotic therapy has proved to be inefficient. However, their explicit clinical efficiency is still questionable; moreover, they contain HLA antigens, which limits their usefulness. If such a drastic treatment is initiated, it should be continued for a few days, according to the short lifespan of these cells.

7.3.4
Associated Local Radiation Injury

The experience of severe radiation accidents which happened in the past shows that bone marrow is rarely the only tissue to be damaged. This was clearly demonstrated by the Chernobyl and Goiânia accidents, where victims suffered from bone marrow injury associated with extended radiation 'burns'. In these cases, the medical management was highly complicated by the associated aplastic-skin effects: when patients with radiation burns (independently of their depth) became immunosuppressed and pancytopenic, skin closure was very difficult to obtain. It is also clear that the therapeutic efforts devoted to supporting the haematological deficiency failed because of burns covering significant areas of the body surface. In addition, the use of haemopoietic growth factors might exacerbate the radiation-induced burns, as has been suggested in the Nesvizh case. On the other hand, it should be noted that the management of extended burns has been enormously improved and their prognosis is not as pessimistic as in the past.

The association of radiation burns and bone marrow radiation injury constitutes a typical medical emergency. The emergency starts during the early phase, when shock is related to plasma leakage: during a secondary phase, infection will be highly life-threatening. The probability of infection increases with the time course of the burns. It is therefore mandatory that the associated burns should be sterile and closed during the critical period of bone marrow depression.

7.4
Specific Treatment of Haemopoietic Injury

7.4.1
General

In the 1970s and 1980s, bone marrow transplantation had become enshrined in the literature as a proposed final successful therapy for the acute radiation syndrome (Wald, 1983; Saenger, 1986; Drum and Rappeport, 1990). There were at least two main reasons sustaining this medical attitude: bone marrow appeared to be the most sensitive organ to whole-body exposure and clinicians had experience with several thousand such procedures, including preconditioning therapy for transplantation. After the Chernobyl accident, which was the first occasion where a large number of heavily irradiated victims had to be treated and where several BMTs were performed, it was concluded the benefits were limited (Gale, 1987; Guskova *et al.,* 1990). In the opinion of the physicians in charge of the victims, this therapy resulted in worsening the conditions of their patients and they advised against its use. However, it is impossible to say for certain whether the transplantations were warranted, as many cases among the most heavily exposed victims developed complications mainly due to the associated burns. Anyhow, it is recognised that a BMT must be regarded as an exceptional and potentially hazardous measure (Gale, 1986). Although BMT may not be completely contraindicated, the particular conditions prevailing in accidental overexposure explain the need for exploring other therapeutic approaches such as growth factor therapy.

7.4.2
Bone Marrow Transplantation

7.4.2.1
General background

Indications for transplanting bone marrow to a radiation casualty rests on high doses received by all areas—doses which are incompatible with spontaneous recovery and result in the absence of any relatively protected areas which might provide a basis for autorepopulation. It may be concluded that the bone marrow transplants will be useful only in a small proportion of victims, probably those receiving doses exceeding 8 Gy (Gale, 1988). At these levels, digestive and possibly neurological syndromes are likely to cause grave concern and may require more urgent treatment than the haemopoietic syndrome.

Among all the transplantation patients who were accidentally irradiated, none of the survivors showed evidence of long-term engraftment. For some patients the graft was rejected. This may be related to the persisting lymphohemopoiesis of the recipient. For some patients the transplantation had a negative impact due mainly to immune deficiencies which contributed to the death of the patient. The main limitations of transplants are: (1) the presence of residual endogeneous haemopoiesis inducing rejection; (2) difficulties in setting up the transplant: these include difficult tissue-typing after irradiation and insufficient availability of a graft; (3) the deleterious effect of the transplant (immunosuppressive treatment and/or GVHD)

1 It has been suggested that incomplete bone marrow ablation should be ‘topped up’ by additional irradiation after the accident in order to allow a safer engraftment of the recipient (for doses between 5 and 10 Gy) (Thomas, 1986b). This appears unrealistic as the data available on the irradiation dose received by the patient are, most of the time, not sufficient. In addition, further immunosuppression

would greatly complicate the clinical course. Thus, allogenic bone marrow transplantation should be used mainly when the recipient's bone marrow is almost entirely ablated. A precise dose evaluation is necessary in order to assess early the possibility of autologous recovery.

2 The transplantations were occasionally performed with poor HLA matching due to the emergency. Furthermore, HLA typing after irradiation exposure is sometimes difficult to realise because of lack of cells (lymphocytes) to type. It has been suggested that high-risk workers (such as nuclear plant firemen) should be HLA-typed before any accident. The traditional serological method of HLA testing can be replaced by the more precise and efficient polymerase chain reaction methods for HLA classes I and II antigens. This technique needs very few cells of any type and it allows one to distinguish between single nucleotide mismatches in donor and recipient who are serologically (phenotypically) matched. Bone marrow sampling and freezing has also been studied. However, practical, ethical and psychological difficulties are rather important; decisions to implement this preventive measure should take into account the individual risk of exposure to lethal doses weighted by the probability of occurrence of the accident.

3 Early studies on haematological syndrome treatment suggested that transitory bone marrow transplantation may help a patient to go through a period of severe radiation-induced aplasia before an autologous reconstitution. However, the risks related to the transplantation itself are high as a GVHD may occur and the use of immunosuppressive drugs may generate their own problems of toxicity.

7.4.2.2
Complications

Apart from consuming enormous technical and professional efforts, the complications which may follow allogeneic BMT may pose major threats to the survival of the patient. These include engraftment failure, GVHD, infections and some other complications of minor importance, due to having either a lower frequency or a lesser degree of potential severity (Drum and Rappeport, 1990).

Engraftment failure Generally, failure of transplanted marrow occurs within a few weeks after BMT (Emerson and Gale, 1988). The failure is related to immune rejection (antibody- or cell-mediated sensitisation to non-HLA transplant antigens); it may be due to a combination of inadequate immunosuppression and/or sensitisation by previous transfusion, which should be avoided when a BMT is likely to be performed. Failure may also be due to serious systemic infection, drugs, lack of adequate marrow progenitors in the graft as well as recovery of recipient cells in case of insufficient depletion, as might have been the case in the Vinca cases (Mathé *et al.,* 1959).

Transplants of T-cell-depleted grafts have been performed in order to prevent GVHD (Fahey *et al.,* 1987). This method succeeds in reducing GVHD incidence but results in an increased risk of graft failure, i.e. 10–20 per cent instead of less than 1 per cent when using conventional transplants. These failures may be related either to the lack of engraftment or to failure after initial engraftment because the radiation doses received are not high enough to destroy all recipient myeloid and lymphoid cells. In this case, the removal of donor T-cells would permit the survival and proliferation of residual recipient haemopoietic cells (Butturini *et al.,* 1986). Cases of graft failure are not well documented. However, it has been shown that specific monoclonal anti-T-cell antibodies can result in decreased incidence of graft failure in T-cell-depleted bone marrow transplants (Cobbold *et al.,* 1986).

Acute GVHD The acute form of GVHD occurs within two months after the transplantation of histocompatible transplants in about half or more of adult recipients, when no specific prevention is performed. GVHD is a major complication following allogenic bone marrow transplantation. Signs compatible with

acute GVHD have been observed in the treatment of radiation victims. Its characteristic features include fever, erythematous rash, diarrhoea and jaundice. In addition, recovery of immune functions is significantly delayed; this deficit encourages the development of opportunistic infections, such as those caused by *pneumococcus, toxoplasma* or *cytomegalovirus*. The overall mortality rate is about 10 per cent. Biopsies of the skin show vacuolar alteration of the basilar epidermis and dyskeratinotic cells in the epidermis, vacuoles at the basement membranes may progress to cleft and frank subepidermal bulla formation. In the liver lymphocytic infiltration and necrosis of the small bile conducts may be observed. Crypt necrosis leading to eventual mucosal denudation occurs in the intestinal tract.

The immunological recognition and responses seen in GVHD are attributed to histocompatibility differences between donor and recipient and are mediated by inflammatory cytokines (TNF- , IL-1, IL-2) and cellular infiltration of effector cells (many of which display a natural killer cell phenotype) in the target tissues resulting into their destruction (Gale and Reisner, 1986). The degree of donor-recipient HLA incompatibility for HLA-A, B, DR and DQ directly correlates with the risk of GVHD following transplantation. However, disease-free survival of haematology patients undergoing a one-locus-mismatched related transplant is not different from that of an HLA genotypically identical sibling transplant. It has been demonstrated that long-term disease-free survival of haematology patients can be achieved with unrelated donor bone marrow transplantation, albeit with significant morbidity associated with GVHD. From the US National Marrow Donor Programme experience, the overall risk of acute high grade GVHD is 64 per cent (McGlave *et al.,* 1994).

Methotrexate and/or cyclosporin after allogenic BMT have shown effectiveness in preventing acute GVHD in patients with haematological malignancies who are to undergo BMT. In studies combining both regimens treatment-related deaths are less frequent as a result (von Bueltzingsloewen *et al.,* 1993).

Opportunistic infections Opportunistic infections are the second cause of death after GVHD. These infections are similar to those observed in any immunodepressed patient. They include initial infections caused by bacteria and viruses and subsequent infections caused by antibiotic-resistant bacteria, yeast, fungi and viruses (Wade, 1993). Sites of infection are primarily the digestive tract, the sinuses, lung and skin. The generalised use of initial antibiotic therapy encourages antibiotic-induced microbial flora shifts. Consequently, the cause of infection-related mortality is mainly due to fungal and viral infections.

Against bacterial infections, the ideal antibiotic regimen should cover a broad spectrum of pathogens, provide additive or synergistic effects against the more virulent gram-negative pathogens, and decrease the emergence of resistant organisms. The infections with gram-negative bacilli are pre-eminent during the period of severe granulopenia which is often associated with the loss of cutaneous and/or mucous membrane layers, when doses to the skin and/or abdomen are high enough.

The difficulties related to diagnosis and treatment of fungal infections have led to an empirical approach, based on the development of an effective regimen for fungal prophylaxis. Classical antifungal agents have proved to be inadequate and systematic use of amphoteracin-B after a positive blood culture and positive culture or biopsy from skin, lung, sinus, and digestive tract remains the gold standard for the treatment of fungal infections. After wide use of this drug, questions still remain on the optimal administration, drug dosage and duration of treatment.

After the first weeks following the exposure, viral pneumonias are the main risk from infection. Herpes group viruses, such as *herpes simplex* and *cytomegalovirus* are of primary importance. Patients with BMT who are seropositive for antibodies to *herpes simplex* have an 80 per cent probability of developing a *herpes simplex* infection within the first five weeks after transplant (Wade, 1993). In addition, these infections may be responsible for considerable discomfort and pain and may limit the patient's ability to maintain oral nutrition. Antiviral drugs, such as acyclovir, have proved to be effective. Among marrow transplant

recipients, *cytomegalovirus* is the most common opportunistic pathogen detected and is a major cause of morbidity and mortality. *Cytomegalovirus* infection is also much more problematic, as there is no effective therapy. This is an additional reason for promoting prophylaxis by preferring *cytomegalovirus*-negative donors for any required component transfusions.

A few months after a BMT, several other opportunistic infections may occur and involve the liver: *cytomegalovirus* infection, hepatitis A or B virus and *Epstein-Barr* virus have been observed. In the case of successful BMT, revaccination should be considered soon after the recovery, as the patient's cellular memory for prior donor infection and immunisation is absent.

Other complications In addition to the complications described above, some other radiation-induced effects may occur and raise important medical problems. They include: radiation pneumonitis, hepatic veno-occlusive disease, gastrointestinal manifestations and deterministic effects in any organ or tissue with thresholds lower than the dose received by the patient.

Pathogenesis of veno-occlusive disease of the liver has not been completely elucidated, since BMT involves the use of high-dose cytoreductive treatment. In addition, the nature of the liver disease may include forms due to GVHD. Hepatic veno-occlusive disease is observed in about half or more of the patients after BMT. Among patients with this disease, 50 per cent develop moderate forms, while 25 per cent develop severe and mild forms, respectively. The severe forms may be complicated by renal and cardiopulmonary failure. It seems that the use of growth factors such as GM-CSF protects against this liver disease (McDonald *et al.,* 1993).

Radiation pneumonitis has lost its predominance since drastic precautions are mandatory when irradiating the thorax (dose fractionation, midlung shielding). However, this severe disease may result from an accidental exposure and complicate the medical course.

As after any radiation exposure of the abdomen at doses in excess of 1 Gy, several gastrointestinal manifestations, such as nausea, vomiting and diarrhoea may be observed in the hours following the exposure. In some cases these non-specific signs may persist and, when particularly severe, represent one end of the spectrum of sequelae which are represented by obstructing strictures, fistulas, perforation and bleeding (Drum and Rappeport, 1990).

7.4.2.3
New trends

In such a fast-moving field, directions of research of specific interest for radiation-induced aplasia include source of HLA-compatible donor cells, transplantation with selected haemopoietic stem and progenitor cells, and new transplant regimens.

Source of HLA-compatible donor cells The availability of a graft is often a problem for bone marrow transplantation. As the size of the modern family tends to diminish, the probability of finding a matched related donor falls. Although the size of the bone marrow registry for unrelated donors is increasing, there are still patients with no matching donors. Furthermore the delays for the donor search and planning the transplant may impair the results.

New sources of haemopoietic stem cells for transplantation are needed. Cord blood may be a useful opportunity. A cord blood bank has recently been established in order to study the feasibility of unrelated transplants. Cord blood samples are collected upon delivery. The sample is then readily available once frozen and typed (Snyder *et al.,* 1994). Cord blood cell transplants are well established in matched related situations for children. GVHD has been seldom observed; however, a delay in platelet recovery was frequently noted. Cord blood transplants have never been used for adult recipients although the number of stem cells

present in the sample may theoretically be sufficient (Thierry *et al.,* 1992). The proven success of such transplants (unrelated and/or towards a large recipient) will condition their usefulness for the treatment of radiation-induced aplasia.

Positive selection of immature haemopoietic cells New techniques for preservation and storage of haemopoietic stem cells have been developed recently. It has been shown that it is possible reproducibly to prepare highly enriched samples of haemopoietic stem and progenitor cells. These cells can be obtained from bone marrow, cord blood or peripheral blood by immunological recognition of a specific marker (CD34). They are isolated from mature cells by reversible binding to a specific device (antibody bound to magnetic beads, biotin-avidin interaction, plastic binding). CD 34^+ enriched samples are small in size, and a few millilitres may contain the same quantity of progenitors and stem cells as a whole litre of bone marrow. These cells have been used clinically in autologous haemopoietic cell transplantation clinical trials with some success (Berenson *et al.,* 1991). The development of this technique may be interesting for banking haemopoietic samples from potentially exposed workers (workers with significant probability of receiving high doses, as was the case for the intervening firemen at the Chernobyl accident).

New transplant regimen New strategies in order to limit the risk of GVHD or prevent graft failure when transplanting cells with a poor HLA compatibility are under development.

The use of a T-cell depleted haploidentical graft has had little success because of the high risk of graft failure. It has been recently shown that it is possible to realise, in leukaemia patients, a successful transplantation with three HLA mismatches with a low GVHD incidence by transplanting a large number of T-cell-depleted bone marrow and peripheral blood stem cells from the donor at the same time (Aversa *et al.,* 1994). The average concentration of myeloid precursors in the final inoculum was 7–10-fold greater than that found in the bone marrow samples alone. In addition, an increased (when compared with standard practice) conditioning regimen was applied to provide both immunosuppression and myeloablation. One patient rejected the graft, sixteen had early and sustained full donor-type engraftment. One patient who received a high quantity of lymphocytes died from acute grade IV GVHD, nine patients died from transplant-related toxicity, and six patients are alive and event-free. This approach may be of interest for treatment of radiation victims when a bone marrow transplantation is needed but only haploidentical HLA marrow donor can be found.

Most T-cell-depletions are negative-selection processes; CD 34+selection is a positive selection process ending with a sample, theoretically almost totally depleted of cells involved in the acute GVHD reaction. In order to limit the risks associated with this disease, it has been suggested that CD 34^+ cells be used in allogenic bone marrow transplantation. The preliminary results have shown the feasibility of the transplant using G-CSF mobilised CD 34^+ peripheral blood immature cells in combination with unmanipulated bone marrow. None of the six patients treated developed more than a grade II GVHD. It was suggested that the use of CD 34^+ selected allogenic cells may circumvent the need for potentially toxic immunotherapy to minimise GVHD (DiPersio *et al.,* 1994).

7.4.3
Haemopoietic Growth Factors

7.4.3.1
General

Haemopoiesis is under the control of growth and differentiation factors (cytokines) which allow the tissue to adapt itself to new situations by continuously modulating its response. Some of these factors are well

known, some others are only hypothetical. Recent advances in the study and large-scale production of these haemopoietic growth factors have allowed their use for therapeutic purposes. The most studied factors are the granulocyte-macrophage colony stimulating factor (GM-CSF) and the granulocyte colony stimulating factor (G-CSF). These factors are small proteins which bind to specific receptors on the plasma membrane of the target cells. They stimulate the proliferation and/or differentiation of haemopoietic progenitors. Furthermore, they act on the functions of mature cells. They have specific uses in haematology, related to their role in the regulation of growth and differentiation of haemopoietic progenitor cells (see Chapter 2). The results of the clinical trials, performed with numerous patients and often randomised, bring important clues about what to expect from growth factor therapy.

Other factors with broader effects, such as the interleukin-1 (IL-1), interleukin-3 (IL-3), interleukin-6 (IL-6), interleukin-11 (IL-11) or stem cell factor (SCF) are only entering preclinical or clinical trials now. Although numerous *in vitro* or *in vivo* experiments suggest a benefit from their effects, their possible uses in therapy are still questionable. The same conclusion applies for some other factors under development, such as PIXY 321, erythropoietin (EPO), macrophage colony stimulating factor (M-CSF) and thrombopoietin (TPO).

Some growth factors have already been used for the treatment of accidental radiation-induced aplasias and lessons have been learnt from their medical management and follow-up.

7.4.3.2
Current situation

The results of clinical trials involving irradiation protocols for G-CSF and GM-CSF, as well as for some other growth factors which may be of direct therapeutic potential for the treatment of radiation-induced haemopoietic syndrome following accidental exposure, are summarised below (Thierry *et al.,* 1995).

G-CSF, GM-CSF G-CSF predominantly acts on the growth of later maturing cells of the granulocyte-monocyte type, but may also act on early precursors. It influences their maturation into neutrophils, stimulates release of precursors, progenitors and mature neutrophils from bone marrow into the peripheral blood, and enhances their chemotactic and phagocytic functions. GM-CSF promotes growth of progenitors of several myeloid lines (including eosinophils, erythrocytes, monocytes, neutrophils and megakaryocytes). Proliferation of granulocytes and monocytes is increased, and granulocyte survival is prolonged. The stimulated granulocytes display an increased expression of the adhesion molecule CD 11b with an accompanying reduced mobility which may limit their effect during infectious processes.

In radiation accident management, the effects of GM-CSF on numerous cell types could be an advantage when compared with the more restricted activity of G-CSF, if they resulted in increased platelet growth and if the raised macrophages had antimicrobial activities. However, the reduced mobility of the neutrophils and the GM-CSF induction of other cytokines by stimulated cells may limit the advantage (Steward, 1993).

Recent advances in the study and large-scale production of these haemopoietic growth factors have allowed their use for therapeutic purposes. Immediately upon injection, a transient neutropenia occurs followed by a sustained rise of leukocyte counts (granulocytes for G-CSF, granulocytes, monocytes and eosinophils for GM-CSF). Subcutaneous infusion appears to be more efficient than bolus intravenous injection in term of leukocytic rise. The half-life of GM-CSF *in vivo* has been shown to be longer for subcutaneous than for intravenous infusion (at least 10 hours instead of 1–3 hours).

Few studies involving radiotherapy and growth factors are published, apart from bone marrow transplantation clinical trials, where total-body irradiation is often part of the conditioning regimen. Studies involving radiochemotherapy show inconclusive results (Bunn *et al.,* 1992; Momin *et al.* 1992). In one trial

in small cell lung cancer patients, the group of patients receiving GM-CSF had more infections with longer duration and a higher incidence of thrombocytopenia than controls. However, hemicorporal irradiation studies suggest that G-CSF and GM-CSF may help to limit the radiation-induced neutropenia (Fushiki and Abe, 1992; Troussard *et al.,* 1992; MacManus *et al.,* 1993).

Growth factors were used in autologous or allogenic related or unrelated donors bone marrow transplantation in order to reduce the duration and degree of neutropenia induced by conditioning, or because of graft failure. For allogeneic bone marrow transplantation, GM-CSF has been used when the conditioning regimen included total-body irradiation. The results demonstrated a positive effect on granu

Table 7.3 Growth factor therapy in the treatment of past radiation accidents

			Growth factor therapy	
Accident	No. patients	Haematological treatment	Growth factor	Result[b]
Chernobyl 1986	?	13BMT 6FLT[a]	GM-CSF?	Bad
Goiânia 1987	8	Transfusions	GM-CSF	Moderate
San Salvador 1989	3	Classical	GM-CSF	Bad
Soreq 1990	1	BMT	GM-CSF IL-3	Good
Nesvizh 1991	1	Transfusions	GM-CSF IL-3	Good

[a] FLT: foetal liver transplantation
[b] Result expressed as biological response (The Goiânia, San Salvador, Soreq and Nesvizh accidents results in 4, 1, 1 and 1 deaths respectively)

lopoiesis with a shorter duration of neutropenia and no obvious effect on GVHD for matched related transplantation. For matched unrelated transplantation, no effect on duration of neutropenia or thrombocytopenia was observed for patients receiving GM-CSF when compared with historical controls. However, a lower incidence of severe infections has been shown for GM-CSF treated patients when compared with historical control patients. The radiation accidents where growth factor therapy has been implemented are listed in Table 7.3.

Some adverse reactions have been described after the clinical use for G-CSF and GM-CSF. At the recommended doses of 5–10 μg kg^{-1} per day both growth factors are well tolerated. The commonest side-effect is bone pain for G-CSF, especially at higher dose (Ganem and Solal-Celigny, 1993). For GM-CSF, side-effects include bone pains, chills, headaches, mild myalgias and fever; for higher doses (60 μg kg^{-1} per day), capillary leak syndrome with generalised oedema, pleural and pericardial effusion were reported as well as large-vessel thrombosis. GM-CSF may also reactivate an autoimmune disease. The development of antibodies directed against the non-glycosylated form of GM-CSF has been described during treatment. These effects appear to be more frequent and intense at higher doses. Following discontinuation of the treatment the adverse reactions disappear rapidly.

Other factors undergoing clinical trials Interleukin-3 exerts functional and proliferative effects on multiple cell types, including granulocytic, eosinophilic, basophilic monocytic and thrombocytic types. Initial phase I studies have demonstrated multiple cell type increases in peripheral blood counts in patients with bone marrow failure. This included dose dependent increases in white blood cell counts. IL-3 is now in phase III clinical trials involving cancer patients, aplastic anaemia and myelodyplastic syndrome.

Preliminary results suggest a clinical benefit for some patients in terms of increase of leukocyte, reticulocyte and platelet counts. The observed increases in platelet and reticulocyte counts induced by IL-3 are only moderate; it is concluded that IL-3 appears to be at least as efficient as GM-CSF or G-CSF with regard to their actions on thrombopoiesis (Ganser *et al.,* 1991). The combined treatment with GM-CSF does not exert superior effects on platelet production compared with IL-3 therapy alone or GM-CSF alone (Oster *et al.,* 1991). Side-effects include fever associated with headache and stiffness of the neck, flushing, local erythema, bone pain, lethargy, nausea and vomiting.

Interleukin-6 is a pleiotropic cytokine involved mainly in the induction of haemopoiesis (especially maturation of megakaryocytes). IL-6 is entering phase I/II clinical trials for treatments using chemotherapy or radiation-induced thrombocytopenia in the case of solid tumours. Preliminary results on phase I trials are contradictory, suggesting that the positive haemopoietic response (observed mainly on platelet recovery) may not be reproducible when IL-6 is given to patients with hypoplastic bone marrow. Side-effects include fatigue, malaise, body aches, sore throat, dizziness, headache, anorexia, fever, sweating and chills. Anaemia was frequently observed.

Interleukin-1 has been reported to enhance haemopoietic growth-factor-induced haemopoiesis and cytokine secretion by T-cells, monocytes, fibroblasts and is entering phase I/II clinical trial as a regulator of haemopoiesis in patients receiving chemotherapy for urogenital cancers. Treatment results suggest that IL-1 is effective in shortening the duration of neutropenia induced by chemotherapy. However, the toxicity of IL-1 limits its clinical utility. Adverse effects include high fever, sleepiness, anorexia, myalgias, arthralgias, headache, gastrointestinal disturbances and hypotension (Crown *et al.,* 1991).

Stem cell factor has been reported to stimulate haemopoietic growth-factor-dependent early haemopoiesis, myelopoiesis, lymphopoiesis, erythropoiesis and mast cell growth. It is now entering phase I/II clinical trial for haemopoietic reconstitution following anticancer chemotherapy. Preliminary results suggest a significant expansion in numbers and proliferative capacity of differentiated and primitive haemopoietic progenitor cells (Tong *et al.,* 1993).

Interleukin-11 has been reported to stimulate growth-factor-dependent haemopoiesis, B-cell differentiation and to inhibit adipogenesis. It is now entering phase I/II clinical trial for the treatment of thrombocytopenia associated with cancer treatment. Preliminary results suggest that IL-11 has a slight thrombopoietic activity and is well tolerated in women with advanced breast cancer undergoing chemotherapy. Side-effects include reversible fatigue, myalgias and arthralgias (Gordon *et al.,* 1993).

PIXY 321 is a fusion protein consisting of recombinant human IL-3 and GM-CSF coupled by a flexible amino acid linker sequence. Preliminary results of phase I clinical trials suggest a reduction of neutropenia and a mild reduction of thrombocytopenia following chemotherapeutic regimen for breast carcinoma treatment. A stimulation of haemopoiesis was observed in patients with bone marrow failure. Side-effects include fever, myalgia, headache, fatigue and bone pain. Mild skin reactions were observed following subcutaneous injection (Collins *et al.,* 1993).

Erythropoietin is a primary physiological regulator of haemopoiesis. It acts by controlling the proliferation, differentiation and maturation of erythroid progenitors. These properties are the bases on which clinical assays have been set up. A survey of the clinical trials indicates that EPO has an established role in the treatment of anaemia associated mainly with renal disease, but may also be useful with refractory anaemia in haematological disorders (such as myelodysplasia), autotransfusion, multiple myeloma and AIDS. It has been suggested that EPO could act on megakaryocyte progenitors; however, data on platelet counts during clinical trials are conflicting since they have sometimes been found unaffected and sometimes significantly increased (Cazzola *et al.,* 1992). Adverse reactions include mild flu-like symptoms, while rise of blood pressure, iron deficiency, seizure, hyperkalaemia, hyperphosphatemia and increased dialyser

clotting are attributable to an increase in red cell mass. As red cell transfusion is a rather easy and safe procedure, EPO has never been used in the treatment of accidental irradiation-induced aplasia.

Macrophage colony-stimulating factor stimulates the survival, proliferation and differentiation and enhances the functions of monocytes and macrophages. It has been suggested that M-CSF may have a role in the treatment of fungal infection. M-CSF is well tolerated when administered to patients who develop invasive fungal infection after BMT. Thrombocytopenia seems a dose-limiting toxicity which may limit the use of M-CSF in the treatment of radiation-induced aplasia (Nemunaitis *et al.,* 1993).

Thrombopoietin is a newly cloned factor which allows the proliferation of megakaryocytes, but also enhances the endomitosis rate of these cells and promotes platelet production. Preliminary experimental results suggest that thrombopenia could be significantly reduced in irradiated animals by thrombopoietin alone. The factor may also have some role in erythropoiesis (perhaps because its protein structure is rather similar to that of erythropoietin). There was an apparent absence of side effect in these experiments. Human toxicology and efficacy data on thrombopoietin are not available and the therapeutic potential of this cytokine should be viewed with guarded optimism. However, the addition of a factor acting on thrombocytopenia to the (short) list of growth factors available for clinical treatment could be considered as a major breakthrough for all patients now submitted to platelet transfusion problems (such as immunisation) (Lock and Foster, 1994).

7.4.3.3
New trends

Although questions have been raised about the real clinical efficiency of growth factors (Quesenberry, 1993), the results of the clinical trials, *in vivo* and *in vitro* radiobiology experiments and also lessons learnt from the management of accidents all suggest that growth factor therapy could be of use after an accidental overexposure to stimulate the remaining haemopoietic stem cells in order to shorten the duration of aplasia. Early administration after the accident may be important as it has been shown, through late compassionate use that, although the growth factor injection resulted in a good initial rebound in circulating levels of myeloid cells, the patient may still have succumbed to infectious processes.

While G-CSF has already demonstrated a proven efficacy against granulocytopenia in numerous clinical and experimental settings, so far, it has not been used for the treatment of radiation-injured patients. G-CSF is well tolerated, has fewer side-effects than GM-CSF or IL-3, and it promotes the production of fully-functional granulocytes. The broader action of GM-CSF and IL-3 and especially their possible role in thrombopoiesis stimulation, added to their proven action on granulopoiesis *in vivo,* could be beneficial for the patients. However, neither the clinical trials, nor the management of accidental irradiations allows the definition of a precise schedule for the treatment of thrombocytopenia by growth factors. In situations where a bone marrow transplantation is indicated and can be performed, these growth factors might be used after the transplantation in order to promote haemopoietic recovery and to limit the risk of infection for the patient without effects on GVHD.

It has been suggested after the Brazil accident (where a victim experienced a late hypoplasia) that, when internal exposure is involved, the use of growth factors would stimulate haemopoiesis-induced progenitors or stem cells to progress in the cell cycle, while the cells are still being irradiated. The combination of haemopoietic growth factors inducing mitosis and simultaneous prolonged radiation exposure might result in the depletion of the stem cell pool. This was not confirmed by observations. This hypothesis could however be important in situations where internal exposure persists during treatment.

Attempts to stimulate autologous bone marrow recovery were conducted in some past accidents prior to allogeneic BMT. This therapeutic attitude is questionable, since it might result in the induction of graft rejection mediated by the recipient's cells.

Interleukin-6, interleukin-11 and SCF are promising haemopoietic growth factors entering clinical trials for the treatment of aplasia. Furthermore, clinical trials for combination of growth factors have been set up. Other growth factors are entering preclinical trials, such as thrombopoietin and leukaemia inhibiting factor (LIF), which is known to increase IL-3-dependent proliferation of early human haemopoietic progenitors and to stimulate thrombopoiesis. However, the doses and the schedules of administration are not yet established, their side-effects are not well identified, long-term effects are mostly unknown, and this may be of particular importance for factors which are known to stimulate the growth of some leukaemia cell lines. Advances in growth factor research suggest that it is possible to design new, more efficient therapies for radiation-injured patients.

Ex-vivo expansion of haemopoietic precursor stem cells and differentiated cells is a new approach in growth factor therapy (Williams, 1993; Haylock *et al.,* 1992). These studies aim to expand the pool of progenitors and stem cells for transplantation (or to expand differentiated cells for transfusion). This is made possible by the development of techniques allowing the selection of a population of haemopoietic progenitors and stem cells from the blood (with stimulation by growth factors prior to stem cell harvesting) or bone marrow using CD 34^+ cell positive selection. The next step consisting of their culture with combinations of growth factors or additional stromal cells is also under development. The use of continuous perfusion cultures may help to solve some of the technical problems of size of the samples and reproducibility of the experiments. This approach is interesting for the treatment of patients with radiation-induced aplasia, either because the cells necessary for *ex vivo* expansion have been banked before the radiation accident or if such cells can be found in the blood or in the marrow and harvested in sufficient quantity after the accidental irradiation, and that it is possible to collect them. Such cells could be available in the blood after various types of irradiation as suggested by results on haemopoietic progenitors in the peripheral blood after therapeutic irradiation (Thierry *et al.,* 1985). Some of the growth factors have restricted use *in vivo* due to their toxic side-effects although their effects on haemopoiesis could be useful. *Ex vivo* experiments could allow their use without adverse reaction. Important research is necessary to adapt the *ex vivo* expansion of haemopoietic precursor and stem cells for transplantation or differentiated cells for transfusion in the treatment of radiation-induced aplasia, as *in vitro* cultures are different for both purposes. To be of therapeutic use the cells produced must retain normal function and regulation. In this respect, potential mitotic aging during expansion may be counterproductive.

Several growth factors (such as GM-CSF) inhibit programmed cell death *in vitro* and in animal models (Brach *et al.,* 1992). This property could be of great interest for the treatment of radiation-induced aplasia, as it may help to enhance the lifetime of the irradiated cells, thus reducing the severity and duration of aplasia. Assays to study the effects *in vivo* of growth factors on apoptosis must therefore be designed with regard to the role of irradiation.

7.5
Combined Injury Complications

Combined injury is defined as the complex of symptoms shown by individuals injured by thermal burns, blast or any other forms of trauma combined with overexposure to radiation. In the past, many lessons have been learnt, especially after some major accidents, but little documentation on the medical effects is available. In general, estimates of combined injury will be adversely affected by degradation of the

environment to the extent that it might occur and from lack of medical support facilities. The first medical experience for combined injury relies on the observations made after the detonation of atomic bombs over Hiroshima and Nagasaki in 1945; the conditions prevailing in these circumstances (predominating blast effect, no beta burns, limited availability of medical facilities) suggest that general rules cannot be derived from the Japanese A-bomb victims.

Nevertheless, it is possible to list some difficulties met in the clinical management of such victims:

1 The latent period of the acute radiation syndrome may be reduced, leaving limited time for the medical response.
2 Pancytopenia will occur sooner than in the case of an isolated bone marrow syndrome.
3 Shock intensity will increase and may be of major medical concern.
4 Resistance to external infection will be rapidly impaired.
5 Healing (wounds and/or burns) will be delayed and impaired.
6 Lethality will be much higher and will occur much more rapidly than normally expected.

In other words, the overall response will generally be greater than what would be predicted by the assumption that the various injuries act independently of the others. These factors allow one to derive a general conclusion for the clinical management of such patients: early evaluation and aggressive resuscitation of a burn (or multitrauma) patient are mandatory. They include ventilation, oxygenation, resuscitation from hypovolaemia, all of them requiring prompt implementation. Any life-saving blood transfusion will be done with all necessary precautions, such as depleting live white cells by previous irradiation of the blood products. As it might be difficult to assess the magnitude of the bone marrow radiation injury, over-shadowed by associated injury symptoms, all efforts should be made to assess clinically and biologically the severity of each component of the combined injury, and to evaluate their dynamics. For instance, the acute radiation syndrome will allow a successful outcome from surgical intervention only during a short period of time, which may be not longer than three or four days after the overexposure. Ideally, all potential sources of infection should be eradicated during this period. In fact, the closure of extended wounds or burns is not necessarily recommended or technically feasible in such a short time. The use of biological wound dressings may allow the host to respond in a way that will minimise morbidity and mortality (Hirsch, 1990).

Combined injuries compromise the prognosis for the victim and contribute to an increase in the probability of early lethality, as well as in later times. In addition, the assessment of bone marrow injury severity may be much less accurate in the case of associated injury: in these circumstances, lymphocytes may be an unreliable indicator, as patients with severe burns (or trauma) often develop lymphopenia. Signs of tissue injuries can mimic and obscure those caused by severe overexposure to radiation.

Injury to organs other than the bone marrow may increase some side-effects of drugs used to correct the pancytopenia, and consequently may set a problem for their appropriate use or for their interruption because of the emergence of adverse effects. For example, administration of growth factors such as GM-CSF and IL-3 may result in fever, skin effects such as pruritus, dry and moist desquamation or even ulceration, vomiting which may require therapy, renal dysfunction and pain. In the case of the Belarus accident, the GM-CSF treatment which was initiated on the first day was interrupted on the seventh day because some clinical signs may have been drug-related and considered as the third grade of complications (IAEA, 1995). The question of whether these effects were due to growth factor alone or to a synergistic action of radiation and growth factor remains unanswered. According to the current and limited experience, decisions to use drugs with side-effects on the skin should be carefully discussed and evaluated.

The combination of whole-body overexposure and localised exposure at high doses with high energy radiation may result in increased severity. In fact, deep and extended necrosis of underlying tissues as occurring after a severe skin radiation burn (for example, a source in the pocket for a few hours) results in toxaemia, with its unavoidable consequences on excretory organs, in particular kidney and liver. These organs, which are already suffering from the whole-body exposure, may not support additional injuries, and consequently may be a source of complication for the hypoplasia or aplasia. These phenomena probably caused the early death of an accidentally irradiated victim in Estonia (Nénot, 1994).

Another complication for bone marrow injury may be caused by associated internal exposure. The combination of external and internal exposure will result, in the case of sufficiently high doses, in a bone marrow which will be depressed or suppressed after the acute (or subacute) exposure with a delay of one to three weeks and which in addition will receive a continuous low dose rate exposure. This latter, if alone, could never result in an acute syndrome and in most of the cases will only increase the probability of stochastic effects. If combined with an acute (or subacute) external overexposure, it may compromise the bone marrow recovery, or, at least, will increase the severity of the critical period, in seriousness and duration. As it is not possible, in terms of severity, to assess the additivity of doses delivered at various dose rates, prognosis and therefore therapeutic decisions will be difficult to evaluate.

7.6
Late Effects

The late effects which may occur after a radiation-induced aplasia are not due to a single phenomenon but correspond to the complex interaction of resulting effects on various bone marrow components, i.e. haemopoietic cells, stromal cells, vascular system, regulatory system, etc. (see Chapters 3, 4 and 5). Modifications to these various tissues and systems may reduce or damage stem cell pools and proliferative capacity, and a prolonged hypoplasia may occur. In addition, exposure of the bone marrow increases the long-term risk of stochastic effects, such as leukaemia. Some other late effects may also occur, directly related to the applied treatment.

7.6.1
Prolonged Hypoplasia

Hypocellular bone marrow with erythroblastic and granulocytic hypoplasia and increase of mast cells may be observed after radiation exposure. The radiation induced myelofibrosis is defined as the replacement of the bone marrow fatty and haemopoietic tissues by a fibrous tissue which is made up of fibroblasts and collagen fibres. Sclerosis represents a later stage of fibrosis in which the collagen fibres may appear as a confluent eosinophillic non-fibrillar material (hyaline fibrosis). Acute and chronic inflammations as well as subendothelial vacuolation may also be observed in the marrow of these patients (Rywlin, 1976). It is generally believed that the vascular system is the main target for later radiation damage in normal tissues. The vascular insufficiency is related to lesions of the slowly dividing cells in the blood vessel walls. Endothelial cell loss or modified endothelial cell function may be involved. The changes that have been reported include blood vessel wall thickening, vessel dilatation and endoarteritis (Hopewell *et al.,* 1993).

Stromal cell depletion may also be directly involved in the late bone marrow radiation pathogenesis and the bone marrow microenvironment may be a target for late effects of irradiation (Withers *et al.,* 1980). The haemopoietic microenvironment is a complex network of stromal cells (myofibroblasts, macrophages, endothelial cells, adipocytes and preadipocytes), accessory cells (T-lymphocytes), extracellular matrix

proteins, adhesion molecules and growth factors, all of which interact with each other. All these components are in close contact with the bone marrow haemopoietic cells. These cellular interactions are important during the proliferation and the differentiation of haemopoietic stem cells. Thus, the cellular and molecular components of the haemopoietic microenvironment play a crucial role in haemopoiesis. One of the major effects of bone marrow exposure at high doses is on the haemopoietic stroma. It may be detected with *in vitro* systems that haemopoietic stromal cells are sensitive to doses around 2.5–5 Gy (Knospe, 1988). However, stromal cells *in vivo* have a good capacity for repair and it has been shown that damage after single exposures of 20 Gy can be repaired; the repair process needs about one year, and is only partial after this delay. Thus, a damaged microenvironment results in impaired haemopoiesis.

Radiation mechanistic studies have been conducted in various cell types, using molecular and cellular biology concepts (UNSCEAR, 1988). Many data are available concerning the radiation effects on cell cycle, radiation-induced gene expression and signal transduction. Changes observed in the second messengers are in accordance with a possible effect of ionising radiation on growth factor production or on adhesion molecule expression, through a cellular signalling cascade. Furthermore, interactions of haemopoietic cells with bone marrow sinusoidal endothelial cells are of potential importance during the homing of haemopoietic cells following bone marrow transplantation and during the egress of mature haemopoietic cells to the circulation. Ionising radiation may modify these interactions.

Macrophages are an important source of growth factors in the bone marrow, such as GM-CSF, G-CSF, IL-6, and are the main source of IL-1 and TNF- . In addition to stromal cells, T-lymphocytes (called accessory cells) are present in the microenvironment. Because T-cells seem to be the only source of IL-3, their role is crucial to the microenvironment. Extracellular matrix proteins are of particular importance in the haemopoietic microenvironment. These proteins also have an important role in inflammatory response. Growth factor binding to extracellular matrix proteins seems an important parameter to study. This binding provides a reservoir of growth factors and protects them from degradation. Irradiation might affect this binding, causing changes in the local concentration of growth factors. Further studies are needed in order to establish the role of the microenvironment in the late effects of radiation at the bone marrow level and to propose therapeutic approaches for the late hypoplasia.

7.6.2
Radiation-induced Leukaemia

There is a well established risk of induction of leukaemia by radiation exposure. This domain, where most of the studies deal with a very large cohort of patients, is not developed further in this chapter. It is, however, important to keep in mind that bone marrow is the most sensitive tissue to radiation-induced cancer, with a mean latent period of 7–10 years for the onset of leukaemia and a lethality risk factor estimated at 50×10^{-4} Sv^{-1} (ICRP, 1991). A leukaemia survey is obviously a part of the long-term follow-up of the patients (Marshall and Mettler, 1990).

7.6.3
Late Effect Related to Treatment

7.6.3.1
Chronic graft-v.-host disease

Chronic GVHD occurs later than acute GVHD, i.e. later than three months after BMT. It is manifested by cholestatic serum chemistry, dermatologic changes similar to scleroderma or dermatomyositis, dry stomatis and keratoconjunctivitis (Drum and Rappeport, 1990). In severe cases, a variety of other organ-specific inflammatory processes may occur. Continued infections via the lung and digestive tract may require specific treatment. Careful and rapid attention should be given to the earliest and most minimal indications of infection. The overall risk of chronic GVHD one year after unrelated donor transplantation is 55 per cent (McGlave *et al.,* 1994).

7.6.3.2
Growth factor therapy

Whether G-CSF or GM-CSF are potentially carcinogenic or indeed whether stimulation with growth factors may result in bone marrow exhaustion or stem cell failure are questionable. It has been reported that, at high dose, GM-CSF trials for myelodysplastic syndrome patients initiated rather than prevented the onset of acute myeloid leukaemia. Adverse reactions with stimulation of leukaemia blast cells under GM-CSF administration have been reported in some sporadic acute myeloid leukaemia cases (Thierry *et al.,* 1995).

7.6.4
New Trends

The development of polymerase chain reaction technology has allowed chimerism studies following transplantation to progress. It is now possible to show the presence of very few donor-type cells in a small sample. The approach which is more sensitive than the techniques used in the past could be used retrospectively to study a possible partial chimerism of patients who have been transplanted because of an accidental exposure (Lawler *et al.* 1989).

Excess of collagen in the bone marrow microenvironment is a constant finding in myelofibrosis. Collagenase inhibitors and stimulators of collagen synthesis (platelet derived growth factor, tumour growth factor beta, epidermal growth factor) have been implicated in idiopathic myelofibrosis (Martyré *et al.,* 1994). The involvement of growth factors in the molecular mechanism of the radiation-induced late effects on bone marrow hypoplasia have to be studied.

Techniques of culture of human mast cells have been recently published. Such long-term cultures, using a stromal cell line obtained after irradiation, may constitute a model for bone marrow irradiation-induced basophilia (Arock *et al.,* 1994).

7.7 Conclusions

It was pointed out in the introduction of this chapter that accidental cases represent a more reliable basis for evaluating the medical management of radiation-induced severe haemopoietic injury than patients with diseases treated by TBI, as they provide data which are more usable and transposable. Nevertheless, the extensive and comprehensive use of these data is limited by the low frequency of accidents and by their disparity. Only a few hundred accidents with health consequences have occurred in half a century. These accidents resulted in about one thousand casualties, including about one hundred deaths, among which bone marrow injury played a prevailing role in most cases. The casualty list shows that about half of the severe injuries are related to the unsafe handling of industrial and medical sealed sources, while the other half originates from the civilian and military nuclear industry (most of them at the Chernobyl accident in 1986, with twenty-eight deaths directly related to radiation exposure) (Nénot, 1990). While not wishing to trivialise the problem, this figure is negligible when set against the total number of safe operations involving radiation sources (such as industrial radiography devices, industrial irradiation facilities, radiotherapeutic devices, etc.).

Nevertheless, this paucity of cases explains the limited interest generated in the medical as well as the political world by radiation accidents—unless an accident concerning a large number of people happens. As this current attitude seems difficult to accept, the only basis for ensuring the best available medical management to future overexposed victims relies on a coherent, permanent and comprehensive radiation accident preparedness. It includes planning, education, research and preparation: keys to effective response in medical emergencies and which should be given the highest consideration prior to accidents involving ionising radiation. The medical response should rest on the experience and lessons learned in serious nuclear and radiological emergencies, which consequently deserve to be reviewed from this perspective. These reviews should also consider the time-scale concerning the availability of data which are essential for a precise diagnosis and appropriate prognosis, and practitioners should be ready to face situations where decisions will need to be taken in the absence of reliable dosimetric results.

References

ANNO, G.H., BAUM, S.J., WITHERS, H.R. & YOUNG, R.W. (1989) Symptomatology of acute radiation effects in humans after exposure to doses of 0.5–30 Gy, *Health Physics,* **56**, 821–38.

APPLEBAUM, F.R., BADGER, C.C., BERSTEIN, I.D. *et al.* (1992) Is there a better way to deliver total body irradiation? *Bone Marrow Transplantation,* **10**, (suppl. 1), 77–81.

AROCK, M., HERVATIN, F., GUILLOSSON, J.J., MENCIA-HUERTA, J.M. & THIERRY, D. (1994) Differentiation of human mast cells from bone marrow and cord blood progenitor cells by factors produced by a mouse stromal cell line, *Annals of New York Academy of Sciences,* **725**, 59–68.

AVERSA, F., TABILIO, A., TERENZI, A. *et al.* (1994) Successful engraftment of T-cell-depleted haploidentical 'three-loci' incompatible transplants in leukaemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum, *Blood,* **84**, 3948–55.

BARANOV, A.E. (1993) Is allogeneic bone marrow transplantation needed in case of severe uniform whole-body irradiation: experience from a recent accident (NESHWISH, Belarus) and previous accident. In AFRRI (eds), *Second Consensus Development Conference on the Treatment of Radiation Injuries, Bethesda, USA,* p. 67.

BARANOV, A.E. & GUSKOVA, A.K. (1990) Acute radiation disease in Chernobyl accidents victims. In R.C.Ricks & S.A.Fry (eds), *The Medical Basis for Radiation Accident Preparedness. II: Clinical Experience and Follow-up since 1979.* New York: Elsevier/ North Holland, pp. 79–87.

BARANOV, A.E., GALE, R.P., GUSKOVA, A.K. *et al.* (1989) Bone marrow transplantation after the Chernobyl nuclear accident, *N. Engl. J. Medicine,* **321**, 205–12.

BARANOV, A.E., SELIDOVKIN, G.D., BUTTURINI, A. & GALE, R.P. (1994) Hematopoietic recovery after 10 Gy acute total body radiation, *Blood,* **83**, 596–9.

BARRET, A.J. (1987) Bone marrow transplantation, *Cancer Treatment Revue,* **14**, 203–13.

BERENSON, R.J., BENSINGER, W.I., HILLS, R.S. *et al.* (1991) Engraftment after infusion of CD 34^+ marrow cells in patients with breast cancer or neuroblastoma, *Blood,* **77**, 1717–22.

BOND, V.P. & SUGAHARA, T. (1969) *Comparative Cellular and Species Radiosensitivity.* Baltimore: Williams & Wilkins.

BRACH, M.A., DEVOS, S., GRUSS, H.J. & HERRMANN, F. (1992) Prolongation of survival of human polymorphonuclear neutrophils by granulocyte-macrophage colony-stimulating factor is caused by inhibition of programmed cell death, *Blood,* **80**, 2920–4.

BUNN, P.A., CROWLEY, J. & HAZUKA, M. (1992) The role of GM-CSF in limited stage SCLC: a randomized phase III study of the Southwest Oncology Group (SWOG), *Proceedings of the American Society of Clinical Oncology,* **11**, 974.

BUTTURINI, A. & GALE, R.P. (1990) Role of hematopoietic growth factors in radiation victims. RhGM-CSF following the Goiânia accident . In D.Browne, J.F.Weiss, T.J. MacVittie & M.V.Pillai (eds), *Treatment of Radiation Injuries.* New York: Plenum Press, pp. 127–32.

BUTTURINI, A., SEEGER, R. & GALE, R.P. (1986) Recipient immune competent T-lymphocytes can survive intensive conditioning for bone marrow transplantation, *Blood,* **68**, 954–6.

BUTTURINI, A., DE SOUZA, P.C., GALE, R.P. *et al.* (1988) Use for recombinant granulocyte-macrophage colony-stimulating factor in the Brazil radiation accident, *Lancet,* ii, 471–5.

CAZZOLA, M., PONCHIO, L., BEGUIN, Y. *et al.* (1992) Subcutaneous erythropoietin for treatment of refractory anaemia in haematologic disorders: results of a phase I/II clinical trial, *Blood,* **79**, 29–37.

COBBOLD, S.P., MARTIN, G., QIN, S. & WALDMAN, H. (1986) Monoclonal antibodies to promote marrow engraftment and tissue graft tolerance, *Nature,* **323**, 164–5.

COLLINS, C., LIVINGSTON, R.B., ELLIS, G. & CARON, D. (1993) Effect of PIXY on haematopoietic recovery after high-dose cyclophosphamide (CTX) etoposide (VP-16) and cisplatin (CDDP) (CEP) in women with breast carcinoma, *Blood,* **82**, 10, 366a, 1447.

COSSET, J.M., SOCIÉ, G., DUBRAY, B., GIRINSKY, T., FOURQUET, A. & GLUCKMAN, E. (1992) Single dose versus fractionated total body irradiation before bone marrow transplantation: radiobiological and clinical considerations, *Proceedings of 1992 Meeting of the American Society for Therapeutic Radiology and Oncology (ASTRO), San Diego, 9–12 November 1992.*

CROWN, J., JAKUBOWSKI, A., KEMENY, N. *et al.* (1991) Phase I/II trial of recombinant human interleukin-1 with and without 5-fluorouracil in patients with gastrointestinal cancer receiving 5-fluorouracil, *Blood,* **78**, 1420–5.

DIPERSIO, J., MARTIN, B., ABBOUD, C., RYAN, D. & BERENSON, R. (1994) Allogenic BMT using bone marrow and CD 34 selected mobilised PBSC: comparison to BM alone and mobilised PBSC alone, *Blood,* **10** (suppl. 1, abstract 351), 91a.

DIXON, B. (1985) The biological and clinical effects of acute whole or partial body irradiation, *J. Society for Radiological Protection,* **5**(3), 121–8.

DOLOY, M.T., MALARBET, J.L., GUEDENEY, G. *et al.* (1991) Use of unstable chromosome aberrations for biological dosimetry after the postirradiation mitosis, *Radiation Research,* **125**, 141–51.

DRUM, D.E. & RAPPEPORT, J.M. (1990) Treatment of whole body radiation accident victims. In F.A.Mettler, C.A.Kelsey & R.C.Ricks (eds), *Medical Management of Radiation Accidents.* Boca Raton: CRC Press, pp. 89–108.

EMERSON, S.G. & GALE, R.P. (1988) Hemopoietic engraftment and differentiation after bone marrow transplantation. In N.G.Testa & R.P.Gale (eds), *Hematopoiesis, Long-term Effects of Chemotherapy and Radiation* (Hematology, vol. 8). New York: Marcel Dekker, pp. 339–55.

FAHEY, J.L., SARNA, G., GALE, R.P. & SEEGER, R. (1987) Immune interventions in disease, *Annals of Internal Medicine,* **106,** 257–74.

FENECH, M. & MORLEY, A.A. (1985) Measurement of micronuclei in lymphocytes, *Mutation Research,* **147**, 29–36.

FERREBEE, J.W. & THOMAS, E.D. (1958) Factors affecting the survival of transplanted tissues. *American Journal of Medical Sciences,* **235**, 369–86.

FLIEDNER, T.M. & NOTHDURFT, W. (1986) Cytological indicators: haematopoietic effects. In A.Kaul, A.Dehos, W.Bögl *et al.* (eds), *Biological Indicators for Radiation Doses Assessment.* Munich: MMV Medizin Verlag, pp. 123–56.

FUSHIKI, M. & ABE, M. (1992) Randomized double blinded controlled study of rHG-CSF in patients with neutropenia induced by radiation therapy. *Proceedings of the American Society of Clinical Oncology,* **11,** 1437.

GALE, R.P. (1986) Dose injury assessment and medical effects at Chernobyl, In V.P.Bond & E.P.Conkrite (eds), *Workshop on Short-term Health Effects of Reactor Accidents: Chernobyl.* Brookhaven National Laboratory, BNL 52030/UC 48 (Biology and Medicine-TIC-4500), pp. 12–13.

(1987) Immediate medical consequences of nuclear accidents: lessons from Chernobyl, *JAMA,* **258**(5), 625–8

(1988) Perspective—Medical response to radiation and nuclear accidents: lessons for the future, *J. National Cancer Institute,* **80**, 995–7.

GALE, R.P. & CHAMPLIN, R.E. (1986) Bone marrow transplantation in acute leukemia, *Clinical Haematology,* **15**, 851–72.

GALE, R.P. & REISNER, Y. (1986) Graft rejection and graft-versus-host diseases: mirror images. *Lancet,* i, 1468–70.

GANEM, G. & SOLAL-CELIGNY, P. (1993) Utilisation en thérapeutique des facteurs de croissance hématopoïétiques. *Edition Techniques, Encyclopédie Medico-Chirurgicale* (ed.), Paris, France, Hématologie, 13070-A-10.

GANSER, A., SEIPELT, G. & HOELZER, D. (1991) The role of GM-CSF, G-CSF, interleukin 3 and erythropoietin in myelodysplastic syndromes, *Am. J. Clinical Oncology (CCT),* **14,** (suppl. 1), S34–9.

GILBERTI, M.V. (1980) The 1967 radiation accident near Pittsburgh, Pennsylvania, and a follow-up report, In K.F.HÜBNER & S.FRY (eds), *The Medical Basis for Radiation Accident Preparedness.* New York: Elsevier/ North Holland, pp. 131–40.

GORDON, M.S., SLEDGE, G.W., BATTIATO, L. *et al.* (1993) The *in vivo* effects of subcutaneously (SC) administered recombinant human interleukin 11 (Neumega™ rhIL-11 growth factor, rhIL-11) in women with breast cancer (BC), *Blood,* **82**, 498a 1976.

GUSKOVA, A.K., BARANOV, A.E. & BARABANOVA, A. (1987) Acute effects of exposure in Chernobyl victims, *Medicine Radiology (Moscow),* **32**(12), 3–18 (in Russian).

GUSKOVA, A.K., NADEZHINA, N.M., BARABANOVA, A.V. *et al.* (1990) Acute effects of radiation exposure following the Chernobyl accident. Immediate results of radiation sickness and outcome of treatment. In D.Browne, J.F.Weiss, T.J.MacVittie & M.V. Pillai (eds), *Treatment of Radiation Injuries.* New York: Plenum Press, pp. 195–209.

HARDING, R.K. & DAVIS, C.J. (1986) Progress in the elucidation of the mechanisms of radiation-induced vomiting, *Int. J. Radiation Biology,* **50,** 947–50.

HAYLOCK, D.N., TO, L.B., DOWSE, T.L., JUTTNER, C.A. & SIMMONS, P.J. (1992) *Ex vivo* expansion and maturation of peripheral blood CD 34^+ cells into the myeloid lineage, *Blood,* **80**, 1405–12.

HELLER, C.G. (1967) Radiobiological factors in manned space flight. In W.H.Langham (ed.), *Report of the Space, Radiation Study Panel of the Life Sciences Committee.* Washington, DC: National Academy of Sciences.

HIRSCH, E.F. (1990) The status of combined injuries. In D.Browne, J.F.Weiss, T.J. MacVittie & M.V.Pillai (eds), *Treatment of Radiation Injuries.* New York: Plenum Press, pp. 141–4.

HOPEWELL, J.W., CALVO, W., JAENKE, R., REINHOLD, H.S., ROBBINS, M.E.C. & WHITEHOUSE, E.M. (1993) Microvasculature and radiation damage. In W.Hinkelbein, G.Bruggmoser, H.Frommhold & E.Wannenmacher (eds). *Recent Results in Cancer Research.* Berlin: Springer-Verlag, 130, pp. 1–16.

HUBER, R., STRENG, S. & BAUCHINGER, M. (1983) The suitability of the human lymphocyte micronucleus assay system for biological dosimetry, *Mutation Research,* **III,** 185–93.

IAEA (International Atomic Energy Agency) (1979) *Biological Dosimetry: Chromosomal Aberrations Analysis for Dose Assessment.* Technical Report Series No. 260. Vienna: IAEA.

(1988) *The Radiological Accident in Goiânia.* Vienna: IAEA.

(1990) *The Radiological Accident in San Salvador.* Vienna: IAEA.

(1993) *The Radiological Accident in Soreq.* Vienna: IAEA.

(1995) *The Radiological Accident in Belarus.* Vienna: IAEA.

ICRP (International Commission on Radiological Protection) (1984) *Non-stochastic Effects of Ionizing Radiation.* Publication 41, **14**. Oxford: Pergamon.

(1991) *1990 Recommendations of the International Commission on Radiological Protection.* Publication 60, **21**. Oxford: Pergamon.

JAMMET, H. (1979) Problèmes posés par les irradiations accidentelles prolongées, *Bulletin de l'Académie Nationale de Médecine,* **163, 1**48–160.

(1984) Irradiation corporelle to tale accidentelle à forte dose. In P.Galle, R.Masse & J.C. Nénot (eds), *Actualités sur les irradiations accidentelles et thérapeutiques,* pp. 166–96.

JAMMET, H., MATHÉ, G., PENDIC, B. *et al.* (1959) Etude de six cas d'irradiation totale aiguë accidentelle, *Revue française d'études cliniques et biologiques,* **4**, 210–25.

JAMMET, H., GONGORA, R., LE GÔ, R., MARBLE, G. & FAES, M. (1968) Observation clinique et traitement d'un cas d'irradiation globale accidentelle, *Proceedings of the First International Congress of Radiation Protection,* Oxford: Pergamon, pp. 1249–90.

JAMMET, H., GONGORA, R., POUILLARD, P., LE GÔ, R. & PARMENTIER, N. (1980) The 1978 Algerian accident: four cases of protracted whole body irradiation. In K.F. Hübner & S.A.Fry (eds), *The Medical Basis for Radiation Accident Preparedness.* New York: Elsevier/North Holland, pp. 113–29.

KNOSPE, W.H. (1988) Long-term bone marrow damage after irradiation. In N.G.Testa & R.P.Gale (eds), *Hematopoiesis, Long-term Effects of Chemotherapy and Radiation* (Hematology: vol. 8). New York: Marcel Dekker, pp. 93–130.

LADNER, H.A. (1991) Reproductive organs. In E.Sherer, C.Streffer & K.R.Trott (eds), *Radiopathology of Organs and Tissues.* Berlin: Springer-Verlag, pp. 433–59.

LAWLER, M., MCCANN, S.R., CONNEALLY, E. & HUMPHRIES, P. (1989) Chimaerism following allogenic bone marrow transplantation: detection of residual host cells using the polymerase chain reaction. *British Journal of Haematology,* **73**, 205.

LITTLEFIELD, L.G., JOINER, E.E., DUFRAIN, R.J., HÜBNER, K.F. & BECK, W.L. (1980) Cytogenetic dose estimates from *in vivo* samples from persons involved in real or suspected radiation exposures. In K.H.Hübner & S.A.Fry (eds), *The Medical Basis of Radiation Accident Preparedness,* New York: Elsevier/North Holland, pp. 375–90.

LLOYD, D.C. & EDWARDS, A.A. (1983) Chromosome aberrations in human lymphocytes: effects of radiation quality, dose and dose rate. In T.Ishihara & M.S.Sasaki (eds), *Radiation Induced Chromosome Damage in Man.* New York: Alan R.Liss, pp. 23–49.

LOCK, S. & FOSTER, D.C. (1994) The structure, biology and potential therapeutic applications of recombinant thrombopoietin, *Stem Cells,* **12**, 586–98.

LUCAS, J.N., TENJIN, T., STRAUME, T. *et al.* (1989) Rapid human chromosome aberration analysis using fluorescence *in situ* hybridation, *Int. J. Radiation Biology,* **56**, 35–44.

MACMANUS, M.P., CLARKE, J., MCCORMICK, D. & ABRAM, W.P. (1993) Use of recombinant granulocyte-colony stimulating factor to treat neutropenia occurring during craniospinal irradiation, *Int. J. Radiation, Oncology, Biology and Physics,* **26**, 845–50.

MARSHALL, J.C. & METTLER, F.A. (1990) Long-term followup of patients involved in radiation accidents. In F.A.Mettler, C.A.Kelsey & R.C.Ricks (eds), *Medical Management of Radiation Accidents.* Boca Raton, CRC Press, pp. 208–18.

MARTYRÉ, M.C., ROMQUIN, N., LE BOUSSE-KERDILES, M.C. *et al.* (1994) Transforming growth factor-beta and megakaryocytes in the pathogenesis of idiopathic myelofibrosis, *British Journal of Haematology,* **88**, 9–16.

MATHÉ, G., JAMMET, H., PENDIC, B. *et al.* (1959) Transfusion et greffe de moelle osseuse homologue chez des humains irradiés à haute dose accidentellement, *Revue française d'études cliniques et biologiques,* **4**, 226–38.

MATHÉ, G., AMIEL, J.L. & SCHWARZENBERG, L. (1964) Treatment of acute total-body irradiation injury in man, *Annals of New York Academy of Sciences,* **114**, 368–92.

MCDONALD, G.B., HINDS, M.S., FISHER, L.D. *et al.* (1993) Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients, *Annals of Internal Medicine,* **118**, 255–67.

MCGLAVE, P., VOGELSANG, G., PETERSDORF, E. & ANTIN, J. (1994)Graft-versus-host disease: clinical course and underlying mechanisms. In E.J.Benz & J.R.McArthur (eds), *Hematology 1994, Education Program (American Society of Hematology),* Nashville, pp. 118–23.

MOMIN, F., KRAUT, M., LATTIN, P. & VALDIVIESO, M. (1992) Thrombocytopenia in patients receiving chemoradiotherapy and G-CSF for locally advanced non small cell lung cancer, *Proceedings of the American Society of Clinical Oncology,* **11**, 983.

NEMUNAITIS, J., SHANNON-DORCY, K., APPELBAUM, F.R. *et al.* (1993) Long-term follow-up of patients with invasive fungal disease who received adjunctive therapy with recombinant human macrophage colony-stimulating factor, *Blood,* **82**, 1422–7.

NÉNOT, J.C. (1990) Overview of the radiological accidents in the world, updated December 1989. *Int. J. Radiation Biology,* **57**, 1073–85.

(1994) Medical report.

NOTHDURFT, W. (1991) Bone marrow. In E.Scherer, C.Streffer & K.R.Trott (eds), *Radiopathology of Organs and Tissues.* Berlin: Springer-Verlag, pp. 113–69.

OLIVEIRA, A.B. (1990) Treatment of infectious complications of the hematopoietic syndrome. In D.Brown, J.F.Weiss, T.J.MacVittie & M.V.Pillai (eds), *Treatment of Radiation Injuries.* New York: Plenum Press, pp. 95–100.

OSTER, W., FRISCH, J., NICOLAY, U. & SCHULZ, G. (1991) Interleukin 3 biological effects and clinical impact, *Cancer,* **67**, 2712–17.

PARMENTIER, N., BOULENGER, R. & PORTAL, G. (1968) Problèmes de dosimétrie lors de l'accident de criticité survenu au réacteur Venus à Mol, en date du 30 Décembre 1965, *Proceedings of the First International Congress of Radiation Protection, Rome, September 5–10, 1966, O*xford: Pergamon, pp. 1231–48.

PARMENTIER, N., NÉNOT, J.C. & JAMMET, H.J. (1980) A dosimetric study of the Belgium (1965) and Italian (1977) accidents. In K.F.Hübner & S.A.Fry (eds), *The Medical Basis for Radiation Accident Preparedness.* New York: Elsevier/North Holland, pp. 105–12.

PARMENTIER, N., NÉNOT, J.C. & PARMENTIER. C. (1990) Two cases of accidental protracted overexposure: aspect of an extensive bone marrow study. In R.C.Ricks & S.Fry (eds), *The Medical Basis for Radiation Accident Preparedness II: Clinical Experience and Follow-up since 1979.* New York. Elsevier, pp. 29–51.

QUESENBERRY, P.J. (1993) Growth factor frenzy, *Experimental Hematology,* **21**, 831–6.

RYWLIN, A.M. (1976) Histopathology of the bone marrow: stromal reactions. In L.P. Cawley (ed.), *Series in Laboratory Medicine.* Boston: Little, Brown, pp. 153–90.

SAENGER, E.L. (1986) Radiation accidents, *Annals of Emergency Medicine,* **15,** 1061–6. (1990) Evaluation of extent of injury. In F.A.Mettler, C.A.Kelsey & R.C.Ricks (eds), *Medical Management of Radiation Accidents.* Boca Raton: CRC Press, pp. 67–88.

SCHIMPFF, S.C. (1990) Infection in radiation accidents: an overview. In D.Brown, J.F. Weiss, T.J.MacVittie & M.V.Pillai (eds), *Treatment of Radiation Injuries.* New York: Plenum Press, pp. 75–85.

SNYDER, E.L., ANDERSON, K., SILBERSTEIN, L. & ADAMSON, J. (1994) Transfusion medicine . In E.Benz & J.R.McArthur (eds), *Hematology 1994, Education Program (American Society of Hematology),* Nashville, pp. 96–106.

STEWARD, W.P. (1993) Granulocyte and granulocyte-macrophage colony-stimulating factors, *Lancet,* **342**, 153–7.

THIERRY, D., JULLIEN, D., RIGAUD, O., HARDY, M., VILCOQ, J.R. & MAGDELENAT, H. (1985) Human blood granulocyte macrophage progenitors (GM-CFU) during extended field radiation therapy, *Acta Radiological Oncology,* **24**, 521–6.

THIERRY, D., HERVATIN, F., TRAINEAU, R. *et al.* (1992) Hemopoietic cell progenitors in cord blood, *Bone Marrow Transplantation,* **9** (suppl. 1), 101–4.

THIERRY, D., GOURMELON, P., PARMENTIER, C. & NÉNOT, J.C. (1995) Hematopoietic growth factors in the treatment of therapeutic and accidental irradiation induced aplasia, *Int. J. Radiation Biology,* **67**, 103–17.

THOMAS, E.D. (1986a) Long-term results of marrow transplantation for leukemia, *Bone Marrow Transplantation,* **1** (suppl. 1), 175–6.

(1986b) Bone marrow transplantation. In V.P.Bond & E.P.Cronkrite (eds), *Workshop on Short-term Health Effects of Reactor Accidents: Chernobyl.* Brookhaven National Laboratory, BNL 52030/UC 48 (Biology and Medicine-TIC-4500), 30–32.

(1990) Total body irradiation regimes for marrow grafting, *International Journal of Radiation, Oncology and Biology Physics,* **19,** 1285–8.

TONG, J., GORDON, M.S., SROUR, E.F. *et al.* (1993) *In vivo* administration of recombinant methionyl human stem cell factor expands the number of human marrow haematopoietic stem cell, *Blood,* **82,** 784–91.

TROUSSARD, X., GINDREY, B., BATHO, A. *et al.* (1992) Double hemibody irradiation with GM-CSF support in multiple myeloma: a pilot study. *Blood,* **80** (suppl. 1), 121a.

TUBIANA, M. & LALANNE, C.M. (1963) Evolution hématologique des malades soumis à une irradiation totale pour transplantation d'organes, *Annales de radiologie,* **6**, 561–80.

UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation) (1988) *Sources, Effects and Risks of Ionizing Radiation.* Annex G: *Early Effects in Man of High Doses of Radiation.* Report to the General Assembly, with annexes. New York: United Nations, pp. 545–612.

US Nuclear Regulatory Commission, (1990) *Health Effects Models for Nuclear Power Plant Accident Consequence Analysis. Low LET Radiation.* Prepared by J.S.Evans, Harvard University. NUREG/CR-4214, SAND85–7185, Rev. I, Part I.

VALVERDE, N.J., CORDEIRO, J.M. & OLIVEIRA, A.R. (1990) The acute radiation syndrome in the ^{137}Cs Brazilian accident, 1987. In R.C.Ricks & S.Fry (eds), *The Medical Basis for Radiation Accident Preparedness II: Clinical Experience and Follow-up since 1979.* New York: Elsevier, pp. 89–107.

VON BUELTZINGSLOEWEN, A., BELANGER, R., PERRAULT, R. *et al.* (1993) Acute graft-versus-host disease prophylaxis with Methotrexate and Cyclosporine after Busulfan and Cyclophosphamide in patients with hematologic malignancies, *Blood,* **81,** 849–55.

WADE, J.C. (1993) Management of infection in patients with acute leukemia, *Hematology/ Oncology Clinics of North America,* **7**, 293–315.

WALD, N. (1983) Diagnosis and therapy of radiation injuries, *Bulletin of the New York Academy of Medicine,* second series, **59**(10), 1129–38.

WILLIAMS, D.A. (1993) *Ex vivo* expansion of hematopoietic stem and progenitor cells— robbing Peter to pay Paul? *Blood,* **81,** 3169–72.

WITHERS, H.R., PETERS, L.J. & KOGELNIK, H.S. (1980) The pathology of late effects of irradiation . In R.E.Meyn & H.R.Withers (eds), *Radiation Biology in Cancer Research.* New York: Raven, pp. 439–48.

YE, G.Y., WANG, G.L., HUANG, S.M. *et al.* (1990) The People's Republic of China radiation accidents, 1980, 1985, 1986, and 1987. In R.C.Ricks & S.Fry (eds), *The Medical Basis for Radiation Accident Preparedness. II: Clinical Experience and Follow-up since 1979.* New York. Elsevier, pp. 53–67.

8
The Pathogenesis of Leukaemia

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8
The Pathogenesis of Leukaemia

8.1
Introduction

There have been many reports to support an association between exposure to ionising radiations and the development of leukaemia (see Chapter 11) and it is generally accepted that the spectrum of leukaemias induced by radiation is similar to the *de novo* disease. This generalisation appears to hold true for most well studied groups and although there are unexplained exceptions, such as the leukaemias induced by radiotherapy for spondylitis where rarer forms of leukaemia are over-represented (Maloney, 1987), there is no reason to assume that radiogenic leukaemias differ fundamentally from those not attributable to ionising radiation. In general, both epidemiological and laboratory investigations indicate that the major effects of radiation exposures are the initiation of malignant changes rather than effects on pre-existing initiated cells. However, this should not be interpreted to mean that radiation has no part to play in other aspects of the development of malignant diseases.

Risk estimates for radiation leukaemogenesis are based primarily on epidemiological studies of exposed populations. These are the only totally secure data of direct relevance to humans. However, most human exposures are to doses significantly lower than in these exposed populations. For practical purposes, therefore, it is usually necessary to extend the estimates of risk to conditions of low dose and low dose rate, and sometimes to radiations of different type, for which direct observation of effects in humans has not been possible. These extrapolations may be assisted by information gained from experimental studies of the underlying biological processes in humans and other mammals. Many of these data are derived from studies of cell killing, and the induction of chromosome aberrations and gene mutations. Consequently, their direct relevance to radiation leukaemogenesis is uncertain.

8.2
Haemopoietic Malignancies

It is not the purpose of this chapter to review the malignancies of the haemopoietic system: such information can be found in standard textbooks of pathology and haematology. Rather, the objective is to present an overview of the major biological features of those leukaemias that are known to be induced by exposure to ionising radiations as a pointer to the mechanistic aspects of the diseases that will need to be investigated in the context of radiation effects.

Extensive investigations of the molecular events in many human malignancies have identified two basic categories of genetic changes in tumour cells (reviewed by UNSCEAR, 1993; Pawson and Hunter, 1994). The first is the *gain-of-function mutation* that alters the activity of genes involved in mediating responses to growth regulatory signals. Such genes may encode growth factors, growth factor receptors, and the components of the signal transduction pathways that transfer signals from the membrane to the nuclear DNA. Generally these genes are known as oncogenes. The second is the *loss-of-function mutation* that reduces the activity of genes normally functioning as inhibitory factors for proliferation and/or differentiation. Collectively these genes are known as tumour suppressor genes. Both types of mutational event have been implicated in leukaemia development.

8.3
The Leukaemias

The commonest neoplastic disorders of the haemopoietic system are the leukaemias. These are neoplastic proliferations of blood cell precursors in the bone marrow and the malignant cells usually enter the circulation where they may be present in large numbers. The leukaemic cells suppress and replace the normal haemopoietic cells in the bone marrow with consequent reduction in normal blood cell production. Unlike carcinomas and sarcomas, the tumour cells do not form distinct tumour masses but proliferate diffusely in the marrow and in the infiltrated tissues.

Although the leukaemias are a markedly heterogeneous group of disorders, they may be divided into four main groups on the basis of whether the clinical course of the untreated disease is acute or chronic, and on whether the major cell types involved are myeloid or lymphoid. Acute and chronic leukaemias may also be char

Table 8.1 The French-American-British classification of acute non-lymphocytic (myeloid) leukaemias

Subtype	Major morphological feature	Leukaemia
M0	Minimal differentiation Myeloblasts without granules	Acute myeloblastic leukaemia
M1	Minimal maturation Myeloblasts with or without few granules	Acute myeloblastic leukaemia
M2	Granulocytic maturation Myeloblasts with granules promyelocytes, few myelocytes	Acute myeloblastic leukaemia
M3	Promyelocytes with prominent granules	Acute promyelocytic leukaemia
M4	Myeloblasts and promyelocytes promonoblasts and monoblasts (>20%)	Acute myelomonocytic leukaemia
M5	Monoblasts	Acute monoblastic leukaemia
M6	Myeloblasts (>50%) Erythroblasts (>30%)	Acute erythroleukaemia
M7	Megakaryoblasts	Acute megakaryoblastic leukaemia

acterised at the cellular level by the extent of differentiation along the haemopoietic lineages. The two main chronic disorders are chronic myeloid leukaemia (CML) and chronic lymphocytic leukaemia (CLL). The two main groups of acute leukaemias are acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML) although, because the latter includes a diversity of malignancies that may affect monocytic,

erythroid and megakaryocytic as well as granulocytic lineages, the more usual clinical classification is acute non-lymphoblastic leukaemia (ANLL). This heterogeneity is reflected in the major classification system used to define AML subtypes, the French-American-British (FAB) system (Bennett *et al.,* 1976), which defines leukaemias on the basis of their major morphological features (Table 8.1). ALL can also be subdivided on the basis of morphological features but the development of immunophenotyping has added considerably to our understanding of these leukaemias (Table 8.2).

Myelodysplastic syndromes are primary disorders having, in common, morphological abnormalities with maturation defects which result in ineffective haemopoiesis. Initially not malignant, there is a variable but, in certain of the disorders, predictable progression to leukaemia: for this reason they are commonly regarded as pre-leukaemic syndromes. The myelodysplastic syndromes are also classified using FAB criteria (Bennett *et al.,* 1982) but the demarcation between different groups (Table 8.3) may often be difficult to achieve because the clinical and haematological features may not always correspond exactly to the definitions provided by the working group. The myeloproliferative disorders are diseases characterised by abnormal, excessive and sustained proliferation of cells in particular bone marrow lineages with a significant probability of evolving into leukaemia. They consist of primary polycythaemia, a proliferative disorder of erythroid series; myelofibrosis, a related condition in which there is also marrow fibrosis and essential thrombocythaemia, a disorder characterised by proliferation of megakaryocytic cells

Table 8.2 The French-American-British classification and immunophenotypes of acute lymphoblastic leukaemias

Subtype	Morphological features		Immunophenotype	Leukaemia
L1	Small homogeneous cell population Regular nucleus, may be indented Fine or clumped chromatin Nucleoli indistinct or not visible Scanty cytoplasm		early B-cell	null ALL
		Pre-B-cell	cALL	
L2	Large heterogeneous cell population Irregular nucleus Fine chromatin 1 or 2 prominent nucleoli Moderately abundant cytoplasm	T-lymphocyte	T-ALL	
L3	Heterogeneous cell population Regular oval/round nucleus Fine chromatin Prominent cytoplasmic vacuoles Prominent nucleoli		B-lymphocyte	B-ALL

Table 8.3 The myelodysplastic syndromes (MDS)

	Blast cells		Ringed sideroblasts in bone marrow (%)	% of MDS	Leukaemic progression (%)
Subtypes	Bone marrow (%)	Blood (%)			
RA	<5	<1	<15	28	12
RA-S	<5	<1	15	4	8

	Blast cells		Ringed sideroblasts in bone marrow (%)	% of MDS	Leukaemic progression (%)
Subtypes	Bone marrow (%)	Blood (%)			
RAEB	5–20	<5	–	23	44
CMMoL	20	<5	–	16	14
RAEB-T	> 20–30	5	–	9	60

RA—refractory anaemia; RA-S—refractory anaemia with ringed sideroblasts; RAEB—refractory anaemia with excess blast cells; CMMoL—chronic myelomonocytic leukaemia; RAEB-T—refractory anaemia with excess blast cells in transformation.

(platelet precursors). All three disorders may exhibit overlapping features, one disorder may evolve into one of the others and all three may evolve into acute non-lymphoblastic leukaemia.

The most comprehensively documented radiogenic leukaemias are those in the Japanese bomb survivors where AML was the most common leukaemia, CML was increased with a shift to a younger age group than normal, the incidence of T-cell leukaemia was higher than in Western populations and that of CLL was not increased at all. The early studies were carried out before the FAB classifications of acute leukaemias and myelodysplastic syndromes were in routine use and when it was not known that adult T-cell leukaemia (ATL) is associated with a virus (HTLV-1) that is prevalent in parts of Japan, including the island of Kyushu where Nagasaki City is located. A re-evaluation of the clinical material (Matsuo *et al.,* 1988) has reclassified some of the leukaemias as ATL and others as myelodysplastic syndromes. Acute leukaemias have been subdivided into FAB subtypes and all subtypes of AML were recorded; most were M1 and M2 (25 and 30 per cent, respectively) or M4 and M5 (12 and 9 per cent, respectively) FAB subtypes. M7 was not detected in the low dose (0–1 Gy) AML group and M3 and M6 were absent in the high dose (>1 Gy) AML group. These findings are based on the earlier TD65 dosimetry but will not be significantly affected by the revised dose estimates. In general, the incidence of disease type has reflected the target population and other exposed groups, including those therapeutically or diagnostically exposed, show similar patterns.

Studies of patients who have received radiotherapy in the absence of chemotherapy indicate that M1/M2 AMLs and MDS are the most common disorders, with less ALL and CML and no evidence for an increased incidence of CLL. Attempts to assess the leukaemia risk for diagnostic exposures (lifetime exposure of 0.02–0.075 Gy) have suggested that both AML and CML occur slightly more frequently among patients who have undergone multiple studies (Preston-Martin *et al.,* 1989). The nature of the dose response relationship and the importance of other risk factors remain controversial.

There is a rapidly expanding literature detailing chromosomal and gene abnormalities in a wide variety of malignant diseases and this is particularly so for the leukaemias (see later). Despite the wealth of detail, however, there is considerable uncertainty about the mechanisms underlying the transition from normal to neo-plastic states. It is generally accepted that the transition is a complex multistep process involving genetic and epigenetic changes that result in the emergence of a dominant malignant clone. This somatic evolutionary process is influenced by genetic, physiological and environmental factors but very little is understood of the detailed events that contribute to leukaemogenesis.

8.4
Clonality and Lineage Studies

An important clue to understanding the process of leukaemogenesis was the observation that malignancy is a clonal proliferation and with rare exception the progeny of a single cell. The first indication of this was the observation in multiple myeloma of the monoclonality of immunoglobulin but the idea became more accepted following the introduction of specific marker studies such as that using glucose-6-phosphate dehydrogenase (G6PD) to investigate X-chromosome inactivation in females (reviewed by Raskind and Fialkow, 1987).

In early embryogenesis, one of the two X-chromosomes in each female somatic cell is inactivated and all the descendants of a cell retain the same inactivation, i.e. the maternally (X^m) or the paternally (X^p) derived X-chromosome. As the cells in most tissues contain an equal number of inactivated X^m and X^p cells, the decision seems to occur more or less at random and females contain two populations of somatic cells, therefore. All cells derived by clonal proliferation will have X^m or X^p inactivated cells but not both. Most of the pioneering work was carried out by examining cells from certain individuals, heterozygous for the X-linked gene G6PD, as the two gene products are readily distinguished by their different electrophoretic mobilities. More recently, X-chromosome DNA polymorphisms which produce changes in endonuclease recognition sites and which can be distinguished as restriction fragment length polymorphisms (RFLPs) have been successfully used in clonality studies (reviewed by Vogelstein *et al.,* 1985).

Myeloid malignancies shown to have a stem cell origin include CML and many, but not all, cases of AML. In addition the preleukaemic myelodysplastic syndromes and myeloproliferative disorders are similarly clonal stem cell disorders. These studies have been reviewed by Fialkow (1990). On the basis of isoenzyme studies, there appear to be at least two forms of AML (Fialkow *et al.,* 1987). In one (mainly adults and elderly patients), multiple myeloid lineages are part of the leukaemic clone and in the other (mainly children and young adults), leukaemic differentiation is expressed mainly in the granulocytic lineage with erythroid cells and platelets being derived from normal stem cells.

Mature B-lymphocytes carry surface immunoglobulin (Ig) which acts as their antigen receptor. Any particular B-cell produces only one specific Ig molecule as a consequence of specific gene rearrangements. The precursors of such a B-lymphocyte also synthesise Ig but the molecule is located in the cytoplasm rather than on the cell surface. Since an individual produces many different B-lymphocytes with differing Ig molecules, the normal situation is one of considerable cellular mosaicism. Evidence for clonality of B-lineage tumours is provided by all cells expressing the same Ig molecule and/or having the same unique Ig gene rearrangement. In virtually all cases of B-cell CLL, the leukaemic cells are monoclonal with respect to Ig production. The conclusion that CLL is a clonal disorder is supported by G6PD studies (Fialkow *et al.,* 1978). T-lymphocytes express receptors for antibody-bound antigens. The production of these receptors is by a mechanism that is very similar to that producing Ig molecules in B-lymphocytes. During the differentiation of the T-lineage there is specific rearrangement of the T-cell receptor and the finding of a unique rearrangement in T-lymphocytes in lymphoid malignancies is strong evidence for clonality (Flug *et al.,* 1985; Waldman *et al.,* 1985). These studies have also contributed to the investigation of the target cells for leukaemic transformation and both B- and T-CLL appear to have arisen in relatively mature cells.

Acute lymphoblastic leukaemia is classified on the basis of morphology, cell surface phenotype and cytogenetic findings. In many cases, therefore, the cells can readily be assigned to the appropriate lymphoid lineage. Gene rearrangement studies help to identify those leukaemias in which the proliferating cells have a precursor phenotype. The common childhood ALL, in which cells express no B-and T-lymphocyte surface markers but an antigen defined as the common (c) ALL antigen, appears to be a disease originating in a cell confined to an early stage of B-lymphocyte development. In some patients, however, many lymphoid

leukaemias, including some cALL cases are more complex. For example, the null ALL phenotype associated with a particular translocation involving chromosomes 4 and ll, has stem cell features and may express myeloid as well as lymphoid markers (Sobol *et al.,* 1987).

It should be noted that not all clonally expanded populations are malignant. Clonal proliferation of B-lymphocytes following antigen stimulation is not a malignancy, for example. It might be argued that this process is a physiological response and this identifies the distinction between clonality and malignancy. Unfortunately it is not so simple. The haemolytic disorder paroxysmal nocturnal haemoglobinuria (PNH) is a clonal expansion of abnormally regulated cells that replaces the normal counterparts (Borst and Greaves, 1987; Rotoli and Luzzatto, 1989). In chronic myeloid leukaemia the growth of the leukaemic clone is not autonomous, the differentiated cells are phenotypically normal and there are normal physiological responses such as the regulated increase in leukocytes in response to infection. CML is a malignancy but PNH is not. However, there are examples of patients with PNH developing leukaemia leading some to consider it a pre-neoplastic condition. Comparisons between the biology of PNH and of CML suggest that the genetic events responsible for clonal expansion differ from those necessary for malignant transformation and this is supported by the observation that benign monoclonal gammopathy is often difficult to distinguish from the initial stages of multiple myeloma, but does not progress in the same manner (Kyle and Just, 1989).

8.5
The Emergence of a Dominant Leukaemic Clone

Although clonal dominance is the situation at diagnosis this does not prove that only one target cell becomes potentially leukaemic following the initiating insult. In many leukaemias it is possible that the earliest deviation from normal is a disordered polyclonal haemopoiesis and the ultimate emergence of a dominant leukaemic clone is due to the self-renewal advantage of a single abnormal stem cell. Clonal evolution may result in a cell line with the capacity to produce its own lineage-specific growth factors and 'autonomous' cell lines may thus arise during the course of leukaemogenesis, even if they are not themselves the origins of the disease.

A number of investigators have conducted detailed analyses of lymphoid leukaemias using panels of various markers including cell-type-specific monoclonal antibodies. The results indicate that, although there are some minimal deviations or asynchronies with respect to maturation stages, the leukaemic cells have the phenotypes of normal lymphoid cells. Since a normal phenotype may be compatible with the cell being leukaemic it is argued that the essential defect is one which disrupts the normal coupling of proliferation and commitment to differentiation (Greaves, 1982). Similar conclusions have been reached following studies of the *in vitro* growth and differentiation of normal and leukaemic myeloid progenitor cells (Sachs, 1982; Metcalf, 1982). Of considerable interest is the observation that the injection of leukaemic myeloid cells into mouse embryos, *in utero,* produced apparently healthy adult mice whose granulocytes contained a leukaemic cell marker (Gootwine *et al.,* 1982). This finding is reminiscent of the extensive studies of the differentiation potential of embryonal carcinoma cells. In certain somatic tissues, these cells readily produce teratocarcinomas but under the influence of normal developmental signals in the blastocyst they are able to contribute to normal embryonic development (Silver *et al.,* 1983). Currently there is much interest in the therapeutic potential of forcing leukaemic cells to respond to differentiation inducers; this is particularly true for acute promyelocytic leukaemia (see below).

The occurrence of leukaemias sharing both lymphoid and myeloid surface antigens does not easily fit the scheme of uncoupling the lineage-specific proliferation and differentiation controls outlined above. Very occasionally, bilineage leukaemias arise where two distinct leukaemic populations, one myeloid the other

lymphoid, are identified. Generally, however, myeloid antigens are detected on cells with B-lymphocyte or T-lymphocyte markers. Greaves and his colleagues (1986) have suggested that these biphenotypic leukaemias represent either leukaemic dysregulation resulting in lineage infidelity of gene expression or lineage promiscuity attributable to the lymphomyeloid stem cell origin of the malignancy.

Our current understanding of the mechanisms regulating the proliferative behaviour of haemopoietic stem cells, although limited, provides a basis for speculative proposals about the principles underlying the emergence of a dominant leukaemic clone and the suppression of normal haemopoiesis in those leukaemias with a stem cell origin. The basic proposition is that the initial lesion produces an abnormal response to growth regulatory mechanisms. A major component of stem cell proliferation control is the local microenvironmental regulation due to a balance between stimulatory and inhibitory factors (see Chapter 2). In the normal steady-state, stem cells may be held for long periods of time in a non-proliferative G_0 state from which a certain proportion is triggered into cell cycle to balance the number of stem cells that have left the population through differentiation. If the effect of a leukaemogenic insult were to change the responsiveness of the target cell by subtly altering some component of the signal transduction pathway, such that it had an increased probability of being triggered into cycle, then the birth rate of these abnormal cells would be greater than that of their normal counterparts. Initially the proliferation of such abnormal cells may be very slow, not only because they are still controlled, albeit imperfectly, but also because increased proliferation may increase sensitivity to differentiation stimuli and thus effect a removal of cells from the population. It has been postulated that a certain time in G_0 is necessary for 'genetic housekeeping' to maximise the probability of an error-free stem cell genome (Lajtha, 1979). This proposal implies that replicating stem cells with shorter G_0 histories may accumulate defects. The consequence of increased probability of recruitment into cycle and the shorter mean time in G_0 may lead to the acquisition of particular mutations that bring about enhanced self-replication and clonal expansion of a preleukaemic stem cell. It is probable that the members of this expanding stem cell clone would be recognised as stem cells, at least initially, by the regulatory cells. This would result in an increase in the local concentration of inhibitory factors and thereby decrease the proliferation of the more responsive normal stem cells. If the normal stem cell population continued to respond to differentiation stimuli, the size of the population would gradually decline and the abnormal stem cells would steadily increase. The end result would be the suppression of normal stem cell function and the emergence of a dominant leukaemic clone.

The scenario described above relates mainly to those leukaemias that originate in members of the stem cell compartment. In the case of many lymphoid leukaemias it is probable that the target cells are not in the stem cell compartment but in cells at various stages of commitment to lymphoid differentiation (Greaves, 1982; Greaves *et al.,* 1986). These cells are long-lived, capable of varying degrees of self-renewal and have considerable proliferative potential. Such cells have characteristics, therefore, that make them potential targets for leukaemic transformation by broadly similar mechanisms.

8.6
Biology of Chronic Myeloid Leukaemia

Chronic myeloid leukaemia (CML) is a haemopoietic stem cell disorder manifested as an excessive proliferation of myeloid cells during the early chronic phase with an inevitable transition to an accelerated phase and blast crisis. The latter stages are marked by the proliferation of blast cells that may be myeloid or lymphoid. There is a rapidly growing literature detailing cytogenetic and molecular features of the disease. These findings may be of value in identifying the general nature of events associated with early and later stages of leukaemia development and are briefly reviewed below.

The characteristic feature of CML is the Philadelphia (Ph) chromosome, a reciprocal translocation between chromosomes 9 and 22 (reviewed by Rowley, 1980). There are a few cases, however, where on the basis of clinical features, patients with typical CML lack the Ph chromosome in the earliest stages of the disease but it is readily detected at later stages (Hayata *et al.,* 1975; Lisker *et al.,* 1980). Such cases suggest that clonal stem cell proliferation can precede the acquisition of the Ph chromosome but that cells with the translocation must have a selective advantage leading to clonal dominance. It is now well established that the translocation results in the head-to-tail fusion of the *bcr* gene on chromosome 22 with the c-*abl* gene derived from chromosome 9. The resultant chimaeric gene is transcribed into a hybrid *bcr-abl* mRNA in which exon 1 of c-*abl* is replaced by 5 *bcr* exons. A *bcr-abl* fusion protein (p $210^{bcr-abl}$) is produced which contains amino acids from the N-terminus of *bcr*. The breakpoints on chromosome 22 are clustered in 5kb, allowing their study by Southern analysis of DNA. Ph negative CML is rare and the frequency of CML without the Ph chromosome but with the *bcr* rearrangement has been estimated to be about 5 per cent of all CML cases (Dube *et al.,* 1989). Thus, *bcr* rearrangement may be considered the diagnostic feature of CML. It should be noted, however, that some investigators have argued that true CML can also occur with neither the Ph chromosome nor the *bcr* rearrangement (Kurzrock *et al.,* 1990).

The aberrant fusion protein has tyrosine kinase activity, is more active than its normal counterpart (p $210^{c\text{-}abl}$) *in vitro* (Kloetzer *et al.,* 1985) and *bcr-abl* constructs have been shown to transform primitive haemopoietic cells in cell culture (McLaughlin *et al.,* 1987; Young and Witte, 1988; Scherle *et al.,* 1990). The malignant potential of the fusion gene has been demonstrated by the induction of a variety of haemopoietic tumours in mice repopulated by transplanting marrow cells infected with *bcr-abl* constructs and the development of lymphoid tumours in *bcr*-v*abl* transgenic mice (Hariharan *et al.,* 1989; Botwell *et al.,* 1988). By manipulation of the constructs, a chronic myeloproliferative disorder remarkably similar to human CML can be induced in mice although the same constructs also induced pre-B lymphomas and acute lymphoblastic leukaemia (Kelliher *et al.,* 1990; Daley *et al.,* 1990). The *bcr-abl*-induced murine CML has been shown to be due to retro viral transfer into a pluripotent stem cell and the resultant CML cells can evolve into an acute leukaemia resembling the blast crisis in the human disease (Daley *et al.,* 1991). An alternative approach to investigating the activities of the *bcr-abl* fusion gene has been to introduce antisense oligonucleotides into CML cells and such experiments have demonstrated the suppression of colony formation *in vitro* of leukaemic, but not normal, haemopoietic progenitor cells (Szczcylik *et al.,* 1991). Some, but not all, CML cell lines also appear to be sensitive to the inhibitory effects of antisense treatments (Skorski *et al.,* 1993; de Fabritiis *et al.,* 1993) and it is unclear to what extent a number of potential problems, including toxicity, efficiency of uptake and intracellular activity of oligomers, may be responsible for differences in results. Generally, all the findings are consistent with a causal connection between the *bcr-abl* gene and the initiation of CML but the mechanism whereby this lesion promotes disease progression is not known and secondary changes are presumed to be of importance.

The clinical symptoms of CML progression to an acute myeloid (~70 per cent) or lymphoid (~30 per cent) disease, include significant weight loss, bone and joint pain, unexplained fever, thrombosis and infections. At the cellular level there are significant increases in blast cells, changes in platelet production and cytogenetic evolution. Chromosomal instability is a fundamental characteristic of CML (Rowley, 1980) and, although about 20 per cent of patients entering the terminal acute phase show no gross chromosomal changes and retain an unchanged Ph chromosome (Rowley and Testa, 1982), it is generally believed that acquired genetic abnormalities are critical events leading to accelerated disease and blast crisis. In blast crisis of myeloid origin there is usually a hyperdiploid karyotype with non-random abnormalities in various combinations; most commonly these are +8, −19, i(17q) and +22q−. The phenotype of lymphoid blast crisis

usually corresponds to that of a precursor-B-cell and it is not yet clear whether there is any specificity of cytogenetic markers corresponding to the lymphoid phenotype.

Although the molecular basis of disease progression is not well characterised, mutations in the c-*myc* (Blick *et al.,* 1987) and *ras* (LeMiastre *et al.,* 1989; Collins *et al.,* 1989) oncogenes have been implicated in some patients and, more frequently, mutations in p53 (Ahuja *et al.,* 1989; Kelman *et al.,* 1989; Imamura *et al.,* 1994) and Rb (Ahuja *et al.,* 1991) tumour suppressor genes. Mutations in the p53 gene are detected in some 20–30 per cent of cases of blast crisis (Imamura *et al.,* 1994) and a changing p53 status in the blast crisis of CML with treatment has been described (Foti *et al.* 1991, 1994; Kurosawa *et al.,* 1994; Guinn *et al.,* 1994a, b). In the study reported by Guinn and colleagues it seems that late chronic phase p53 mutant cells were depleted by treatment to below the level of detection. Subsequent resistance to treatment was accompanied by blast crisis with a different p53 mutation. The patient was transplanted but relapsed and it was the p53 mutation previously detected in the chronic-phase that was found at this time (Guinn *et al.,* 1994b). It is probable that detection of p53 mutations in the late chronic phase reflects increasing genomic instability and may be predictive of progression to blast crisis, therefore. Genomic instability as a frequent feature in the evolution of CML from chronic phase to blast crisis has been suggested by Wada and colleagues (1994) on the basis of their findings of instability of variable number of tandem repeats (VNTR) sequences. Instability of microsatellite repeats was not found by Silly and colleagues (1995) and more extensive studies are required to evaluate the importance of somatic instability of repeat sequences in leukaemogenesis. It is possible that there may be differences in the replication/repair mechanisms of tandem repeats and repeats of the di-, tri- or tetranucleotides in microsatellites.

A number of studies have suggested that abnormalities of growth factor production and/or responsiveness may be important in promoting transition from the chronic phase. It has been suggested that there may be autocrine and/or paracrine stimulation of proliferation by IL-1 in the advanced stages of the disease. This proposal is based on the observations of constitutive expression of IL-1 mRNA and protein in many blast crisis cell lysates (Wetzler *et al.,* 1990), the suppression of proliferation of CML-cells *in vitro* by soluble IL-1 receptors and IL- neutralising antibodies (Estrov *et al.,* 1991) and the observations that cultured marrow stromal cells, derived from haematologically normal individuals or chronic phase CML patients, produce few cytokines while stromal cells derived from blast crisis marrow constitutively produce GM-CSF, IL-1 and IL-6 (Wetzler *et al.,* 1991).

More recently it has been found that macrophage inflammatory protein (MIP)-1 , a chemokine with inhibitory effects on primitive haemopoietic cells (reviewed by Wright and Pragnell, 1992; Lord, 1995) has no effect on the proliferative status of CML progenitor cells in long-term cultures (Eaves *et al.,* 1993). A defect in the responsiveness to this, or a similarly acting factor, might explain the deregulated proliferation of cells with normal differentiative properties in chronic phase CML. The effects of two other inhibitory factors for primitive haemopoietic cells, transforming growth factor (TGF)- and the tetrapeptide NAc-Ser-Asp-Lys-Pro-OH (Seraspenide™) have also been investigated. CML cells respond normally to the inhibitory effects of TGF- (Cashman *et al.,* 1992) but not to the tetrapeptide (Cashman *et al.,* 1994). It is interesting that Cashman and her colleagues (1994) found that the inhibitory effects of the tetrapeptide on normal cells was antagonised by the addition of MIP-1 , an antagonist of the action of MIP-1 (Broxmeyer *et al.,* 1991, 1993). Therefore, it is possible that the apparent differential effects of the tetrapeptide on normal and CML progenitors cells may be a secondary consequence of the differential responsiveness of these cells to MIP-1 .

8.7
Biology of Acute Leukaemias

Acute leukaemias constitute approximately 10 per cent of all human cancers and are the most common malignancy in children and young adults. They can be divided by age incidence into those occurring in childhood, ~80 per cent of which are ALL, and those diagnosed in adults (>15 years), ~85 per cent of which are ANLL. Many are associated with consistent chromosome abnormalities and there is a rapidly growing literature detailing the molecular events associated with the cytogenetic features (see, for example, Berger, 1992; Cole-Sinclair *et al.,* 1994; Pedersen-Bjergaard and Rowley, 1994; Johansson *et al.,* 1994; Rabbits, 1994). In many cases these cytogenetic/molecular changes are associated with characteristic subtypes. In AML, the cytogenetic abnormalities may correlate with FAB subtype but patients with a particular FAB subtype may or may not show a particular cytogenetic abnormality. The functional significance of the discrepancy is not yet apparent and may remain unexplained until a biological subdivision, rather than a morphological classification, is available.

Mutation of *ras* oncogenes is the most commonly detected molecular abnormality in acute myeloid leukaemia (20–30 per cent of cases) with a predominance of N-*ras* activation (Farr *et al.,* 1988). The precise role of *ras* oncogenes remains unclear, however, as a mutation detected at presentation may either be absent or replaced by a different mutation at relapse (Farr *et al.,* 1988, 1991) and in some cases of AML not all leukaemic cells have the mutation (Toksoz *et al.,* 1987; Shen *et al.,* 1987) possibly due to the acquisition of the mutation being associated with impaired clonal maturation (Bashey *et al.,* 1992). Although these data suggest that *ras* activation occurs after leukaemia initiation, *ras* mutations are frequently found in myelodysplastic syndromes and those patients with a *ras* mutation are more likely to evolve into AML (Yunis *et al.,* 1989). In some MDS patients the mutation can be detected in both myeloid and lymphoid cells, implying that the mutation arose in a stem cell and implicating *ras* activation as an early (possibly initiating?) event in these particular cases. Other oncogenes have been less systematically studied but elevated expression of c-*myc* or c-*myb* weakly correlated with each other, were unrelated to FAB subtype and associated with resistant disease. Conversely, expression of c-*fms,* c-*fes* and c-*fos* were highly correlated and associated with good response to therapy (Preisler and Raza, 1987; Preisler *et al.,* 1989).

The p53 tumour suppressor gene status in leukaemia has been investigated by several investigators (reviewed by Prokocimer and Rotter, 1994; Imamura *et al.,* 1994). Only about 15 per cent of cases of AML and 5 per cent of MDS have aberrant p53, but these patients tend to have multiple chromosomal abnormalities, a poor response to treatment and short survival. In ALL the frequency of abnormality is to some extent correlated with disease subtype; 3 per cent in cALL, 50 per cent in L3-ALL (Burkitt's type) and rare in T-ALL (but significantly increased in relapsed T-ALL). More recently, it has been reported that the Wilms' tumour suppressor gene, WT-1, is expressed in approximately 80 per cent of AML samples, but not in normal bone marrow or blood cells, but there was no correlation with FAB subtype, immunophenotype or response to treatment (Brieger *et al.,* 1994). The significance of this observation is not yet clear but WT-1 expression appears to provide a useful marker for leukaemic cells. The retinoblastoma (RB) tumour suppressor gene (normally expressed in haemopoietic cells) has been reported to be inactivated in approximately 20–25 per cent of AML cases (Tang *et al.,* 1992). No correlation between inactivation of the RB gene and FAB subtype, cytogenetic characteristics or clinical response was found. The significance of these various findings is not yet clear.

It is becoming increasingly clear that chromosomal translocations resulting in tumour-specific fusion proteins are a major feature of the acute leukaemias. Very often, the proteins involved are transcription factors indicating that aberrant transcriptional regulation is an important aetiological feature. Illustrative examples of fusion genes associated with chromosomal translocations are shown in Table 8.4. This is by no

means an exhaustive list and a review of these molecular events is beyond the scope of this chapter. Transcription factors may be regarded as having specific domains associated with dimerisation, DNA binding and trans-activation functions (Harrison, 1991) and the creation of fusion genes alters one or more of these functions. Recent reviews of this subject include those by Cleary (1991), Rabbits, (1991, 1994) and Cole-Sinclair and colleagues (1994).

A major feature in the acute leukaemias is the breakdown of the co-ordinated regulation of self-replication and commitment to differentiation leading to the proliferation of abnormal cells, and a number of observations have suggested that haemopoietic growth factors may play a role in leukaemic cell proliferation. Prior to the purification and molecular cloning of these factors it had been shown that AML progenitor cells from many patients would proliferate in the presence of crude colony-stimulating activity such as conditioned media from cell lines or feeder cells. The clones generated were shown to be leukaemic by karyotypic analysis but the clone size was very small, the cells showed little or no maturation and the frequency of clonogenic cells was highly variable from one patient to another (reviewed by Metcalf, 1984). Later, it was shown that the colony-stimulating factors (see Chapter 2) were the major growth stimulatory activities and a variety of other factors have been shown to enhance or inhibit these responses (reviewed by Lowenberg and Touw, 1992). At present there is no clear relationship between growth factor responsiveness and FAB subtype (Delwel *et al.,* 1988) but cells from about 20 per cent of t(8; 21) AMLs respond to IL-5 (Touw *et al.,* 1991). This latter finding again suggests that a biological rather than a morphological classification is necessary for mechanistic investigations. One difficulty in the interpretation of these types of experiment is the lack of convincing data that clonogenic cultures of leukaemic cells represent the clonogenic stem cells of the disease: rather, they may identify with more mature members of the leukaemic clone. Leukaemic haemopoiesis retains a basic hierarchical structure (a caricature of normal haemopoiesis) and consequently, the leukaemic stem cells are a minority population within the total leukaemic cell mass. An

Table 8.4 Examples of translocation breakpoints and gene fusions in acute leukaemias

Translocation	Affected genes		Leukaemia type
t(1; 19)(q23; p13.3)	PBX1 (1q23)	E2A (19p13.3)	ALL
t(17; 19)(q22; p13)	HLF (17q22)	E2A (19p13)	ALL
t(15; 17)(q21-q11–22)	PML (15q21)	RARA (17q21)	M3-AML
t(11; 17)(q23; q21.1)	PLZF (11q23)	RARA (17q21)	M3-AML
t(4; 11)(q21; q23)	MLL (11q23)	AF4 (4q21)	ALL/pre-B-ALL/AML
t(9; 11)(q21; q23)	MLL (11q23)	AF9 (11q23)	ALL/pre-B-ALL/AML
t(11; 19)(q23; p13)	MLL (11q23)	ENL (19p13)	pre-B-ALL/T-ALL/AML
t(X; 11)(q13; q23)	MLL (11q23)	AFX1 (Xq13)	T-ALL
t(1; 11)(q32; q23)	MLL (11q23)	AF1P (1p32)	ALL
t(6; 11)(q27; q23)	MLL (11q23)	AF6 (6q27)	ALL
t(11; 17)(q23; 21)	MLL (11q23)	AF17 (17q21)	AML
t(8; 21)(q22; q22)	AML1 (21q22)	ETO (8q22)	AML
t(6; 9)(p23; q34)	DEK (6p23)	CAN (9q34)	AML

expected feature of a leukaemic stem cell is self-replication capacity and this is not detected in the currently available clonogenic assays. Whether this is due to deficiencies in the culture conditions is not clear, but at

present we probably do not have sufficient information about the most ancestral members of the leukaemic clone. Nevertheless, much valuable information about the biology of acute myeloid leukaemia has been obtained from cell culture studies including the inability of leukaemic cells to mature into terminally differentiated, postmitotic cells. This is a characteristic feature of the breakdown of the linkage between loss of self-replicative capacity and commitment to differentiation and is of central importance to the maintenance of the leukaemic state. Evidence in support of this contention is obtained from the exceptional examples where maturation of leukaemic blasts is associated with extinction of clonogenicity (Sachs, 1978; Metcalf, 1984) and in a specific mouse model of myeloid leukaemia where inhibition of leukaemia development was achieved by injection of haemopoietic growth factors (Lotem and Sachs, 1981).

A notable exception to the maturation block that currently is attracting much interest is the specific subgroup of acute promyelocytic leukaemia (M4-AML) with the balanced translocation t(15; 17) (Rowley *et al.,* 1977). In these leukaemias the chromosomal breakpoints are in the retinoic acid receptor (RAR) gene on chromosome 17, a member of the nuclear steroid/thyroid hormone receptor super-family and in a novel gene on chromosome 15 called PML (for promyelocytic leukaemia) that is a zinc finger transcription factor. The 15; 17 translocation fuses the genes of PML and RAR to generate two reciprocal fusion transcripts PML-RAR and RAR -PML (de The *et al.,* 1990, and reviewed by Lavau and Dejean, 1994). A striking feature of the leukaemic cells with this particular molecular lesion is their capacity to respond to retinoic acid by granulocytic differentiation (Breitman *et al.,* 1998; Chomienne *et al.,* 1990), a response that has been exploited to induce complete (but transient) remission in most patients (Huang *et al.,* 1988; Castaigne *et al.,* 1990 and reviewed by Chen *et al.,* 1995). It is anticipated that molecular studies of the genes involved, together with studies of differentiation induction, will contribute to understanding the molecular basis of the lesion in this particular leukaemia. It remains to be seen, however, whether inducing agents can be found to obtain therapeutic benefit and mechanistic information in other acute leukaemias with fusion genes.

In a number of experimental models, the induction of autocrine stimulation by aberrant haemopoietic growth factor secretion, or growth factor receptor function, has been implicated in myeloid leukaemogenesis (reviewed by Metcalf, 1994). At the same time, it is important to note that while transgenic models of growth factor over-expression may produce a myeloproliferative syndrome, aberrant and excessive proliferation does not produce leukaemia. Additional steps that bring about the differentiation block appear to be obligatory for evolution to acute leukaemia. In those cases where human AML cells exhibit a certain level of spontaneous proliferation in culture, it can be shown that they have the ability to secrete haemopoietic growth factors (GM-CSF, G-CSF and M-CSF) and other cytokines (IL-1, IL-6 and TNF). Generally this is not a constitutive production but is inducible *in vitro* by, for example, IL-1 which upregulates the transcription and stability of the appropriate mRNA (Delwel *et al.,* 1989). As this property does not necessarily correlate with an ability to proliferate autonomously *in vitro,* it is uncertain how often AML cells actually produce growth factors *in vivo*.

Despite considerable effort, there is very little progress in understanding the mechanisms involved in the regulation of ALL cell proliferation. This is due largely to the limited success in developing clonogenic cultures for both B- and T-ALL cells and the lack of characterisation of growth regulatory factors present in the conditioned media commonly used for such assays. Factors known to stimulate the more mature stages of B- and T-lymphocyte development, induce DNA synthesis in cultures of ALL cells very infrequently, even though the cells may express high affinity receptors for such factors (Touw *et al.,* 1989, 1990a, b).

It has been reported that MIP-1 significantly suppresses the proliferation of clonogenic AML cells (Ferrajoli *et al.,* 1994) but, perhaps not surprisingly, this does not seem to be a universal finding (Defard *et al.,* 1994). Defard and colleagues also found that some AML cells responding to MIP-1 and TGF- are

insensitive to the tetrapeptide Seraspenide™ suggesting heterogeneity in the responses of AML cells to inhibitory factors. This probably reflects the heterogeneity of the disease. Interestingly, whereas very low levels of MIP-1 mRNA are detectable in normal bone marrow nucleated cells, high levels have been detected in the cells obtained from patients with aplastic anaemia and preleukaemic myelodysplastic syndromes (Maciejewski *et al.,* 1992) and in freshly obtained unstimulated AML and ALL cells (Yamamura *et al.,* 1989), consistent with the view that leukaemic cells are largely unresponsive to MIP-1 . This raises the possibility that the myelosuppression in leukaemia (and, possibly, the bone marrow failure observed in some leukaemic and preleukaemic disorders) is a consequence of the over-expression of MIP-1 and that a number of distinct lesions may lead to a similar dysregulation of proliferation in different leukaemias.

The myelodysplastic syndromes are known to be induced by ionising radiation and certain chromosomal abnormalities are common (Pedersen-Bjergaard, 1992). These include monosomy 5 or 5q-, monosomy 7 or 7q-, trisomy 8, and loss of the Y-chromosome. Certain chromosome aberrations found in AML are rarely found in MDS suggesting that patients with these abnormalities have not developed their disease from a pre-existing MDS; these include t(8; 21), and t(15; 71). Generally, progression to AML is associated with additional genetic changes. The 5q- is one of the most frequent cytogenetic abnormalities observed in MDS and AML (Mitelman, 1991) and it is generally assumed that the deleted region encodes a tumour suppressor gene but the precise lesion is not known (Westbrook and Keinanen, 1992; Boultwood *et al.,* 1994). Leukaemic progression is uncommon except when the aberration is found in combination with other chromosomal defects (Hoelzer *et al.,* 1984).

8.8
Experimental Radiation Leukaemogenesis

Radiation-induced leukaemias have been observed in a variety of experimental animals but susceptibility and type of disease varies markedly between species and strains (see Chapter 10). The leukaemogenic effects of a given radiation dose have also been observed to be influenced by a variety of host or constitutional factors for reasons that are not fully understood.

Mice have been far more extensively studied than any other species and thymic lymphomas, sometimes developing into generalised lymphoma and lymphoid leukaemia have been the focus of most of these studies. One of the mechanisms by which radiation may induce thymic lymphoma involves activation of latent endogenous viruses (reviewed by Kaplan, 1967) and the extent to which this type of mechanism may be involved in the pathogenesis of other radiogenic lymphomas and leukaemias is not resolved. Animal models provide useful systems for studying the pathogenesis of radiation-induced leukaemias and to characterise cellular and molecular events that are early or late features of the disease process.

The first systematic study of radiation-induced myeloid leukaemia demonstrated that in RF mice a 2–6 per cent spontaneous frequency was increased to 30–40 per cent by a single whole-body exposure to 1.5–3 Gy X-rays (Upton *et al.,* 1958). Since then a number of mouse strains with negligible spontaneous incidence of the disease have been found to be susceptible to myeloid leukaemia development following X-irradiation. Among these strains are SJL/J (Haran-Ghera *et al.,* 1967), CBA/H (Major and Mole, 1978) and C3H (Hayata *et al.,* 1983) and they provide valuable models to investigate leukaemias that are unequivocally related to the radiation exposure.

The clonal origin of radiation-induced myeloid leukaemia has been studied in female C3H mice, heterozygous for the X-linked enzyme phosphoglycerate kinase (Pgk), where the two variants of the enzyme, Pgk-1a and Pgk-1b can be distinguished by electrophoretic mobility. Spleen cells obtained from

control mice were found to produce two bands on electrophoresis whereas cells from leukaemic spleens gave a single band indicating that the leukaemias were monoclonal diseases (Bessho and Hirashima, 1987). Monoclonality was confirmed in colonies of cells derived from myelomonocytic progenitors detected as granulocyte/macrophage colony forming cells (GM-CFC). Examples of leukaemic mice in which erythrocytes were of the same or different isoenzyme type to the leukaemic cells were reported, suggesting that in some animals the disease originated in a single cell capable of differentiating into both myelomonocytic and erythroid cells, whereas in others the target cell appeared to be restricted in its differentiation potential. That there might be different target cells for murine AML is similar to the situation in the human leukaemias revealed by isoenzyme studies (see above).

Approximately 20–25 per cent of CBA/H, SJL/J or C3H mice exposed to 3 Gy whole-body X-irradiation develop AML with latent periods of 6–30 months and a peak incidence at 18 months. Anaemia is the characteristic presenting symptom and it is usually a matter of days before terminal disease. By daily physical examination and taking monthly blood samples we have, in some cases, been able to identify leukaemic CBA/H mice in advance of terminal illness (Wright and Lorimore, 1990). No evidence of leukaemic haemopoiesis in peripheral blood could be detected earlier than 4–6 weeks before terminal disease and there is little evidence for a defined preleukaemic syndrome. Although the CBA/H strain is highly inbred and whole-body X-irradiation is a homogeneous insult to the haemopoietic tissues, we have found a spectrum of myeloid leukaemia subtypes. These leukaemias can be classified using the FAB criteria and of the primary leukaemias we have examined in detail, M2 and M4 have been the most common subtypes, M5 were less common, a few examples of M4, M0 and M1 and no cases of M3 or M7 have been diagnosed. More than 80 per cent of these leukaemias had a terminal or interstitial deletion of one homologue of chromosome 2, confirming other studies (Silver *et al.,* 1987; Rithidech *et al.,* 1993), and this finding is also characteristic of the myeloid leukaemias induced in the other susceptible strains (Trakhtenbrot *et al.,* 1988; Hayata, 1983; Hayata *et al.,* 1983, and reviewed by Haran-Ghera, 1989). The interstitial deletions commonly involve the C2-E5/F1 region and terminal deletion breakpoints are clustered at C2 and C3/D with a few leukaemias exhibiting breaks in the E and F bands. Overall, the data are consistent with the possibility that the C2-E5 region of chromosome 2 might contain one or more genes that are involved in AML development and, by analogy with human diseases, may encode a tumour suppressor gene.

One of the earliest studies by Azumi and Sachs (1977) described chromosome 2 deletions in six leukaemia-derived cell lines that failed to show differentiation response to MGI, a macrophage and granulocyte differentiation inducer subsequently shown to be interleukin-6 (Sachs, 1990). In four cell lines that responded to MGI, there was no evidence of chromosome 2 aberrations. Five MGI-responsive mutant cell lines derived from an unresponsive cell line with a loss of part of one chromosome 2, one normal chromosome 12 and two translocated chromosomes 12, maintained the abnormal chromosome 2, but lost either the one normal or one of the translocated chromosomes 12. The results suggested that chromosomes 2 and 12 encode genes that are involved in the differentiation of myeloid leukaemic cells and the observed differentiation response was controlled by the balance between these genes. Unfortunately, the molecular basis of these findings remains unknown.

Aberrations involving chromosome 2 are detectable in bone marrow within days of a potentially leukaemogenic dose of X-irradiation (Breckon *et al.,* 1991; Fennelly *et al.,* 1995) and can be readily found in bone marrow cultures established from the marrow of 80–100 per cent of animals four months after irradiation (Trakhtenbrot *et al.,* 1988), significantly before the first appearance of AML. It has been suggested that chromosome 2 breakage at such sites is an initiating event in radiation-induced AML (Silver *et al.,* 1987) although it is evident that the chromosome 2 aberrations in the absence of additional changes are insufficient for the development of AML. The high frequency of breaks on chromosome 2 has been

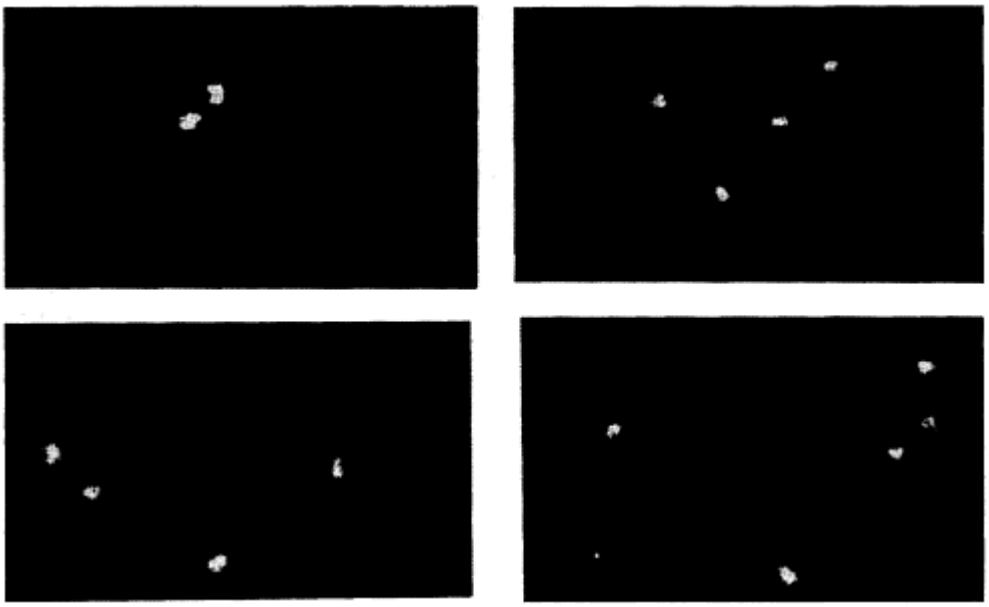

Figure 8.1 Fluorescence *in situ* hybridisation analysis reveals the instability of the Y-chromosome in a primary, radiation-induced acute myeloid leukaemia in a CBA/H mouse (*see also* Colour Plate 4)

attributed to the presence of radiation sensitive sites, characterised by interstitial telomeric repeat sequences (Breckon *et al.,* 1991; Bouffler *et al.,* 1993) although the precise molecular nature of the lesion in the chromosome 2 aberrations remains unclear. It was reported that in the CBA/H colony there were, unexpectedly, four genotypic variants of the interstitial telomere $(TTAGGG)_n$-like sequence arrays and that almost all radiation-induced AMLs were in one of the four variants (Silver and Cox, 1993). These variants were not detected in CBA/H mice from other laboratories and when the experiments were repeated the original observations could not be confirmed (R. Cox, personal communication). We have not detected the reported variants in the breeding or foundation stocks at the MRC Radiobiology Unit. A possible explanation is that these variants arose within a particular breeding line and have not been maintained in subsequent lines.

Recently, using arbitrarily primed-PCR analysis of DNA prepared from X-ray-induced AMLs in CBA/H mice, we have identified a sequence that was deleted in several leukaemias. The corresponding probe hybridised to the Y-chromosome which is deleted in ~50 per cent of our AMLs and, compared with the characteristic early loss of chromosome 2, is a late event in radiation leukaemogenesis (Fennelly *et al.,* 1995). We have isolated, and sequenced, corresponding genomic clones and have shown that they contain at least two forms of repeated Y-chromosome-specific endogenous retroviral sequences (MuRVY and IAP-E-related). Fluorescence *in situ* hybridisation of AML cells using genomic clones indicate that the Y-chromosome is highly unstable in murine AMLs and has identified a high incidence of subclonal variants (Figure 8.1). The presence of at least five hundred copies of endogenous retro viral sequences on the murine Y-chromosome (Hutchison and Eicher, 1989; Eicher *et al.,* 1989), including potentially active long terminal repeat (LTR) sequences, raises the possibility that the rearrangement of the Y-chromosome sequences activates or suppresses gene expression at other chromosomal loci.

Recurrent secondary loss of the sex chromosome occurs very frequently in the specific M2 t(8; 21)(q22; q22) subtype of human AML in which the translocation is between the AML1 and ETO genes, both of which encode transcription factors. In a recent survey of 7111 human acute leukaemias (Johansson *et al.,* 1994; Johansson, personal communication) it has been documented that 432/558 (77 per cent) of leukaemias of this subtype contain secondary chromosome abnormalities. In males, 76 per cent (205/271) of the secondary abnormalities involve the loss of the Y-chromosome and in females 59 per cent (95/161) involve loss of an X-chromosome. By contrast, sex chromosome loss in other AML subtypes (and in ALL subtypes) occurs with an incidence of less than 5 per cent (Johansson *et al.,* 1994; Mertens *et al.,* 1993). Increasingly, human X-chromosome abnormalities are being linked to leukaemia and preleukaemic disorders and commonly involve Xp22 and Xq28 (Goyns *et al.,* 1993; Thick *et al.,* 1994; Rack *et al.,* 1994). In addition to our study in which ~50 per cent of the CBA/H leukaemias had a deleted Y-chromosome by cytogenetic analyses (Fennelly *et al.,* 1995), in the study of C3H and RFM leukaemias by Hayata and his colleagues (1983), loss of Y-chromosome was observed in 36/46 male cases. Anecdotally, the incidence of radiation-induced AML in female CBA/H mice is much lower than in males (Major, 1979) and there is very limited information for the other strains. However, the loss of an X-chromosome has been reported in leukaemias induced by irradiation in 2/3 C3H and 1/2 B6C3F1 female mice (Hayata *et al.,* 1983). Thus, the radiation-induced murine AMLs may be of value for investigating this recurrent secondary loss of the Y- or X-chromosome and is relevant to the human situation.

We have found that our AP-PCR probe also cross-hybridised to a polymorphic locus in the inbred CBA/H colony which maps to the pseudoautosomal region (PAR) of the sex chromosomes. Inaccurate recombination at this particular ~10 kbp PAR locus occurs in 7–11 per cent of meioses and results in variant polymorphic bands. Analysis of AML DNA prepared from twenty independently induced CBA/H AMLs revealed that, compared with their incidence in the CBA/H colony, one particular genotype is significantly under-represented (20 per cent of expected) and a second is over-represented (3 times higher than expected). These findings suggest that a specific meiotic recombination event confers a predisposition or resistance to radiation leukaemogenesis in CBA/H mice and suggest new approaches to studying the genetic basis of predisposition to radiation-induced AML.

8.9
Radiation-induced Genomic Instability

The growing body of evidence that a key feature of the evolution of malignancy is genetic instability raises important questions about the contribution of instability to radiogenic diseases. Furthermore, the extent to which genetic factors (predisposition) determine the expression of instability and the susceptibility of individuals to radiation oncogenesis is a central issue, not only for mechanistic understanding but also for risk estimation.

The biological consequences of exposure to ionising radiation include gene mutation, chromosome aberrations, cellular transformation and cell death. These effects are attributed to the DNA-damaging effects of the irradiation resulting in irreversible changes during DNA replication or during the processing of the DNA damage by enzymatic repair processes. Accordingly, it has been widely accepted that most of these changes take place during the cell cycles immediately following exposure. It has been apparent for many years, however, that radiation-induced cytotoxicity may be delayed for up to six generations of cell replication (Puck and Marcus, 1956; Elkind and Sutton, 1959; Trott and Hug, 1970) with death occurring randomly among the progeny cells (Thompson and Suit, 1969). More recently, an enhanced death rate in the progeny of irradiated cells has been shown to persist for many generations. This phenomenon of delayed

reproductive death has been attributed to the induction of 'lethal mutations' arising in the descendants of cells surviving irradiation (Seymour *et al.,* 1986; Gorgojo and Little, 1989) and Brown and Trott (1994) have reported considerable clonal heterogeneity with respect to the decrease in plating efficiency. Evidence is now accumulating that cells initially surviving irradiation and capable of proliferation may produce some descendants in which *de novo* chromosome aberrations (Kadhim *et al.,* 1992, 1994; Sabatier *et al.,* 1992; Martins *et al.,* 1993; Holmberg *et al.,* 1993; Marder and Morgan, 1993) and specific gene mutations (Little *et al.,* 1990) arise. Thus, ionising radiation may induce a transmissible genomic instability, producing a variety of cellular effects that become manifest after many cell cycles in the progeny of the irradiated cells.

We have reported a high frequency of non-clonal cytogenetic abnormalities in the clonal descendants of murine and human haemopoietic stem cells surviving low doses of -particle irradiation (Kadhim *et al.,* 1992, 1994). The data are compatible with -particles inducing lesions in stem cells that result in the transmission of chromosomal instability to their progeny. Current investigations of additional inbred mouse strains are revealing genotype-dependent quantitative differences in the expression of instability (Table 8.5) and we have suggested (Kadhim *et al.,* 1994) that inter-individual variation in experiments using human bone marrow reflect a similar genetic component to the instability.

When ionising radiation passes through biological tissue it forms highly structured tracks as a consequence of the deposition of energy (see Chapter 3). Generally, a distinction can be made between sparsely and densely ionising radiations on the basis of the average energy transferred per unit length of track (the linear energy transfer, or LET). A particular feature of the irradiation of tissues by high-LET radiations such as -particles is that the entire insult is concentrated into a relatively small number of separate densely ionising tracks with very limited ranges. At low doses, any individual cell in a tissue is likely to receive no dose, or, if it happens to be in the path of a track, to receive a substantial dose of radiation. For -particle-emitters, therefore, the problem of whether low doses might produce biologically important effects reduces to assessing the effectiveness of a single track, or a very small number of tracks, in producing appropriate damage in the relevant target cell. The delayed cytogenetic abnormalities reported after -particle irradiation should be indicative of changes that can occur, in a decreased proportion of cells, at much lower doses. At any arbitrarily low dose to a cell population, those few cells that are intersected by an -particle would individually receive a single track dose of ~0.5 Gy, comparable to the doses we have delivered experimentally (Goodhead, 1988; Lorimore *et al.,* 1993). Similar considerations do not apply to X-irradiations: with decreasing dose the individual cells receive approximately corresponding decreases to very low levels. Thus, extrapolation of current observations to low doses may be substantially different for the two types of radiation.

Although genomic instability is a feature of the development of certain leukaemias, it is unclear at present whether radiation-induced instability is implicated in radiogenic leukaemias. A causal connection between chromosome instability and the induction of leukaemia is found in the human disorder Fanconi anaemia; a genetic disease in which there is chromosomal instability, progressive bone marrow

Table 8.5 Genotype-dependent differences in the expression of chromosomal instability in the clonal progeny of murine haemopoietic stem cells in bone marrow suspensions exposed to -particles

Genotype of bone marrow cells	-irradiation (Gy)	Metaphases with chromosome aberrations (%)
CBA/H	0	2
	0.5	21
DBA/2	0	2
	0.5	13

Genotype of bone marrow cells	-irradiation (Gy)	Metaphases with chromosome aberrations (%)
C57B1/6	0	<1
	0.5	3
(C57B1/6×DBA/2) F1	0	<1
	0.5	2

failure and a significant predisposition to acute myeloid leukaemia (Auerbach, 1992). This prompts the hypothesis that chromosomal instability, whether genetically determined or induced by low-dose irradiation, could produce changes in the haemopoietic stem cells of some individuals that may contribute to the subsequent development of leukaemia. It is interesting that in a recent study of Fanconi anaemia at different stages of the disease, including aplastic anaemia, MDS and AML, it was evident that monoclonal haemopoiesis was a frequent finding in the course of the disease and preceded the onset of neoplasia in some cases. The genetic mechanisms underlying Fanconi anaemia-associated leukaemogenesis, however, appear to be independent of N-*ras* and p53 mutations (Venkatraj *et al.,* 1994) suggesting a mechanism distinct from that commonly seen in MDS and AML.

In the clonal analyses, at the time when cytogenetic abnormalities were demonstrated, there is a readily detected incidence of apoptotic cells in the colonies derived from clonogenic cells in marrow suspensions exposed to -particles (Kadhim *et al.,* 1995). It is known that lethally irradiated cells may undergo several cell divisions before they lose their ability to divide and time lapse cinematographic studies by Thompson and Suit (1969) demonstrated that cell death occurred randomly among the progeny of lethally irradiated cells. As apoptotic cells were seen in all clones examined it is unlikely that they represent this delayed death of lethally irradiated cells. Stem-cell-derived colonies contain differentiating cells that become postmitotic and it is difficult to compare these colonies with clones derived from established cell lines. The apoptotic cells are a minority population and are seen alongside actively proliferating cells. If the apoptosis is occurring in cells with any proliferative potential, it is possible that they are cells in which instability-derived lesions in DNA are incompatible with further proliferation. Alternatively, delayed apoptosis may result from signal autonomy, a cellular state where a cell simultaneously engages in incompatible pathways of proliferation and cell cycle arrest and in response to these conflicting signals initiates an apoptotic response (Hibner and Coutinho, 1994). Whether this is the basis of lethal mutations is a matter for speculation. It is of interest that there may be a genotype-dependent inverse correlation between the incidence of delayed chromosomal aberrations and death by apoptosis in haemopoietic cells. The genetic background that produces an apoptotic response may well eliminate potentially leukaemic cells and effectively reduce the probability of leukaemia development.

References

AHUJA, H., BAR-ELI, M., ADVANI, S.H., BENCHIMOL, S. & CLINE, M.J. (1989) Alterations in the p53 gene and the clonal evolution of the blast crisis of chronic myelocytic leukemia, *Proceedings of the National Academy of Sciences, USA,* **86**, 6783–7.

AHUJA, H., JAT, P.S., FOTI, A., BAR-ELI, M. & CLINE, M.J. (1991) Abnormalities of the retinoblastoma gene in the pathogenesis of acute leukaemia, *Blood,* **78**, 3259–68.

AUERBACH, A.D. (1992) Fanconi anemia and leukemia, tracking the genes, *Leukemia,* **6**, (suppl. 1), 1–4.

AZUMI, J.I. & SACHS, L. (1977) Chromosome mapping of the genes that control differentiation and malignancy in myeloid leukemic cells, *Proceedings of the National Academy of Sciences, USA,* **74**, 253–7.

BASHEY, A., GILL, R., LEVI, S. *et al.* (1992) Mutational activation of the N-ras oncogene assessed in primary clonogenic culture of acute myeloid leukemia (AML): implications for the role of N-*ras* mutation in AML pathogenesis, *Blood,* **79**, 981–9.

BENNETT, J.M., CATOVSKY, D., DANIEL, M.T. *et al.* (1976) (The French-American-British (FAB) Co-operative Group), Proposals for the classification of the acute leukaemias, *Br. J. Haematology,* **51**, 189–99.

(1982) (The French-American-British (FAB) Co-operative Group), Proposals for the classification of the myelodysplastic syndromes, *Br. J. Haematology,* **33,** 451–8.

BERGER, R. (1992) The cytogenetics of haematological malignancies, *Clinical Haematology,* **5**, 791–814.

BESSHO, M. & HIRASHIMA, K., (1987) Single cell origin of radiation-induced myeloid leukemia in mice with cellular mosaicism, *Experimental Hematology,* **15**, 589 (abstr.).

BLICK, M., ROMERO, P., TALPAZ, J., BERAN, M. & GUTTERMAN, J.U. (1987) Molecular characteristics of chronic myelogenous leukemia in blast crisis, *Cancer Genetics and Cytogenetics,* **27**, 349–56.

BORST, P. & GREAVES, D.R. (1987) Programmed rearrangements altering gene expression, *Science,* **235**, 658–67.

BOTWELL, D.D.L., CORY, S., JOHNSON, G.R. & GONDA, T.J. (1988) Comparison of expression in hemopoietic cells by retroviral vectors carrying two genes, *J. Virology,* **62**, 2464–73.

BOUFFLER, S., SILVER, A., PAPWORTH, D., COATES, J. & COX, R. (1993) Murine radiation myeloid leukemogenesis: relationship between interstitial telomere-like sequences and chromosome 2 fragile sites, *Genes Chromosomes and Cancer,* **6**, 98–106.

BOULTWOOD, J., LEWIS, S. & WAIANSCOAT, J.S. (1994) The 5q-syndrome, *Blood,* **84**, 3253–60.

BRECKON, G., PAPWORTH, D. & COX, R. (1991) Murine myeloid leukaemogenesis: a possible role for radiation-sensitive sites on chromosme 2, *Genes Chromosomes and Cancer,* **8**, 8–14.

BREITMAN, T., COLLINS, S.J. & KEENE, B.R. (1981) Terminal differentiation of promyelocytic leukaemia cells in primary cultures in response to retinoic acid, *Blood,* **57**, 1000– 4.

BRIEGER, J., WEIDMANN, E., FENCHEL, K., MITOU, P.S., HOELZER, D. & BERGMAN, L. (1994) The expression of the Wilms' tumour gene in acute myelocytic leukemias as a possible marker for leukemic blast cells, *Leukemia,* **8**, 2138–43.

BROWN, D.C. & TROTT, K.R. (1994) Clonal heterogeneity in the progeny of HeLa cells which survive X-irradiation. *International Journal of Radiation Biology,* **66,** 151–5.

BROXMEYER, H.E., SHERRY, B., COOPER, S. *et al.* (1991) Macrophage inflammatory protein (MIP)-1 abrogates the capacity of MIP-1 to suppress myeloid progenitor growth, *J. Immunology,* **147**, 2586–94.

BROXMEYER, H.E., SHERRY, B., LU, L. *et al.* (1993), Comparative analysis of the human macrophage inflammatory protein family of cytokines (chemokines) on proliferation of human myeloid progenitor cells, *J. Immunology,* **150**, 3448–58.

CASHMAN, J.D., EAVES, A.C. & EAVES, C.J. (1992) Granulocyte-macrophage colony-stimulating factor modulation of the inhibitory effect of transforming growth factor- on normal and leukemic human hematopoietic progenitor cells, *Leukemia,* **6**, 886–92. (1994) The tetrapeptide AcSDKP specifically blocks the cycling of primitive normal but not leukemic progenitors in long-term culture: evidence for an indirect mechanism, *Blood,* **84**, 1534–42.

CASTAIGNE, S., CHOMIENNE, C., DANIEL, M. *et al.* (1990) ALL-trans retinoic acid as a differentiation therapy for acute promyelocytic leukaemia. I: clinical results, *Blood,* **76**, 1704–9.

CHANG, W.P. & LITTLE, J.B. (1992) Persistently elevated frequency of spontaneous mutations in progeny of CHO clones surviving X-irradiation; association with delayed reproductive death phenotype, *Mutation Research,* **270**, 191–9.

CHEN, S-J., WANG, Z-Y. & CHEN, Z. (1995) Acute promyelocytic leukemia: from clinic to molecular biology, *Stem Cells,* **13,** 22–31.

CHOMIENNE, C., BALLERINI, P., BALITRANT, N. *et al.* (1990) ALL-trans retinoic acid in acute promyelocytic leukemia. II: *in vitro* studies of structure-function relationship, *Blood,* **76**, 1710–17.

CLEARY, M.L. (1991) Oncogenic conversion of transcription factors by chromosomal translations, *Cell,* **66**, 619–22.

COLE-SINCLAIR, M.F., FORONI, L. & HOFFBRAND, A.V. (1994) Genetic changes: relevance for diagnosis and detection of minimal residual disease in acute lymphoblastic leukaemia, *Clinical Haematology,* **7**, 183–233.

COLLINS, S.J., HOWARD, M., ANDREWS, D.F., AGURA, E. & RADICH, J. (1989) Rare ocurrence of N-*ras* point mutations in Philadelphia chromosome positive chronic myeloid leukemia, *Blood,* **73**, 1028–32.

DALEY, G.Q. & BALTIMORE, D. (1988) Transformation of an Interleukin-3 dependent hematopoietic cell line by the chronic myelogenous leukaemia-specific $P210^{BCR/ABL}$ protein, *Proceedings of the National Academy of Sciences, USA,* **85**, 9312–16.

DALEY, G.Q., VAN ETTEN, R.A. & BALTIMORE, D. (1990) Induction of chronic myelogenous leukemia in mice by the P210BCR/ABL gene of the Philadelphia chromosome, *Science,* **247**, 824–30.

(1991) Blast crisis in a murine model of chronic myelogeneous leukaemia, *Proceedings of the National Academy of Sciences, USA,* **88**, 11335–8.

DE FABRITIIS, P., AMADORI, S., CALABRETTA, B. & MANDELLI, F. (1993)Elimination of colonogenic Philadelphia-positive cells using BCR-ABL antisense oligonucleotides . *Bone Marrow Transplantation,* **12**, 261–6.

DEFARD, M., LEMOINE, F.M., KHOURY, E. *et al.* (1994) Heterogeneity in the response of acute leukemia cells to the negative regulators Seraspenide, macrophage inflammatory protein 1 (MIP-1) and transforming growth factor (TGF), *Blood,* **84** (suppl. 1), 617a (abstr.).

DELWEL, R., SALEM, M., PELLENS, C. *et al.* (1988) Growth regulation of human acute myeloid leukaemia: effects of five recombinant hematopoietic growth factors in a serum-free culture system, *Blood,* **72**, 1944–9.

DELWEL, R., VAN BUITENEN, C., SALEM, M. *et al.* (1989) Interleukin-1 stimulated proliferation of acute myeloblastic leukemia cells by induction of granulocyte macrophage colony-stimulating factor release, *Blood,* **74**, 586–93.

DE THE H., CHOMIENNE, C., LANOTTE, M., DEGOS, L. & DEJEAN, A. (1990) The t(15; 17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor gene to a novel transcribed locus, *Nature,* **347**, 558–61.

DUBE, J., DIXON, J., BECKETT, T. *et al.* (1989) Location of breakpoints within the major breakpoint cluster region (bcr) in 33 patients with bcr rearrangement-positive chronic myeloid leukaemia (CML) with complex or absent Philadelphia chromosomes, *Gene Chromosomes and Cancer,* **1**, 106–11.

EAVES, C.J., CASHMAN, J.D., WOLPE, S.D. & EAVES, A.C. (1993) Unresponsiveness of primitive chronic myeloid leukemia cells to macrophage inflammatory protein 1 , an inhibitor of primitive normal cells, *Proceedings of the Academy of Sciences, USA,* **90**, 12015–19.

EICHER, E.M., HUTCHINSON, K.W., PHILLIPS, S.J., TUKER, P.K. & LEE, B.K. (1989) A repeated segment on the mouse Y chromosome is composed of retroviral-related Y-enriched and Y-specific sequences, *Genetics,* **122**, 188–92.

ELKIND, M.M. & SUTTON, H. (1959) X-ray damage and recovery in mammalian cells in culture, *Nature,* **184**, 1293–5.

ESTROV, Z., KURZROCK, R., WETZLER, M. *et al.* (1991) Suppression of chronic myelogenous leukemia colony growth by interleukin-1 (IL-1) receptor antagonists and soluble IL-1 receptors: a novel application for inhibitors of IL-1 activity, *Blood,* **78**, 1476–84.

FARR, C.J., SAIKI, R.K., ERLICH, H.A., MCCORMICK, E. & MARSHALL, C.J. (1988) Analysis of *ras* gene mutations in acute myeloid leukaemia by polynerase chain reaction and oligonucleotide probes, *Proceedings of the National Academy of Sciences, USA,* **85**, 1629–33.

FARR, C., GILL, R., KATZ, F., GIBBONS, B. & MARSHALL, C.J. (1991) Analysis of *ras* gene mutations in childhood myeloid leukaemia, *Br. J. Haematology,* **77**, 323–7.

FENNELLY, J., CRABTREE, G., MACDONALD, D. *et al.* (1995) Complex Y chromosome aberrations are a recurrent secondary event in adiation induced murine acute myeloid leukemia, *Leukemia,* **9**, 506–12.

FERRAJOLI, A., TALPAZ, M., ZIPF, T.F. *et al.* (1994) Inhibition of acute myelogenous leukemia progenitor proliferation by macrophage inflammatory protein-1 , *Leukemia,* **8**, 798–805.

FIALKOW, P.J. (1990) Clonal development and stem cell origin of leukaemias and related disorders. In M.D.Henderson & T.A.Lister (eds), *Leukemia* (5th edn). New York: Saunders, pp. 35–54.

FIALKOW, P.J., NAJFELD, V., REDDY, A.L., SINGER, J. & STEINMANN, L. (1978) Chronic lymphocytic leukaemia: clonal origin in a committed B-lymphocyte progenitor, *Lancet,* **2**, 444–6.

FIALKOW, P.J., SINGER, J.W., RASKIND, M.D. *et al.* (1987) Clonal development, stem cell differentiation and clinical remissions in acute nonlymphocytic leukemia, *New Engl J. Medicine,* **317**, 368–73.

FLUG, F., PELICCI, P.G., BONETTI, F., KNOWLES II, D.M. & DALLA-FAVERA, R. (1985) T-cell receptor gene rearrangements as markers of lineage and clonality in T cell neoplasms, *Proceedings of the National Academy of Sciences, USA,* **82**, 3460–4.

FOTI, A. & CLINE, M.J. (1994) Sequential relapse of blastic crisis may involve different clones of cells with different molecular abnormalities, *Br. J. Haematology,* **87**, 627–30.

FOTI, A., AHUJA, H.G., ALLEN, S.L. *et al.* (1991) Correlation between molecular and clinical events in the evolution of chronic myelocytic leukemia in blast crisis, *Blood,* **77**, 2441–4.

GOODHEAD, D.T. (1988) Spatial and temporal distribution of energy, *Health Physics,* **55**, 231–40.

GOOTWINE, E., WEBB, C.G. & SACHS, L. (1982) Participation of myeloid leukaemia cells injected into embryos in haematopoietic differentiation in adult mice, *Nature,* **299**, 63–5.

GORGOJO, L. & LITTLE, J.B. (1989) Expression of lethal mutations in progeny of irradiated mammalian cells, *Int. J. Radiation Biology,* **55**, 619–30.

GOYNS, M.H., HAMMOND, D.W., HARRISON, C.J., MENASCE, L.P., ROSS, F.M. & HANCOCK, B.W. (1993) Structural abnormalities of the X-chromosome in non-Hodgkin's lymphoma, *Leukemia,* **7**, 848–52.

GREAVES, M.F. (1982) Target cells, cellular phenotypes and lineage fidelity in human leukaemia, *J. Cellular Physiology* (suppl. 1) 113–26.

(1986) Differentiation-linked leukemogenesis in lymphocytes, *Science,* **234**, 697–704.

GREAVES, M.F., CHAN, L.C., FURLEY, A.J.W., WATT, S.M. & MOLGAARD, H. V. (1986) Lineage promiscuity in hemopoietic differentiation and leukemia, *Blood,* **67**, 1–11.

GUINN, B.B., PADUA, R.A., BURNETT, A.K. & MILLS, K.I. (1994a) p534 mutations indicate a changing clonal evolution in a portion of chronic myelocytic leukemia patients, *Blood,* **84**, 3591.

GUINN, B.B., SMITH, M.C., PADUA, R.A., BURNETT, A.K. & MILLS, K.I (1994b) The role of p53 mutations in the switch to blast crisis in chronic myeloid leukaemia, *Br. J. Haematology,* **86**, 49 (abstr.).

HARAN-GHERA, N. (1989) Radiation induced deletion of chromosome 2 in myeloid leukemogenesis. *Current Topics in Microbiology and Immunology,* **149**, 35–41.

HARAN-GHERA, N., KOTLER, M. & MESHORER, A. (1967) Studies on leukaemia development in the SJL strain of mice, *J. National Cancer Institute,* **39**, 653–61.

HARIHARAN, I.K., ADAMS, J.M. & CORY, S. (1988) BCR-ABL oncogene renders myeloid cell line factor independent: potential autocrine mechanism in chronic myeloid leukaemia, *Oncogene Research,* **3**, 387–99.

HARIHARAN, I.K., HARIS, A.W., CRAWFORD, M. *et al. (*1989) A BCR-v-ABL oncogene induced lymphomas in transgenic mice, *Molecular and Cellular Biology,* **9**, 2798–805.

HARRISON, M.L. (1991) A structural taxonomy of DNA-binding domains, *Nature,* **353**, 715–19.

HAYATA, I. (1983) Partial deletion of chromosome 2 in radiation-induced myeloid leukemia in mice. In T.Tishihar & M.A.Sasaki (eds), *Radiation-induced Chromosome Damage in Man.* New York: Alan R, Liss, pp. 277–97.

HAYATA, I., KAKATI, S. & SANDBERG, A.A. (1975) Another translocation related to the Ph-1 chromosome, *Lancet,* **1**, 1300.

HAYATA, I., SEKI, M., YOSHIRA, K. *et al.* (1983) Chromosomal aberrations observed in 52 mouse myeloid leukaemias, *Cancer Research,* **43,** 367–73.

HIBNER, U. & COUTINHO, A. (1994) Signal antonymy: a mechanism for apoptosis induction, *Cell Death and Differentiation,* **1**, 33–7.

HOELZER, D., GANSER, A. & HEIMPEL, H. (1984), 'Atypical' leukemias: preleukaemia, smouldering leukaemia and hypoplastic leukaemia, *Recent Results in Cancer Research,* **93**, 69–101.

HOLMBERG, K., FALT, S., JOHANSSON, A. & LAMBERT, B. (1993) Clonal chromosome aberrations and genomic instability in X-irradiated human T-lymphocyte cultures, *Mutation Research,* **286,** 321–30.

HUANG, M., TE, Y., CHEN, S. *et al.* (1988) Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia, *Blood,* **71**, 567–71.

HUTCHISON, K.W. & EICHER, E.M. (1989) An amplified endogenous retroviral sequence on the murine Y chromosome related to murine leukemia viruses and virus-like 30S sequences, *J. Virology,* **63**, 4043–6.

IMAMURA, J., MIYOSHI, I. & KOEFFLER, H.P. (1994) p53 in hematologic malignancies, *Blood,* **84,** 2412–21.

JOHANSSON, B., MERTENS, F. & MITELMAN, F. (1994) Secondary chromosomal abnormalities in acute leukemias, *Leukemia,* **8**, 954–62.

KAPLAN, H.S. (1967) On the natural history of the murine leukaemias, *Cancer Research,* **27**, 1325–40.

KADHIM, M.A., MACDONALD, D.A., GOODHEAD, D.T., LORIMORE, S.A., MAARSDEN, S.J. & WRIGHT, E.G. (1992) Transmission of chromosomal instability after plutonium -particle irradiation, *Nature,* **355**, 738–40.

KADHIM, M.A., LORIMORE, S.A., HEPBURN, M.D., GOODHEAD, D.T., BUCKLE, V.J. & WRIGHT, E.G. (1994) Alpha-particle-induced chromosomal instability in human bone marrow cells, *Lancet,* **344**, 987–8.

KADHIM, M.A., LORIMORE, S.A., TOWNSEND, K.M.S., GOODHEAD, D.T., BUCKLE, V.J. & WRIGHT, E.G. (1995) Radiation-induced genomic instability: delayed cytogenetic aberrations and apoptosis in primary human bone marrow cells. *Int. J. Radiation Biology,* **67**, 287–93.

KELLIHER, M.A., MCLAUGHLIN, J., WITTE, O.N. & ROSENBERG, N. (1990) Induction of a chronic myelogenous leukemia-like syndrome in mice with v-ABL and BCR/ ABL, *Proceedings of the National Academy of Sciences, USA,* **87**, 6649–53.

KELMAN, Z., PROKOCIMER, M., PELLER, S. *et al.* (1989) Rearrangements in the p53 gene in Philadelphia chromosome positive chronic myelogenous leukemia, *Blood,* **74**, 2318–24.

KLOETZER, W., KURZROCK, R., SMITH, L. *et al.* (1985) The human cellular ABL gene product in the chronic myelogenous leukemia cell line K562 has an associated tyrosine protein kinase activity. *Virology,* **140,** 230–8.

KUROSAWA, M., OKABE, M., KUMIEDA, Y. & MIYAZAKI, T. (1994) p53 gene mutation in the chronic phase was not detected in the myeloid crisis of a chronic myelocytic leukemia case, *Blood,* **83**, 2750.

KURZROCK, R., KANTARJIAN, H.M., SHTALRID, M., GUTTERMAN, J.U. & TALPAZ, M. (1990) Philadelphia chromosome-negative chronic myelogenous leukaemia without breakpoint cluster region rearrangements: a chronic myeloid leukaemia with a distinct clinical course, *Blood,* **75**, 445–52.

KYLE, R.A. & JUST, J.A. (1989) Monoclonal gammopathies of undetermined significance, *Seminars in Hematology,* **26**, 176.

LAJTHA, L.G. (1979) Stem cell concepts, *Differentiation,* **14,** 23–34.

LAVAU, C. & DEJEAN, A. (1994) The t(15;17) translocation in actute promyelocytic leukaemia, *Leukemia* (suppl. 2), S9-S15.

LEMIASTRE, A., LEE, M.S., TALPAZ, M. *et al.* (1989) Ras oncogene mutations are rare late stage events in chronic myelogenous leukemia, *Blood,* **73**, 889–91.

LISKER, R., CASAS, L., MUTCHINIK, O., PEREZ-CHAVEZ, F. & LAMBARDINI, J. (1980) Late appearing Philadelphia chromosome in two patients with chronic myelogenous leukemia, *Blood,* **56**, 812–14.

LITTLE, J.B., GORGOJO, L. & VETROS, H. (1990) Delayed appearance of lethal and specific gene mutations in irradiated mammalian cells, *Int. J. Radiation Oncology, Biology and Physics,* **19,** 1425–9.

LORD, B.I. (1995) MIP-1 *:* biological and clinical perspectives. *Forum: Trends in Experimental and Clinical Medicine* (in press).

LORIMORE, S.A., GOODHEAD, D.T. & WRIGHT, E.G. (1993) Inactivation of haemopoietic stem cells by slow alpha-particles, *Int. J. Radiation Biology,* **63,** 655–60.

LOTEM, J. & SACHS, L. (1981) *In vivo* inhibition of the development of myeloid leukaemia by injection of macrophage- and granulocyte-inducing protein, *Int. J. Cancer,* **278**, 375– 86.

LOWENBERG, B. and TOUW, I.P. (1992) Haemopoietic growth factors in acute myeloblastic and lymphoblastic leukaemia, *Bailliere's Clinical Haematology,* **5**, 599–618.

LUZZATO, L. & FORONI, L. (1986) DNA rearrangements of cell lineage specific genes in lymphoproliferative disorders, *Progress in Hematology,* **14**, 303–32.

MACIEJEWSKI, J.P., LIU, J.M., GREEN, S.W. *et al.* (1992) Expression of the stem cell inhibitor (SCI) gene in bone marrow and peripheral blood from normal individuals and patients with bone marrow failure, *Experimental Hematology,* **20**, 1112–17.

MAJOR, I. (1979) Induction of myeloid leukaemia by whole-body single exposure of CBA male mice to X-rays, *Br. J. Cancer,* **47**, 285–91.

MAJOR, I.R & MOLE, R.H. (1978) Myeloid leukaemia in X-ray irradiated CBA mice. *Nature,* **272**, 455–6.

MALONEY, W.C. (1987) Radiogenic leukemia revisited, *Blood,* **70**, 905–8.

MARDER, B.A. & MORGAN, W.F. (1993) Delayed chromosomal instability induced by DNA damage, *Molecular and Cellular Biology,* **13,** 6667–77.

MARTINS, M.B., SABATIER, L., RICOUL, M., PINTON, A. & DUTRILLAUX, B. (1993) Specific chromosome instability induced by heavy ions: a step towards transformation of human fibroblasts, *Mutation Research,* **285**, 229–37.

MATSUO, T., TOMONAGA, M., BENNETT, J.M. *et al.* (1988) Reclassification of leukaemia among A-bomb survivors in Nagaski using French-American-British (FAB) classification for acute leukemia, *Japan. J. Clinical Oncology,* **18,** 91–9.

MCLAUGHLIN, J., CHIANESE, E. & WITTE, O.N. (1987) Transformation of immature haemopoietic cells by the p210 BCR/ABL oncogene product of the Phildelphia chromosome, *Proceedings of the National Academy of Sciences, USA,* **84,** 6558–62.

MERTENS, F., JOHANSSON, B. & MITELMAN, F. (1993) Age- and gender-related heterogeneity of cancer chromosome aberrations, *Cancer Genetics and Cytogenetics,* **70**, 6–11.

METCALF, D. (1982) Sources and biology of regulatory factors active on mouse myeloid leukaemia cells, *J. Cellular Physiology,* (suppl. 1), 175–83.

(1984) *The Hemopoietic Colony Stimulating Factors.* Amsterdam: Elsevier.

(1994) Hemopoietic regulators and leukemia devlopment: a personal retrospective, *Advances in Cancer Research,* **63,** 41–91.

MITELMAN, F. (1991) *Catalog of Chromosome Aberrations in Cancer* (4th edn). New York: Wiley/Liss.

PAWSON, T. & HUNTER, T. (eds) (1994) *Oncogenes and Cell Proliferation,* Current Opinion in Genetics and Development, vol. 4.

PEDERSEN-BJERGAARD, J. (1992) Radiotherapy- and chemotherapy-induced myelodysplasia and acute myeloid leukaemia: a review, *Leukaemia Research,* **16,** 61–5.

PEDERSEN-BJERGAARD, J. & ROWLEY, J.D. (1994) The balanced and unbalanced chromosome aberrations of acute myeloid leukemia may develop in different ways and may contribute differently to malignant transformation, *Blood,* **83**, 2780–6.

PREISLER, H.D. & RAZA, A. (1987) Protooncogene expression and the clinical characteristics of acute nonlymphocytic leukemia, *Seminars in Oncology,* **14**, 207–16.

PRIESLER, H.D., RAZA, A., LARSON, R. *et al.* (1989) Protooncogene expression and the clinical characteristics of acute nonlymphocytic leukemia: a Leukemia Intergroup pilot study, *Blood,* **73**, 255–62.

PRESTON-MARTIN, S., THOMAS, D.C., YU, M.C. & HENDERSON, B.E. (1989) Diagnostic radiography as a risk factor for chronic myeloid leukaemia and monocytic leukaemia, *Br. J. Cancer,* **59**, 639–44.

PROKOCIMER, M. & ROTTER, V (1994) Structure and function of p53 in normal cells and their aberrations in cancer cells: projections on the haemopoietic cells lineages, *Blood,* **84**, 2391–411.

PUCK, T.T. & MARCUS, P.I. (1956) Action of X-rays on mammalian cells, *J. Experimental Medicine,* **103**, 653–66.

RABBITS, T.H. (1991) Translocations, master genes and differences between the origins of acute and chronic leukemias, *Cell,* **67**, 641–4. (1994) Chromosomal translocations in human cancer, *Nature,* **372**, 143–9.

RACK, K.A., CHELLY, J., GIBBONS, R.J. *et al.* (1994) Absence of the XIST gene from late-replicating isodicentric X chromosomes in leukaemia, *Human Molecular Genetics,* **3**, 1053–9.

RASKIND, W.H. & FIALKOW, P.J. (1987) The use of cell markers in the study of human hematopoietic neoplasia, *Advances in Cancer Research,* **49**, 127–67.

RITHIDECH, K.N., BOND, V.P., CRONKITE, E.P. & THOMPSON, M.H. (1993) A specific chromosomal deletion in murine leukaemic cells induced by radiation with different qualities, *Experimental Hematology,* **21,** 427–31.

ROTOLI, B. & LUZZATTO, L. (1989) Paroxysmal nocturnal hemoglobinuria, *Seminars in Hematology,* **26**, 201–47.

ROWLEY, J.D. (1980) Ph -positive leukemia including chronic myelogenous leukemia, *Clinics in Haematology,* **9**, 55–86.

ROWLEY, J.D. & TESTA, J.R. (1982) Chromosome abnormalities in malignant haematological diseases, *Advances in Cancer Research,* **36**, 103–48.

ROWLEY, U.J., GOLOMB, H.M. & DOUGHERTY, C. (1977) 15/17 translocation, a consistent chromosomal change in acute promyelocytic leukaemia, *Lancet,* i; 549–50.

SABATIER, L., DUTRILLAUX, B. & MARTIN, M.B. (1992) Chromosomal instability, *Nature,* **357**, 548.

SACHS, L. (1978) Control of normal cell differentiation and the phenotype reversion of malignancy in myeloid leukaemia, *Nature,* **274**, 535–9.

(1982) Normal development programmes in myeloid leukaemia: regulatory proteins in the control of growth and differentiation, *Cancer Surveys,* **1**, 321–42.

(1990) The control of growth and differentiation in normal and leukemic blood cells, *Cancer Research,* **65,** 2196–206.

SZCZYLIK, C., SKORSKI, T., NICOLAIDES, N.C. *et al.* (1991) Selective inhibition of leukemia cell proliferation by BCR-ABL antisense oligodeoxynucletides, *Science,* **253**, 562–5.

SCHERLE, P.A., DORSHKIND, K. & WITTE, O.N. (1990) Clonal lymphoid progenitor cell lines expressing the BCR/ABL oncogene retain full differentiative function, *Proceedings of the National Academy of Sciences, USA,* **87**, 8908–12.

SEYMOUR, C.B., MOTHERSILL, C. & ALPER, T. (1986) High yields of lethal mutations in somatic mammalian cells that survive ionizing radiation, *Int. J. Radiation Biology,* **50**, 167–79.

SHEN, W.P.V., ALDRICH, T.H., VENTA-PEREZ, G., FRANZA, B.R. & FURTH, M. E. (1987) Expression of normal and mutant *ras* protein in human acute leukaemia, *Oncogene,* **1**, 157–65.

SILLY, H., CHASE, A., MILLS, K.I. *et al.* (1995) No evidence for microsatellite instability or consistent loss of heterozygosity at selected loci in chronic myeloid leukaemia blast crisis, *Leukemia* (in press).

SILVER, A. & COX, R. (1993) Telomere-like DNA polymorphisms associated with genetic predisposition to acute myeloid of leukemia in irradiated CBA mice, *Proceedings of the National Academy of Sciences, USA,* **82**, 1407–10.

SILVER, L.M., MARTIN, G.R. & STRICKLAND, S. (eds) (1983) *Teratocarcinoma Stem Cells.* Cold Spring Harbor Laboratory.

SILVER, A.R.J., BOULTWOOD, J., BRECKON, G. *et al.* (1987) Interleukin-1 gene deregulation associated with chromosomal rearrangement. A candidate initiating event for murine radiation myeloid leukaemogenesis? *Molecular Carcinogenesis,* **2**, 226–33.

SKORSKI, Y., SZCZYLIK, C., MALAGUARNERA, L. & CALABRETTA, B. (1993) Gene targeted specific inhibition of chronic myeloid leukemia cell growth by BCR-ABL antisense oligodeoxynucleotides, *Folia Histocheica et Cytobiologica,* **29**, 85–9.

SOBOL, R.E., MICK, R., ROYSTON, I. *et al.* (1987) Clinical importance of myeloid antigen expression in adult acute lymphoblastic leukemia, *New Engl J. Medicine,* **316**, 1111–17.

TANG, J-L., YEH, S-H., CHEN, P-J., LIN, M-T., TIEN, H-F. & CHEN, Y-C. (1992) Inactivation of the retinoblastoma gene in acute myelogenous leukemia. *Br. J. Haematology,* **82**, 502–7.

THICK, J., MAK, Y-F., METCALFE, J. & TAYLOR, A.M.R. (1994) A gene on chromosome Xq28 associated with T-cell prolymphocytic leukemia in two patients with Ataxia Telangiectasia, *Leukemia,* **8**, 564–73.

THOMPSON, L.H. & SUIT, H.D. (1969) Proliferation kinetics of X-irradiated mouse L cells studies with time lapse photography. II, *Int. J. Radiation Biology,* **15**, 347–62.

TOKSOZ, D., FARR, C.J. & MARSHALL, C.J. (1987) *Ras* gene activation in a minor proportion of the blast population in acute myeloid leukaemia, *Oncogene,* **1**, 409–13.

TOUW, I., GROOT-LOONEN, J., BROEDERS, L. *et al.* (1989) Recombinant haemotopoietic growth factors fail to induce a proliferative response in precursor B acute lymphoblastic leukemia, *Blood,* **66**, 556–61.

TOUW, I., POUWELS, K., VAN AGTHOVEN, A.J. *et al.* (1990a) Interleukin 7 is a growth factor of precursor B and T acute lymphoblastic leukaemia, *Blood,* **75**, 2097–101.

(1990b) Role of interleukin-7 in the growth of acute lymphoblastic leukemia, *Bone Marrow Transplantation,* **6**, (suppl. 1), 36–8.

TOUW, L, DONATH, K., POUWELS, *et al.* (1991) Acute myeloid leukemias with chromosomal abnormalities involving the 21q22 region identified by their *in vitro* responsiveness to interleukin 5, *Leukemia,* **5**, 687–92.

TRAKHTENBROT, L., KRAUTHGAMER, R., REZNITZKY, P. & HARAN-GHERA, N. (1988) Deletion of chromosome 2 is an early event in the development of radiation-induced myeloid leukemia, *Leukemia,* **2**, 545–50.

TROTT, K.R. & HUG, O. (1970) Intraclonal recovery of division probability in pedigrees of single X-irradiated mammalian cells, *Int. J. Radiation Biology,* **17**, 483–6.

UNSCEAR (1993) *Sources and Effects of Ionizing Radiations.* Report to the General Assembly, with annexes. New York: United Nations, pp. 551–618.

UPTON, A.C., WOLFF, F.F., FURTH, J. & KIMBAL, A.W. (1958) A comparison of the induction of myeloid and lymphoid leukaemia in X-irradiated RF mice, *Cancer Research,* **18,** 842–8.

VENKATRAJ, V.S., GAIDANO, G. & AUERBACH, A. (1994) Clonality studies and N-ras and p53 mutation analysis of hemopoietic cells in Fanconi Anemia, *Leukemia,* **8**, 1354–8.

VOGELSTEIN, B., FEARON, E.R., HAMILTON, S.R. & FEINBERG, A.P. (1985) Use of restriction fragment length polymorphisms to determine the clonal origin of human tumors, *Science,* **277**, 642–4.

WADA, C., SHIONOYA, S., FUJINO, Y. *et al.* (1994) Genomic instability of microsatellite repeats and its association with the evolution of chronic myelogenous leukemia, *Blood,* **83,** 3449–56.

WALDMANN, T.A., DAVIS, M.M., BONGIOVANNI, K.F. & KORSMEYER, S.J. (1985) Rearrangements of genes for the antigen receptor on T cells as markers of lineage and clonality in human lymphoid neoplasms, *New Engl J. Medicine,* **313,** 776–83.

WESTBROOK, C.A. & KEINANEN, M.J. (1992) Myeloid malignancies and chromosome 5 deletions, *Bailliere's Clinical Haematology,* **5**, 931–42.

WETZLER, M., KURZROCK, R., ESTROV, Z. *et al.* (1990) Constitutive expression of interleukin-1 beta correlated with interferon-alpha resistance in chronic myelogenous leukaemia, *Proceedings of the Annual Meeting of the American Association for Cancer Research,* **31,** 179a (abstr.).

WETZLER, M., KURZROCK, R., LOWE, D.G., KANTARJIAN, H., GUTTERMAN, J.U. & TALPAZ, M. (1991) Alteration in bone marrow adherent layer growth factor expression: a novel mechanism of chronic myelogenous leukemia progression, *Blood,* **78**, 2400– 6.

WRIGHT, E.G. & LORIMORE, S.A. (1990) Radiation-induced murine leukaemia: a model for acute myeloid leukaemia. In L.Sachs, N.G.Abraham, A.S.Levine, C.J.Wiedemann & G.Konwalinka (eds), *Molecular Biology of Hematopoiesis.* Andover: Intercept Ltd, pp. 607–18.

WRIGHT, E.G. & PRAGNELL, I.B. (1992) Stem cell proliferation inhibitors, *Clinics in Haematology,* **5**, 723–39.

YAMAMURA, Y., HATTORI, T., OBARU, K. *et al.* (1989) Synthesis of a novel cytokine and its gene (LD78) expression in hemopoietic fresh tumor cells and cell lines, *J. Clinical Investigation,* **84,** 1707–12.

YOUNG, J.C. & WITTE, O.N. (1988) Selective transformation of primitive cells by the *bcr/abl* oncogene expressed in long-term lymphoid or myeloid cultures, *Molecular and Cellular Biology,* **8**, 4079–87.

YUNIS, J.J., BOOT, A.J.M., MAYER, M.G. & BOS, J.L. (1989) Mechanisms of *ras* mutation in myelodysplastic syndrome, *Oncogene,* **4**, 609–14.

9
Genetic Effects of Ionising Radiation with respect to Leukaemia

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9
Genetic Effects of Ionising Radiation with respect to Leukaemia

9.1
Introduction

The haemopoietic cells of the bone marrow are among the most sensitive cells in the body to the damaging effects of ionising radiation (IR) (Young and Alter, 1994). The principal target is the genetic material in the nucleus of each cell (UNSCEAR, 1993). Although the initial effects of IR-related bone marrow damage includes cell toxicity, the most important long-term outcomes are myelodysplasia and leukaemia (Young and Alter, 1994). Leukaemia is the generic description of a group of malignant diseases of the blood-forming cells. Differences in cellular phenotype (Foon and Todd, 1986; van Dongen *et al.,* 1987), chromosomal rearrangements (Pui *et al.,* 1990; Rabbitts, 1994) and descriptive epidemiology of these diseases (Linet, 1985; Cartwright *et al.,* 1990) suggest that they may be aetiologically distinct.

There is general agreement that leukaemia can be caused by exposure to ionising radiation (Miller, 1964; Modan and Lubin, 1974; Kohn and Fry, 1984), but the risk posed by levels of IR close to background has been a matter of some dispute, estimated frequencies requiring extrapolation from incidence rates at high doses (UNSCEAR, 1993; Cardis *et al.,* 1994). The problem of assessing low dose radiation effects and the attempts of various risk methods to resolve it have been much debated by the various radiation protection agencies (BEIR V, 1990; UNSCEAR, 1993; NRPB, 1993). One recent estimate suggests a relative risk of 2. 2 Sv^{-1} for all except chronic lymphocytic leukaemia (Cardis *et al.,* 1994). Since lifetime cumulative exposures in the general population are considerably lower than this, there is some reason for the belief that IR is probably not the most important cause of leukaemia. Other agents, including chemical carcinogens such as benzene, and viruses, of which Epstein-Barr virus and human T-leukaemia virus may not be the only examples, have also been implicated. Nonetheless, IR continues to occupy a central position in studies and speculation about the aetiology of leukaemia, as it does in genetic diseases (Schull *et al.,* 1980). It is the potential of heredity, as well as other co-factors, to determine differences in risk among individuals that is the subject of this chapter.

The availability of relatively accurate measures of radiation exposure, at least in comparison with the other suspected leukaemogens such as infections and carcinogenic chemicals, has been an important factor in identifying and monitoring leukaemia rates in populations at risk. Not everyone who is exposed to the same radiation dose seems to develop leukaemia, and this requires an explanation. The simplest is that leukaemia is the result of chance interaction between radiation source and a target cell. Whilst this has attractions in terms of linear dose-response relationships, an equally plausible possibility is that host factors play a significant perhaps even rate-limiting role (Taylor and Birch, 1995).

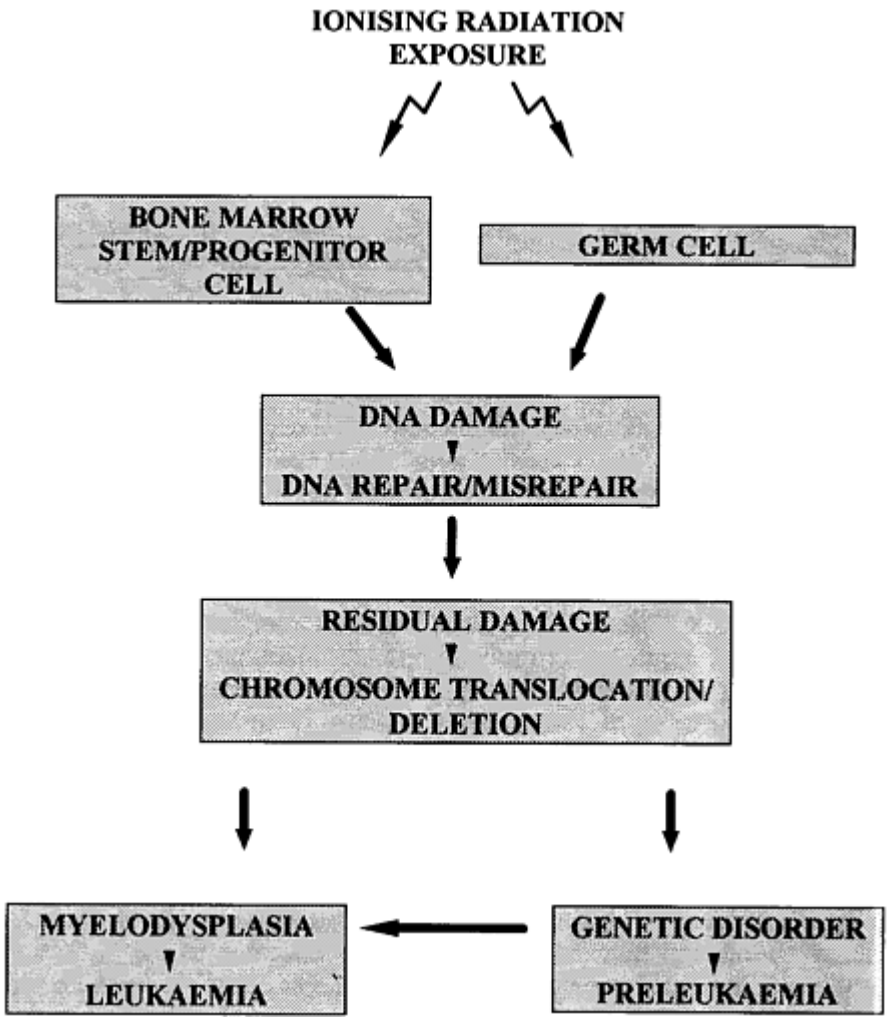

Figure 9.1 Schematic representation of pathways involved in radiation damage to bone marrow, leading to leukaemia

The target of IR-induced damage, and the determinants of cellular recovery both reside in the genetic material of the radiation damaged cell. Some of the pathways involved in this process are shown in Figure 9.1. However, the notion that the physical and chemical structure of DNA alone is the target of IR damage, (albeit with perhaps small variations from one person to another) and is sufficient to determine the risk of developing leukaemia, seems altogether too simplistic. A more likely possibility is that hereditary variation determines how an individual's bone marrow will respond to IR, both through the direct action of DNA damage repair genes, and through genes controlling accessory functions. None of this should be surprising since leukaemia, like other cancers, seems to require mutations in several genes, and these are unlikely to occur simultaneously following exposure to IR. We shall examine here how heredity might influence this process.

9.2
Cells, Chromosomes and Genes

The bone-marrow is responsible for providing most of the cellular constituents of the peripheral blood. The important cells in this process are multipotent, self-renewing haemopoietic stem cells, and their progeny, the lineage-committed progenitor cells which mature into functioning, terminally differentiated blood cells such as lymphocytes, neutrophils, erythrocytes and platelets. The genes which control cell division, differentiation and specialised functions reside within the nucleus. They are carried by the twenty-two pairs of autosomal chromosomes and the single pair of sex chromosomes found in each cell (Gelehrter and Collins, 1990). The chromosomes are the visible evidence of genetic material carried by deoxyribonucleic acid (DNA). The DNA in the stem cells and their progenitors provides the substrate which is damaged by ionising radiation, leading ultimately to leukaemia (Young and Alter, 1994).

Genetic information controlling cellular differentiation and function is carried in coded form as a series of packaged molecular instructions called genes (Vogel and Motulsky, 1986; Gelehrter and Collins, 1990; Klug and Cummings, 1994). These determine the structure of the proteins which actually carry out various cellular functions. The genes are compartmentalised into stretches of protein-coding DNA called exons, separated by non-coding sequences, or introns. The genetic code itself consists of a four-nucleotide base alphabet whose 'letters' are made up of adenine, thymine, guanine and cytosine. The words of the genetic code consist of three letters or codons which either specify an amino acid, transcriptional start and stop signals, or regulatory signals. Since there are sixty-one potential codons but only twenty amino acids, there is redundancy in the genetic code. Genes are distributed along the chromosomes which are the units of replication either by mitosis in somatic cells, or by meiosis in the germ cells (Klug and Cummings, 1994).

9.3
Mechanisms of Leukaemogenesis

Leukaemias are usually characterised by an increase in the frequency of a single haemopoietic cell type which progressively occludes the bone marrow and spills over into the peripheral blood. The clinical ramifications of leukaemia are associated with loss of haemopoietic function and the physical effect of too many cells in the blood circulation. The genetically defective nature of leukaemia cells is easily recognised by the visible alteration of chromosome number and integrity, of which non-random rearrangements are the most important (Rowley, 1982; Williams *et al.,*

Table 9.1 Representative clonal chromosomal translocations in leukaemia

Leukaemia	Chromosome	Gene 1	Gene 2
B-ALL	t(8; 14)(q24; q32)	c-MYC	IgH
T-ALL	t(l; 14)(p32; q11)	TAL1	TCR-
T-ALL	t(7; 9)(q35; q34)	TCR-	TAL2
CML	t(9; 22)(q34; q11)	c-ABL	BCR
pre-B ALL	t(1; 19)(q23; p13)	PBX1	E2A
APL	t(15; 17)(q21; q11)	PML	RARA
pre-B ALL	t(4; 11)(q21; q23)	AF4	MLL
pre-B ALL	t(9; 11)(p22; q23)	AF9	MLL
AML	t(8; 21)(q22; q22)	AML1	ETO

Leukaemia	Chromosome	Gene 1	Gene 2

Source: Adapted from Rabbitts, 1994.

1984; Mitelman, 1991). Typically these include translocations, deletions and insertions (Table 9.1). The translocations in leukaemias usually involve exchanges between non-homologous autosomes and are the most specific manifestation of the non-random genetic change and clonality of leukaemia (Raimondi, 1993; Rabbitts, 1994). Examination of the bone marrow from a patient with leukaemia often reveals two cell populations, one carrying a non-random translocation and other alterations, and the other, a normal karyotype. This is specific evidence that the bone marrow of a leukaemia patient is a chimera of malignant and normal cells.

The chromosome translocations which characterise leukaemias are productive in the sense that they drive the phenotypic changes associated with the disease. Specifically, they either activate proto-oncogenes or generate new genes, referred to as fusion genes. Translocation (t) of chromosome 9q (p and q are the short and long arms respectively of chromosomes) and 22q, gives rise to the 'Philadelphia chromosome', typical of chronic myeloid leukaemia (CML). Pre-B common acute lymphobastic leukaemia involves t(1; 19), null-ALL t(4; 11), the M2 form of AML t(8; 21) and M3 AML t(15; 17), among many others. It is worth noting that the protein product of a fusion gene is a leukaemia-specific molecule, with no counterpart in normal haemopoietic cells.

Translocations are not the only non-random chromosomal changes encountered in leukaemias. Deletions of segments or whole chromosomes also occur, including chromosome 5q (Willman *et al.,* 1993), 9p (Ogawa *et al.,* 1994), and duplications such as trisomy 21 (Watson *et al.,* 1993). These changes suggest that the development of leukaemia is a genetically complex, multistep evolutionary process at the clonal level. The genetic events that precede chromosomal alteration are not understood with any certainty. It is likely that DNA damage is involved and that translocation is the outcome of the failure or inappropriate functioning of repair mechanisms. Although there is no convincing evidence that leukaemic translocations can be transmitted in the germ line, constitutional chromosomal abnormality carries an increased risk of leukaemia, possibly by increasing the level of background genetic instability. The ability of IR to cause chromosomal damage to the germ cells could be regarded as increasing the risk of leukaemia in the offspring of the genetically damaged parent, though this phenomenon is probably rare. Most concepti with gross chromosomal damage either do not implant, or are spontaneously aborted.

9.3.1 Initiation, Promotion and Progression

The multiple steps which lead to leukaemia can be described in terms often used in relation to cancer, namely initiation, promotion and progression (Figure 9.2). Initiation is the key event in leukaemia, since it is the first genetic event, and starts a process which eventually leads to malignant transformation. The genetic nature of initiation is suggested by the fact that it is probably irreversible, but the precise identity of initiating genes in various leukaemias is not clear.

An initiated bone marrow cell must possess some form of growth advantage over its unaffected neighbours if it is to survive and develop into leukaemia. This may involve genetic events, leading to delayed apoptosis or increased proliferative capacity. Any external stimulus which increases this response is referred to as a promoter and, unlike initiation, promotion can be reversed by removal of the stimulus. Promoters themselves have low oncogenic potential but can enhance the yield of neoplasms among already

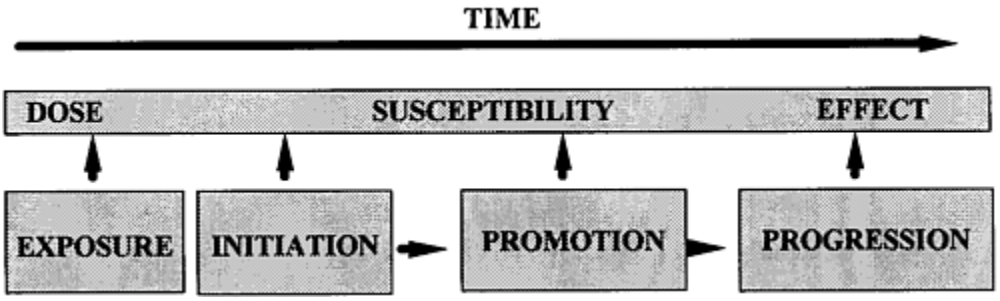

Figure 9.2 Schematic representation of the multiple steps leading to leukaemia and the relationship of the steps to susceptibility

initiated cells. The enhanced survivability of initiated cells is consolidated by progression, which consists in secondary genetic changes which modify proliferation and cell death.

9.3.2
Tumour Suppressor Genes

Cytogenetic and molecular evidence suggests that the key genetic events in leukaemogenesis are mediated by non-random chromosomal translocations. However, the loss of genetic material may also be a crucial event. Evidence of the mutational inactivation of genes was originally obtained from studies of the tumorigenicity of somatic cell hybrids made by fusing normal and tumour cells (Stanbridge, 1990). Loss of specific chromosomal material by non-tumorigenic hybrids rendered them tumorigenic, suggesting that normal genes exist which suppress malignancy. Cytogenetic and molecular studies have now shown that non-random loss of genetic material occurs in many types of solid tumour and, as already mentioned, in leukaemias. These genes are referred to generically as tumour suppressor (TS) genes. TS genes often appear to be growth regulatory genes whose loss of function leads to the release of cells from normal growth controls. Loss of TS gene function is usually, but not always, recessive at the cellular level and requires the deletion of both copies of the gene. Studies of several inherited, mainly childhood, cancers have established that apparent dominantly-inherited cancers such as retinoblastoma are associated with germ-line hemizygous mutations in TS (Cowell and Hogg, 1992) and a second 'hit' or mutation in the relevant target cells resulting in the homozygous loss of TS gene function (Figure 9.3). The identity of TS genes which are important in leukaemia is less certain than in solid tumours and their role as initiators of leukaemogenesis is still unclear. However, if the loss of TS function can cause leukaemia, the ability of ionising radiation to induce gene deletions could be an important mechanism in the causation of leukaemia.

9.3.3
Mutations

Estimates of the number of functional genes in the human genome vary, but there seem to be between 60 and 100×10^5. The genes are distributed among the twenty-three pairs of chromosomes in each cell and, if the supercoiled DNA contained by these chromosomes were stretched out, each cell would measure about 2 m. The total number of cells in the human body is about 10^{14}, so the total length of DNA is about 2×10^{11} km. Allowing that a proportion of the cells are haploid germ cells, the physical length of DNA in each person is still remarkable. Changes in DNA sequence underlie evolution, so it is not altogether surprising that it is an

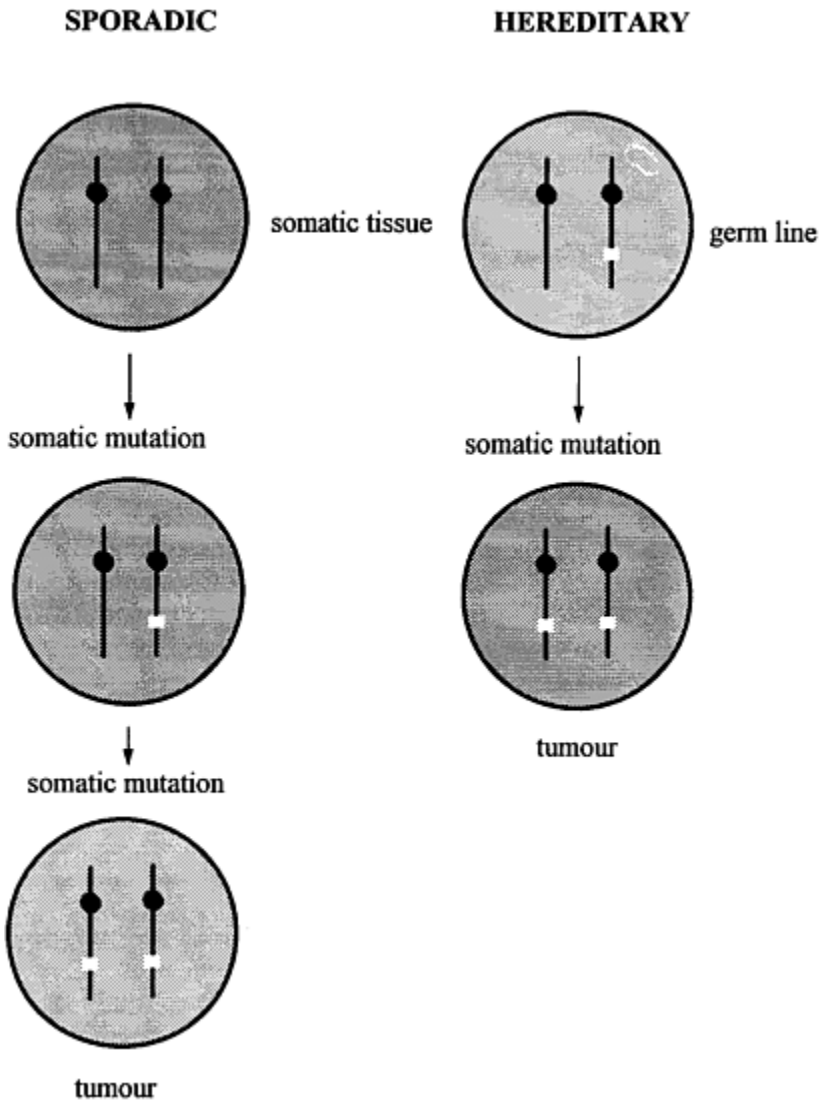

Figure 9.3 Loss of tumour suppressor gene function in sporadic and hereditary tumours, involving the Knudson 'two-hit' hypothesis. TS mutation is denoted by a gap in a chromosome. Note that a TS mutation is transmitted in the germ line in hereditary cancer. (Adapted from Ponder, 1992)

unstable molecule. The tendency of DNA to degrade means that efficient enzymatic repair mechanisms are required to excise, correct and repair errors (Lindahl, 1993). Hereditary defects in repair enzymes reveal how genes are disrupted by the failure to maintain integrity. A number of these disorders, including Fanconi anaemia, ataxia telangiectasia and Bloom syndrome carry a high risk of leukaemia which is enhanced by hypersensitivity to external agents (Cohen and Levy, 1989). The precise meaning of the term 'spontaneous' in relation to DNA degradation is a misnomer since it reflects our ignorance about the cause. It is

conceivable that the stability of DNA is affected by background radiation and naturally occurring genotoxins, and that recovery from this damage is under hereditary control.

Acquired and inherited alterations in DNA sequence are called mutations (Cooper and Krawczak, 1993). They may modify the structure or function of a protein, or its regulation. Mutations are normally deleterious, leading to a loss of homeostatic control over specific cellular functions, but often with knock-on effects. Gene mutation is probably the single most important initiating event in cancer and leukaemia. Mutations in germ cells also give rise to inherited disorders if the mutated sequence is transmitted to offspring. An important consequence of mutation is that it is to all intents and purposes irreversible and its rate is increased by exposure to DNA-damaging agents such as IR. However, the mutagenic consequences of IR in terms of the risk of leukaemia does not require its continued presence.

The number of cells in the bone marrow of a 15 kg child approximates to about 10^{10} kg^{-1} (Lipton and Nathan, 1987), and haemopoietic cells therefore account for a significant proportion of the body's cells (about 1.5×10^{11}). Although this number of bone marrow cells represents a considerable amount of target DNA for IR damage, the actual target pool comprising stem cells and progenitors is only a small fraction of this. The effects of IR on normal haemopoietic and peripheral blood cells have been known for many years from the disruption of normal chromosome structure. Cytogenetic analysis continues to be an important measure of radiation damage and can mimic the type of chromosomal alteration seen in leukaemia.

The risk of incurring a radiation-induced initiating mutation may depend on the target that a key gene presents. Genes clearly differ in size, in sequence and perhaps in mutability. Some genes have mutation hotspots, while others seem to be mutated only rarely. In addition, the effect that a mutation has on a cellular phenotype depends where it occurs, either in an intron, exon, a promoter or at an intron-exon boundary. Other factors may be involved. Differences in chromosomal radiosensitivity may be determined by homogeneously staining chromosomal regions called fragile sites. These areas of instability, which are revealed in cells cultured under stress, may increase the risk of leukaemia, though this has yet to be confirmed. Destabilisation of dinucleotide $(CA)_n$ repeat sequences has also been detected in several tumour types, suggesting that generalised defects in genomic stability could also increase the risk of leukaemia.

DNA damage incurred by bone marrow after IR exposure is a potential leukaemia hazard only in the person carrying that damage. It does not affect the next generation unless it occurs in the germ cells. Although it has been estimated that about 5–10 per cent of all newborns carry new germ-line mutations, most occur in non-coding sequences, or are recessive and have little phenotypic effect. The question of preleukaemic germ cell mutation is considered below.

9.4
Radiation-induced Leukaemia

The causative link between ionising radiation and leukaemia has been known for a long time (see Chapter 11). One of the first pieces of evidence came from the relatively unrestricted medical use of X-rays earlier this century. By 1942, twenty cases of leukaemia had been reported among radiologists (Henshaw and Hawkins, 1944), representing a 1.7-fold increase in frequency among physicians. Since radiologists were more likely to develop leukaemia than other physicians, this estimate was revised to a 9 times greater risk (March, 1944). Looked at another way, 4.7 per cent of radiologists were likely to develop leukaemia compared with 0.5 per cent of other physicians, though a number of confounding factors led to a revised estimate of a 3-fold excess risk (Gilliam, quoted by Miller, 1964).

Following these early indications of the leukaemogeneic effects of radiation, there have been a number of studies of the hazards presented by the medical application of radiation in specific treatments, including

ankylosing spondylitis, thymoma, gynaecological disorders, tinea capitis, polycythaemia vera and general diagnostic X-rays (reviewed by Modan and Lubin, 1974; and see Chapters 3 and 11). Of 14538 patients treated for ankylosing spondylitis with a mean bone marrow dose of 3.7 Gy the relative risk of leukaemia was 9.5 (Court Brown and Doll, 1965). The first cases appeared within two years of radiotherapy and the incidence reached a peak at five years. The risk of myeloid leukaemia was higher than for lymphoid, and no cases of chronic lymphoid leukaemia were observed. In the USA it was estimated that over 200 million radiological examinations are carried out each year, with an average dose to the bone marrow of each person of 8×10^{-4} Gy (Kohn and Fry, 1984). It has also been estimated that perhaps 1 per cent of leukaemia in the general population might be due to diagnostic radiography (Gunz and Atkinson, 1964), though others have suggested that this figure may be appreciably higher (Stewart *et al.,* 1962). The BEIR V committee (BEIR V, 1990) established that the latent period from radiation exposure to leukaemia depends on the age at exposure and also on the haematological type of leukaemia. Although an influence of host genetic susceptibility has yet to be formally demonstrated, it seems a reasonable possibility.

The question of increased sensitivity to IR in relation to age was raised by the Oxford Survey of Childhood Cancers in the 1950s which established that prenatal exposure of the foetus to diagnostic X-rays increased the risk of leukaemia (Stewart *et al.,* 1958). Mortality from leukaemia was 40 per cent higher when mothers had been X-rayed during pregnancy, with a relative risk of 2 between 5 and 7 years. Following this work, several other studies of the effects of prenatal radiation were carried out, some of which contradicted, while others confirmed the Oxford results. One reason for this was that sample sizes in some studies were too small to have sufficient statistical power. However, it is difficult to avoid the conclusion, on the basis of the low doses and modest relative risks involved, that there is increased, but selective susceptibility to the leukaemogenic effects of X-rays in the prenatal period. That this susceptibility might be influenced by co-factors is suggested by the results of Bross and Natarajan (1972) who found a markedly increased risk of leukaemia in prenatally irradiated children in relation to postnatal immunological deficits and infectious diseases (Figure 9.4). There was no detectable effect of radiation in the absence of postnatal infection and the relative risk of leukaemia with infection alone was 1.7–3.7. In contrast, the combined risk of IR and infection was 2.8–24.6, depending on the type of infection. This interaction between IR and infection could be interpreted as indicating that radiation itself is not the primary cause of leukaemia, but suppresses immune responses to some leukaemogenic infectious agent. Another conclusion is that the susceptibility of the foetus *in utero* to IR may be quite high, since the radiation doses used in X-radiography of the foetus were quite small.

The most detailed and long-term examination of the leukaemogenic effects of radiation has been carried out as part of the Atomic Bomb Casualty Commission's (ABCC) (now known as the Radiation Effects Research Foundation, RERF) Life Span Cohort Study among survivors of the Japanese A-bomb explosions at the end of the Second World War (UNSCEAR, 1993). The ABCC identified leukaemia as one of the earliest malignant diseases and a survey of the LSS Cohort results (BEIR V, 1990) estimated that among 2185335 person-years follow up, there were 202 deaths from leukaemia. Mortality rates were significantly increased at exposures of 0.4 Gy, and were maximal following an approximately linear dose-response increase at 3–4 Gy. Above this dose, the 'cell-sterilising' effects of IR probably contributed to the reduction in the number of preleukaemic cells. Excess mortality from leukaemia reached a peak at about ten years after the bombs, and declined thereafter at a rate of about 10.7 per cent per year. Despite the linear dose-response, the total number of leukaemias was relatively small, with a relative risk of 10 after ten years, and then declining. Of course, competing causes of mortality, especially in the early years after the bombs could have reduced the true incidence of leukaemia, but even so, the LSS cohort data suggest that there may have been differences in IR-induced leukaemia susceptibility among the exposed population.

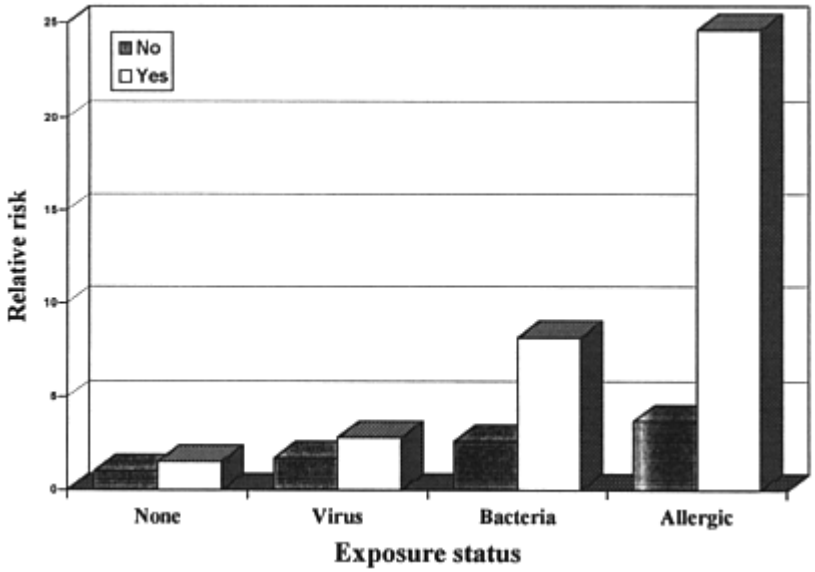

Figure 9.4 Relative risks of leukaemia in relation to intra-uterine radiation exposure and indicators of susceptibility in the 1–4 year age group. No—control; Yes—irradiated. (Data from Bross and Natarajan, 1972)

The experiences gained with these and other groups of individuals exposed to significant levels of radiation have resulted in the development of comprehensive radiation protection measures, based on accurate physical dosimetry and progressive reduction in cumulative exposures to IR. With the abolition of nuclear weapons testing as a source of environmental radiation and, barring unforseen accidents of which Windscale and Chernobyl were the main but not the only examples, levels of IR exposure in the general population are unlikely to be responsible for more than a very small fraction of spontaneous leukaemia. The difficulty of attributing the causes of spontaneous leukaemia occurring under low-level exposure will make it even more difficult to derive accurate estimates of radiogenic leukaemia in the future.

9.5
Heredity and Heritability

Before considering the role of heredity in human leukaemia it is useful to consider the meaning of heredity and heritability. It is self-evident that each individual in an outbred population, with the exception of identical twins, is different from every other due to the random assortment of genes inherited from each parent (Levitan, 1988; Gelehrter and Collins, 1990). Any disease occurring in that individual, whether or not caused by IR, therefore occurs on a unique and complex genetic background and in this sense all diseases have a hereditary contribution (Edwards, 1969). What differs between individuals and also among diseases is the extent of heritability or the degree by which the disease phenotype is determined by inherited, compared with environmental, factors (Falconer, 1990). In the case of IR-induced leukaemia, where an IR dose-outcome relationship probably applies, it could be said that heritability is more likely to contribute following exposure to low doses such as those within the normal background range. Evolution is likely to have provided the bone marrow with sufficient protection in the form of repair enzymes to cope with this type of exposure. At high levels of IR exposure, hereditary susceptibility/resistance mechanisms are likely

to be swamped by the externally-derived damage. It is worth bearing in mind that other hereditary factors may also be important in protection against IR-induced leukaemia, such as immunity to potential leukaemogenic viruses.

9.5.1
Heredity and Disease

The effect of IR on the genetic material in the germ cells and its ability to cause inherited disease has been a central theme of radiation-risk assessment for many years. This has involved estimates of the extra number of cases of different categories of inherited and chromosomal disorder under given conditions of radiation exposure (Vogel, 1992; Ehling, 1991). These measurements involve complex mathematical modelling which takes account of dose, dose rate and type of radiation (Sankaranaryanan, 1988). However, underlying all of these calculations is the need for exact measures of background rates of hereditary disease: there have been few comprehensive surveys. It has to borne in mind that rates of genetic disease vary widely in different populations and are affected by assortative mating such as inbreeding, which is quite common in certain communities. What emerges from the estimates of IR-induced genetic disease is that the hereditary effect of low to moderate radiation exposure is minimal in comparison with the population burden of hereditary disease (UNSCEAR, 1993). The extra cases caused by IR exposure in comparison with natural incidence rates is so small in some diseases that it is difficult to distinguish from natural statistical variation. Many of the data have been collected from studies of incidence rates in dominant and X-linked inherited disorders, where the phenotypic consequences of *de novo* germ-line mutation will be seen in the first generation after germ cell mutation (BEIR V, 1990). In the case of leukaemia, a few dominant and sex-linked single gene disorders are associated with an increased risk but these are quite rare. Of more concern is the contribution of these mutations to the risk of leukaemia when present in heterozygous form. These include the genes for Fanconi anaemia and ataxia-talangiectasia whose frequency of heterozygous carriers in the population may be quite high (perhaps 1/100–1/300). There is also a possibility that IR could induce new mutations in these recessive genes, and this would be undetected phenotypically.

9.5.2
Dominant, Recessive and X-linked Disorders

Before our present understanding of the role of gene mutation as the cause of inherited disease, the hereditary basis of these diseases was, and still is, assessed from their frequency and distribution among family members. It could be seen that certain diseases affected siblings alone, males but not females, or parents as well as children. Statistical analyses of disease frequency among families revealed patterns of segregation between unaffected and affected individuals that approximated to the predictions of Mendelian genetics (Levitan, 1988). Such disorders are often referred to as Mendelian or single gene disorders. Using molecular mapping and cloning techniques, the genes involved in many of these disorders, such as cystic fibrosis, Duchenne muscular dystrophy, Huntington's disease and others have been isolated and sequenced. The evidence clearly shows that they are caused by mutations in single genes.

Mendelian single gene disorders can be divided into three main groups: dominant, recessive and X-linked (Levitan, 1988; Gelehrter and Collins, 1990). In dominant disorders, the disease phenotype affects members of each generation and, therefore, shows a 'vertical' inheritance pattern. In a two-generation family with one affected parent, about half of the children will also be affected. Only the offspring of the affected children are normally considered to be themselves at risk of developing the disorder; offspring of the

unaffected children will normally not be affected. Molecular genetic analysis has confirmed that dominant mutations are able to exert their effect in heterozygous form. In the unlikely event that a person inherits two copies of a dominant mutation, the disease phenotype is usually so severe that it is lethal. Dominant mutations for the most part affect structural proteins and in several cases cause diseases with a late onset. The introduction of a new dominant mutation into a family either by IR or by spontaneous germ cell mutation will be seen as a sporadic case in a first generation offspring and could be overlooked if it is not a phenotypically well defined disorder.

Recessive diseases usually only occur among siblings, rather than parents, and exhibit a 'horizontal' pedigree. Mendelian genetics predict that both parents will carry a single copy of a mutated disease gene, that is they are heterozygous, and this has been confirmed by molecular analysis. The disease phenotype is expressed only when both copies of the gene have been inherited by an affected child. Heterozygous carriers of recessive mutations usually show no phenotypic manifestation of the disease. The only evidence that they carry a recessive mutation, that is as an obligate carrier, is seen when an affected child is produced with another mutation carrier. Mendelian predictions indicate that one in four children of parental carriers will have a recessive disorder; half will carry one copy of the gene mutation, but be unaffected, and one in four will be normal. An inherited X-linked disease arises when a gene on the X-chromosome is mutated. Because X-linked mutations are usually recessive, the disorder will affect only male offspring of female mutation carriers. Females are usually unaffected even though the mutated gene is on an active X-chromosome in approximately half of the cells of a female. The analysis of X-linked disorders is of particular interest to radiation geneticists, since the disease phenotype is manifest in first generation male offspring of female germ cell mutation carriers. Since a number of X-linked diseases are lethal, the analysis of male/female offspring ratios in irradiated populations is of particular interest in long-term outcome studies, such as the Hiroshima-Nagasaki cohort. On the other hand, several X-linked inherited disorders are associated with an increased risk of leukaemia/lymphoma.

9.5.3
Heredity and Radiation-induced Leukaemia

Ionising radiation has been a convenient means of inducing leukaemia in experimental animals, as well as being a causative agent in its own right (Yokoro *et al.,* 1986). This requirement provided much of the impetus for the development of high-leukaemia inbred mouse strains in the 1920s and 1930s which clearly showed genetic differences in leukaemia susceptibility. Despite this, progress in identifying the hereditary contribution in human leukaemia has been considerably more limited (Taylor and Birch, 1995). This is not surprising since human populations are for the most part highly outbred and exposed to much lower doses of IR than those required to induce leukaemia in animals. Determining the hereditary contribution in human leukaemia is likely to become even more difficult in the future as IR exposure levels are progressively reduced. On the other hand, the availability of molecular techniques means that IR-exposed human populations can be studied in more detail with respect to the frequency of specific susceptibility genes. Such molecular epidemiological approaches offer an important way of establishing susceptibility gene frequencies as well as somatic mutational events. It is likely that both will reveal non-uniform frequencies which are influenced by heredity as well as by radiation.

At present the nature of hereditary variation that contributes to the risk of IR-induced leukaemia is a matter of speculation. It could, for instance, include inherited differences in DNA repair capacity, especially under IR-induced stress, the capacity of bone marrow to recover from long-term IR damage, or the ability to deal with infections and other sequelae of radiation exposure. It is unlikely in the normal individual to be

determined by a single gene, but could involve natural allelic variants and heterozygous recessive mutations. Whether it is heredity that determines the time and other factors which determine the transition from bone marrow dysplasia to leukaemia has yet to be determined. The task of assessing these hereditary contributions poses great difficulties. Leukaemia is not normally amenable to classical pedigree analysis since familial cases are very rare. Since some individuals seem to succumb to IR-induced leukaemia while others, exposed to the same dose, do not, it may be possible to predict a person's risk from some measure of cellular response, such as chromosome damage, cell-cycle delay, growth arrest or micronucleus formation. Further detailed analysis will require the application of molecular techniques to identify radiation-induced somatic mutation, and hereditary differences.

9.5.4
Germ Cell Mutation and Leukaemia

The hypothesis that ionising radiation can cause mutations in the germ cells of a parent which, when passed to a child, results in leukaemia was proposed by Gardner *et al.* (1990) following studies of the Seascale leukaemia cluster. Many of the issues raised by this and associated studies are discussed in a volume of collected papers (Beral *et al.,* 1993). That there was an increased frequency of childhood leukaemia in the village of Seascale, in Cumbria, England, in the 1950–1980s is not disputed (Gardner and Winter, 1984; Cook-Mozaffari *et al.,* 1989a, b). Although clusters of leukaemia and lymphoma have been reported elsewhere (Heath and Hasterlick, 1963), they do not, unlike the Seascale cluster, seem to occur specifically around nuclear facilities (Bithell *et al.,* 1994). One of the difficulties in explaining the cause of the Seascale cluster is the significance to be attached to the proximity of the village to the nuclear installation at Sellafield (Beral, 1990). The theory that the cluster was caused by ionising radiation seems to be supported by the apparent relationship between IR dose received by the father of the affected child in the period prior to the conception (paternal preconceptional irradiation, PPI). Since the affected children did not themselves seem to have been in contact with radiation, the logical conclusion, in the absence of an alternative explanation, was that the leukaemias were caused by an effect of radiation on paternal germ cells. The early age of onset of childhood leukaemia, like other types of childhood cancer caused by inherited mutations (Li, 1993), seemed to add plausibility to the germ cell mutation theory of childhood leukaemia though with considerable reservations (Greaves, 1990). However, the germ cell mutation interpretation has been contested on several grounds (Evans, 1990a, b; Narod, 1990; Doll *et al.,* 1994):

1 Radiation workers who had received similar doses but lived outside Seascale did not show the same high frequency of children with leukaemia (Parker *et al.,* 1993).
2 The radiation dose-response relationship for the Seascale leukaemias involved only four cases and is open to statistical argument.
3 There were increased relative risks associated with other occupations not involving radiation.
4 There is no direct evidence that childhood leukaemia originates from a germ cell mutation, unless it is part of an inherited syndrome with a distinct phenotype.
5 The germ cell mutation rate calculated to be required to give rise to the Seascale cluster is far higher than in any known hereditary disease (Wakeford *et al.,* 1994).

Looked at another way, levels of paternal radiation exposure were too low to account for this number of leukaemias (Abrahamson, 1990). The failure of epidemiological studies undertaken in the vicinity of other nuclear installations (Urquhart *et al.,* 1991; McLaughlin *et al.,* 1993a, b) to corroborate the Gardner findings

led to increased scepticism about the interpretation placed on the PPI theory. Most attempts to explain the Seascale cluster now suggest that it was due either to chance, or to some other confounding cause based on local population conditions. Of these, the influx of people into Seascale to work at the Sellafield plant, bringing with them various infectious agents contributing to the risk of childhood leukaemia has been suggested (Kinlen, 1988, 1993). The absence of a similar effect in relation to other nuclear plants, such as Chalk River in Canada (McLaughlin *et al.,* 1993a, b) where population exchanges are not dissimilar, suggests that this may not be the only explanation. More likely is an interaction between radiation and perhaps local geographical conditions. It is worth recalling the large increase in the relative risk of leukaemia in prenatally irradiated children associated with postnatal infectious history (Bross and Natarajan, 1972). Further studies of this interaction are clearly required.

Despite these findings there is still a possibility that unrecognised effects of IR on germ cells could cause leukaemia. Some of these effects are discussed elsewhere, but the simplest explanation is that mutations could occur in recessive 'bone marrow genes' leading to an increased risk of leukaemia. Since this would probably require a second mutation or 'hit', initiation could occur by mutational inactivation of the remaining allele in a bone marrow target cell. The mechanism which results in a second mutation in a bone marrow progenitor could involve antigen-driven proliferation caused by postnatal infection, as postulated by Greaves for childhood common ALL (Greaves, 1988; Greaves and Alexander, 1993). This means that the leukaemia risk in heterozygous carriers of recessive mutations in bone marrow genes could be significant.

9.6
Modifiers and Non-classical Inheritance

Recent molecular studies of genetic disorders have shown that the relationship between genotype and phenotype is not quite as simple as it once appeared. The phenotype may not only depend on the position and type of mutation, but other modifying factors may also be involved.

9.6.1
Imprinting

The risk of inherited disease in an affected family does not always comply with Mendelian predictions, and is often ascribed to the little understood mechanism of penetrance. By taking the frequency of an inherited disease in several families and calculating this as a proportion of the number predicted from Mendelian genetics, it is possible to arrive at a measure of the penetrance of the disease-causing mutation. A disease which is 100 per cent penetrant occurs in all carriers of the mutation. This means that in a disease with 50 per cent penetrance only half of the people with a disease-associated gene mutation develop the disease. In a slightly different way, two people may have the same disease mutation but exhibit different levels of disease severity. This phenomenon is called expressivity. Both penetrance and expressivity may be the manifestation of genomic imprinting (Hall, 1990).

Genomic imprinting is a relatively recently recognised epigenetic modifier which affects the way inheritance influences gene expression. As an epigenetic process it usually acts at the gametic level causing inactivation of one of the parental alleles (Peterson and Sapienza, 1993). Evidence of imprinted human genes comes from a number of sources, mainly associated with the expression of disease phenotypes, and the extent to which imprinting affects normal gene expression is not yet clear. Some of the clearest evidence comes from uniparental disomy (UPD). In rare situations, the expression of a recessive disease in the offspring of parents where only one is the carrier of the gene can be attributed to doubling of the disease-

carrying chromosome and loss of the homologous normal chromosome from the other parent. Such examples of unexpected recessives need to be investigated carefully because it is possible that a recessive disease where there is only one parental carrier could be due to germ-line mutation of a wild-type parental gene.

Imprinting can also be seen when part of a parental chromosome is deleted, giving rise to a chromosomal disorder. The clearest examples of this is deletion of chromosome 15q11–13 which gives rise to Prader-Willi syndrome when the paternal segment is deleted, and Angelman syndrome when the maternal segment is lost. Other examples of imprinting are being described in conditions associated with foetal and infant growth disorders, suggesting that gene expression modification of this type probably plays a part in early development. Evidence is now emerging that genomic imprinting has a role in some cases of inherited cancer such as retinoblastoma and Wilm's tumour. The possibility that imprinting could affect susceptibility to radiation-induced leukaemia in the mouse is suggested by differences in the frequency of tumours in reciprocal crosses between X-ray resistant and susceptible strains (Lilly and Duran-Reynals, 1985). An analogous process in human CML was suggested from the results of chromosome 9/22 translocation studies which showed that the Philadelphia chromosome usually consists of a maternal chromosome 9 and a paternal chromosome 22 (Haas *et al.,* 1992). However, recent evidence has cast doubt on this interpretation (Floretos *et al.,* 1994) and the question of whether the chromosome translocations in leukaemias show parent-of-origin effects due to imprinting awaits further clarification. Bearing in mind the effect that IR can have on gene expression, it would not be surprising if it influenced leukaemia susceptibility through imprinting.

Other indications which suggest that imprinting could influence the risk of leukaemia are seen in the form of chromosomal deletions. Genes of a non-deleted chromosome may be rendered pseudo-homozygous if the undeleted gene is imprinted. Moreover duplications of individual chromosomes, such as chromosome 21, or of haploid or near haploid sets are known to occur in some types of leukaemia (Onodera *et al.,* 1992; Shannon, 1992). Their effects on the expression of the leukaemic phenotype may be influenced by the imprinted status of the duplicated chromosomes. Interestingly, the occurrence of bone marrow defects and leukaemia in patients with Down's syndrome has been attributed to reduced heterozygosity by duplication of chromosome 21 (Abe *et al.,* 1989; Niikawa *et al.,* 1991), an effect that could be influenced by imprinting.

9.6.2
Fragile Sites and Dynamic Mutation

Fragile sites are regions of intrinsic chromosomal instability which are revealed in cultured cells such as lymphocytes under conditions of nutritional deficiency and stress (Sutherland, 1977). Empirical observations that fragile sites occur near the translocations found in leukaemias (LeBeau and Rowley, 1984) suggested that these might explain the propensity of chromosomes for breakage at specific points, leading to leukaemia (Yunis, 1984). However, if sites of chromosome structural weakness were to increase the risk of leukaemia or cancer, this would be counterproductive from an evolutionary standpoint, and it has been argued that the association is probably coincidental (Shabtai *et al.,* 1985; Miro *et al.,* 1987; de Ambrosis *et al.,* 1992). However, it has been necessary to reappraise the role of fragile sites following the isolation and sequencing of the FMR gene causing fragile X mental retardation (Davies, 1991; Richards and Sutherland, 1992). FMR is an inherited disease which mainly afflicts boys and which has been shown to be due to an increase in the number of a trinucleotide CCG repeat sequence from 6–60 copies to > 60 in affected boys. This unusual genetic change is also accompanied by increased severity of FMR with each generation, a phenomenon called *anticipation* which can be related to a progressive increase in CCG repeat copy number. Expansion of trinucleotides is a unique form of genomic instability, now known as *dynamic mutation*.

Trinucleotide repeat expansion is known to be responsible for several inherited diseases, including Huntington's disease, myotonic dystrophy and others (Richards and Sutherland, 1992). To date, there is no evidence that any of the inherited bone marrow disorders associated with leukaemia are caused by expanded trinucleotide repeats. However, there is evidence of a trinucleotide repeat in the AF-9 gene on chromosome 9 which is involved in the t(9; 11) translocation (Rabbitts, 1994). Further studies are needed to establish the radiation sensitivity of sites of dynamic mutation before their significance in leukaemia can be fully understood.

9.7
Multifactorial Inheritance—Quantitative Traits

The majority of common diseases such as diabetes, rheumatoid arthritis, some types of heart and metabolic diseases, and certain neurological and psychiatric disorders involve significant hereditary as well as environmental components, and are usually described as multifactorial diseases. However, such diseases may show only limited evidence of familial occurrence, even though the risks are increased among first-degree compared with second-degree relatives. Measuring the frequency of a disease in relation to kinship can be a useful measure of heritability in multifactorial disease. To understand the genetics of multifactorial disease, however, it has been necessary to develop various mathematical models. Useful though these are, they are mostly descriptive since neither the hereditary nor environmental components can be defined in quantitative terms. One of the most widely used multifactorial models is the threshold model (Falconer, 1990; Klug and Cummings, 1994). This assumes that the underlying risk of a disease is normally distributed but that the disease itself is triggered at a sharply defined threshold. This means that the disease risk is bimodal with two outcomes, either affected or unaffected. The relative contributions of different genes and environment at the threshold point is thought to vary from one individual to another. The sum of the heritable factors is known as liability and it follows that in a stable environment, the major contribution to the risk of multifactorial disease will mainly be genetic liability. It is possible that the genetic background to disorders involving the bone marrow could be multifactorial. For most of the population, exposure to low levels of IR has little impact on bone marrow disease because genetic liability is well below the threshold. However, as the IR dose increases, more people will be closer to the threshold, and the number likely to suffer DNA damage will increase. The larger the dose, the less the genetic liability will determine whether the threshold is exceeded. Since leukaemia is the indirect outcome of bone marrow damage, additional levels of genetic liability may be involved in initation/progression. In single gene diseases such as ataxia telangiectasia, the threshold may be exceeded by the effect of the AT mutation itself, so that the relative contribution of environmental IR to the development of leukaemia in these cases may be quite small.

Attempts to measure genetic liability in multifactorial disease are hindered by the need to identify all of the genes involved. A simpler approach in leukaemia is to measure the amount of DNA damage caused by exposure to a given dose of IR and relate this to the risk of leukaemia. Since it is difficult to screen bone marrow samples from healthy donors, this approach is dependent on screening peripheral blood lymphocytes for chromosomal damage, cell-cycle delay, micronucleus formation or mutation frequency. A molecular approach would be to identify genetic markers common to patients with the same leukaemia and determine their frequency in the normal and IR-exposed population, a method termed *association analysis*.

9.8
Predisposition and Susceptibility

Inherited mutations in single genes, as well as contributions from multiple genes can both cause hereditary disease. The terms *predisposition* and *susceptibility* are often used interchangeably to imply that an individual has an increased liability of developing a disease. It is possible to use a more precise genetic definition of these terms (Greenberg, 1993). For instance an inherited mutation which causes a disease in 100 per cent of mutation carriers is fully penetrant, exceeds the threshold without an environmental component and can be regarded not only as predisposing to the disease but also to predetermine it. No known inherited single gene mutation or set of multiple genes predetermines leukaemia. In other words, not every person carrying an inherited gene mutation which predisposes to leukaemia will, even following radiation exposure, develop leukaemia, with the possible exception of ataxia telangiectasia.

Predisposition and susceptibility are therefore more useful in defining genetic liability in qualitative genetic terms. Assuming that IR-related stress is constant, we can define a leukaemia-predisposing gene as a constitutional (inherited or germ-line) mutation which increases the chance that a person will develop leukaemia. A predisposing gene is a mutation which is both necessary and sufficient to cause disease, since without it the disease will not occur. Such genes are moderately or highly penetrant, they are associated with a family history and they can be tracked indirectly by linkage analysis if the location of the gene but not its sequence is known, or directly by mutation analysis if the gene sequence is known. The role of mutagenic agents such as IR in disease predisposition can be to increase the chance of developing a predisposing mutation in the germ line. In diseases with minimal environmental contribution, predisposing mutations may be the rate-limiting factor.

A susceptibility gene differs from a predisposing gene in being neither necessary nor sufficient to cause disease. Its contribution often involves other genes which contribute to overall liability and an environmental contribution is important and potentially rate-limiting. Susceptibility genes exhibit only weak penetrance. They are usually normal allelic variants of polymorphic genes and they show weak evidence of familial disease. Susceptibility genes cause many of the fairly common multifactorial diseases. However, diseases caused by multiple susceptibility genes may not easily be distinguishable phenotypically from weakly penetrant predisposing single genes. This may require the use of direct DNA analysis to distinguish between the two where this is feasible. Susceptibility genes are detected by population association rather than linkage analysis, using molecular markers in linkage disequilibrium with the disease allele, or by identifying the disease-associated allele itself. The HLA genes are among the best-defined susceptibility genes. HLA alleles are associated with increased risks of many diseases which have an immunological and infectious aetiology. It is possible that susceptibility genes are able to determine how the bone marrow responds to and recovers from radiation exposure, but their role in progression to leukaemia is less easy to define. The possibility is that genetic variations in the repair of damaged DNA could be a determining factor, or that the genes controlling regeneration of haemopoietic cells in the bone marrow or secondary infections are the key elements.

This simplistic distinction between predisposition and susceptibility is not easy to apply to carriers of autosomal recessive predisposing gene mutations. Heterozygous carriers often exhibit no phenotypic effect of such mutations, or if they do, the effects are very mild. This is because recessive genes often code for enzymes, and cells can accommodate a reduction in enzyme level determined by a gene dosage effect. However, there has been a question mark over the effect of external stress on the ability of heterozygous cells to perform normal functions. In the case of cancer and leukaemia, much attention has focused on the autosomal-recessive radiation-hypersensitivity disorder, ataxia telangiectasia (AT). There is quite good evidence to suggest that heterozygous carriers are at increased risk of developing breast cancer, whilst

homozygotes tend to develop leukaemia or lymphoma. Since it has been difficult to show a statistical difference in radiation sensitivity between heterozygous AT cells and normal cells, it is not yet clear whether the radiation risk facing AT homozygotes and heterozygotes is the same. Similar reasoning has been applied to the risks of leukaemia faced by heterozygous carriers of the Fanconi anaemia gene(s), another DNA instability disorder. It is thus possible that a heterozygous predisposing gene could behave more like a susceptibility gene rather than a predisposing gene. Since many relatively rare autosomal recessive diseases have quite high frequencies of heterozygotes in the population, there is a possibility that carriers of two or more mutations in different genes could be at significantly increased risk from environmental stress.

9.9
Hereditary Basis of Human Leukaemia

There has been much recent discussion about the ability of IR to cause leukaemia in the offspring of exposed parents. This has focused attention on the question of whether human leukaemia itself has a hereditary component, and what form it might take (Taylor and Birch, 1995). Since there is no doubt that germ cells are sensitive to IR and that some cases of inherited disease may be caused in this way, might it not be possible to explain some types of leukaemia in the same way? Assuming that such a mechanism, if it exists, is not a unique property of IR exposure, there is thus the question of what evidence actually supports a role for heredity in leukaemia.

9.9.1
Family Studies

As already discussed, the classic approach to determining the hereditary contribution to a disease is to find out whether it occurs among close relatives, that is whether it shows a familial pattern. There are several reasons why diseases occur in families. One of these is pure chance, though in a rare disease like leukaemia this is unlikely to occur with any significant frequency. Another non-genetic explanation is that two people in the same family share a common environment. Leukaemia due to a shared environment could emulate kinship since there would a decreased probability of more distant relatives being affected.

The criteria used to distinguish a heritable from a shared environmental aetiology is that cases in siblings caused by the same genes would be expected to arise at roughly the same age, and the age difference at diagnosis should be less than age itself at diagnosis. Evidence of a concordant subtype can be taken as an indication of a heritable aetiology. Irrespective of whether a leukaemia has a single gene or multifactorial aetiology, the frequency should be highest among first-degree relatives and decline successively in second- and third-degree relatives as genetic distance from the affected individual (proband) increases. In a single-gene disorder, the risk among siblings and offspring should be easily predictable, assuming that factors such as penetrance do not complicate the picture. In leukaemia, however, there are a number of additional complications. Since the disease is rare, only a small proportion of all cases might be expected to occur in families. This means that there are likely to be formidable problems with ascertainment because of the difficulty of finding familial cases, as well as the complications of lifetime risks and family size. The number of potential familial cases may easily be underestimated because there are too few siblings, and in this respect the literature probably under-represents the true frequency of familial leukaemia.

Despite these reservations, epidemiological case-control and cohort studies have reported an increased frequency of leukaemias among relatives, particularly siblings (Gunz *et al.,* 1975; Eriksson and Bergstrom, 1987). The risk is highest in patients with CLL, and lowest in CML (Gunz, 1977; Linet *et al.,* 1989;

Cuttner, 1992). Most studies of childhood leukaemia have focused on siblings, so an accurate picture of vertical transmission in leukaemia is still lacking (Draper *et al.,* 1977; Green, 1986). Individual families with two or more cases of leukaemia among siblings, parents and children, or in more distant relatives do occur (Anderson, 1951; Reilly *et al.,* 1952; Heath and Moloney, 1965). Some of the early reports probably included families with an underlying inherited bone marrow disorder, and it is conceivable that many of the anecdotal family cases that have also been reported have some form of bone marrow dysfunction (Lee *et al.,* 1987; Gramatovici *et al.,* 1993). Prospective investigations to detect constitutional bone marrow defects in leukaemia families are all too infrequent so it is difficult to assess their overall significance. Myelodysplasia in families is known to occur, in some cases with a background chromosomal defect (Li *et al.,* 1978; Lee *et al.,* 1987; Clark *et al.,* 1988). The amount of inherited preleukaemia in the population will not be evident unless it progresses to frank leukaemia. Testing of bone marrow or peripheral blood lymphocyte responses to stress is little known in routine practice unless an underlying genetic disorder is already suspected (Auerbach *et al.,* 1989). Until such studies are more commonplace, it may be impossible to assess the true significance of familial preleukaemic bone marrow disorder and its relationship to the risk posed by IR.

9.9.2
Twin Studies

Twin studies have been used to assess the hereditary component of a number of disorders (Levitan, 1988). The advantage is that since monozygotic twins (MZ) are genetically completely identical, any predominating genetic factor will exert the same effect in both twins. The main approach is to measure the disease frequency in both compared with only one of monozygotic twins. However, since MZ twins also share the same environment it is necessary to control for this by comparing MZ with dizygotic twin (DZ) rates. Twin studies have revealed a high rate of leukaemia among MZ twins, but the onset of MZ leukaemia tends to be much earlier, usually in the infant period, than in DZ twins and non-twin siblings (Keith and Brown, 1971; Keith *et al.,* 1975). The explanation for this seems to be that leukaemia in MZ twins is a monoclonal disease (Chaganti *et al.,* 1979; Ford *et al.,* 1993). It arises in one of the twin pair *in utero* and the leukaemic stem cells are transferred to the other twin through the anastomosed placental blood vessels, with the result that, at birth, both twins are affected. For this reason, the use of twinning data to reach conclusions about the genetic component of early onset leukaemia is somewhat unsound. However, a number of cases of discordant CML in adult identical twins have been documented, confirming the findings of family studies that the heritable component of CML may be smaller than in other leukaemias (Goh and Swisher, 1965; Goh *et al.,* 1967).

9.9.3
Consanguinuity

The ascertainment of familial leukaemia presents the specific problem that two or more cases in the same family are required. Without sufficient numbers of siblings or other family members, Mendelian segregation patterns and multifactorial risks cannot easily be determined. Rare single gene disorders, as well as multifactorial diseases, have a greater chance of occurring in the offspring of spouses who are related, usually as first cousins (Bittles, 1990; Bundey *et al.,* 1990). Consanguineous spouses have the same ancestor and will therefore have a proportion of their genes in common. Offspring of such unions will be homozygous for about 6.25 per cent of their genes, and this means that there is quite a high probability that

a mutated recessive gene will be homozygous. In a multifactorial disease, there is a good chance that a significant number of the genes involved will be the same in consanguineous parents (Levitan, 1988). Despite the decline in rates of consanguinity in the developed countries over the past century, there are large populations worldwide who practise consanguineous marriage for religious or social reasons (Bittles *et al.,* 1991). Some of these populations include migrants to the developed countries, such as Pakistanis who have moved to the UK (Balarajan and Raleigh, 1992). In some cases, recessive diseases which are otherwise quite rare in the indigenous population, can be relatively common in the immigrants. In the case of UK Pakistanis, no detailed data on the frequency of recessive diseases are yet available but there is clearly a significant number of cases of thalassaemia in some communities and the possibility of an increased frequency of Fanconi anaemia, both disorders of the bone marrow. There is also evidence of an increased risk of Hodgkin's disease and other cancers in UK Asians, but the relationship of this to consanguinity has not yet been determined (Stiller *et al.,* 1991).

9.10
Chromosomal and Congenital Abnormalities

The role of chromosomal translocations in leukaemia has already been discussed and it was suggested that available evidence indicates that such rearrangements are not transmitted in the germ line. However, constitutional chromosomal anomalies in general are not uncommon (Epstein, 1986) and are estimated to occur in about 1 in 250 live births (Baird *et al.,* 1988). Whether leukaemic translocations would be developmentally lethal (Knudson, 1985) or whether the mechanisms of chromosome rearrangement in leukaemia and the germ line are fundamentally different is not clear. The fact that transgenic mice containing activated oncogenic gene constructs can survive until birth (Adams *et al.,* 1985) suggests that constitutional leukaemic translocations in humans might not be lethal. A more likely explanation is that leukaemia-chromosome recombination mechanisms are restricted to haemopoietic cells. However, the ability of IR to induce double-strand breaks in DNA suggests that the possibility of germ-line effects should not be completely excluded.

Individual and familial cases of constitutional chromosomal rearrangement associated with leukaemia do occur (Markkanen *et al.,* 1987; de Greef *et al.,* 1982; Kroisel *et al.,* 1992). Some of these cases may be purely coincidence, but where there is more than one leukaemia in a family associated with a chromosomal abnormality, a causal relationship probably exists. A number of these reports include cases of constitutional deletion and leukaemia (Lee *et al.,* 1987; Paul *et al.,* 1987; Shannon *et al.,* 1989), suggesting that deletion of certain TS genes can increase the risk of familial cases. This is particularly relevant when considering the ability of IR to cause large deletions in DNA.

The best-documented constitutional chromosome anomaly with an increased risk of leukaemia is Down's syndrome, or trisomy 21 (Fong and Brodeur, 1987). Most estimates suggest that the risk of leukaemia in children with Down's syndrome is about twenty times higher than in the normal population (Rosner and Lee, 1972). The reason for this increased risk of leukaemia is not certain, though there appear to be abnormalities of bone marrow function in Downs's syndrome (Hayashi *et al.,* 1988; Miyashita *et al.,* 1991). There has been much discussion about the ability of preconceptional IR to cause Down's syndrome; of twelve studies listed in the 1982 UNSCEAR report (UNSCEAR, 1982), four described an increase while eight failed to show an effect. This is an important issue because any increase in the rate of Down's syndrome will indirectly increase the number of people at risk from IR-induced leukaemia. Of equal importance is the possibility that IR may increase the risk of germ cell non-disjunction and deletion. It has been suggested that non-disjunction-predisposing genes could increase the risk of Down's syndrome (Alfi

et al., 1980), but a role in leukaemia has not been determined. The possibility that IR could affect genetic imprinting mechanisms by decreasing chromosome 21 functional zygosity, increasing the risk of leukaemia in Down's syndrome, deserves more study. At present there is no conclusive proof to suggest that Down's syndrome originates by a mechanism other than spontaneous, mainly maternal gametic non-disjunction. Since this is a maternal age-related phenomenon, a role for environmental factors such as IR on oogenesis cannot yet be formally excluded.

Leukaemia and cancer have been reported in association with a number of rare congenital abnormalities and birth defects, such as Rubinstein-Taybi syndrome, and Poland anomaly (Mulvihill, 1975; Mann *et al.,* 1993; Taylor and Birch, 1995). In some cases, such as Rubinstein-Taybi syndrome (Jonas *et al.,* 1978), chromosomal microdeletions may be involved. The possibility that leukaemia might be the outcome of a contiguous gene deletion effect involving a structural anomaly and a closely linked TS gene requires further study. While there is still uncertainty about the frequency of congenital abnormalities caused by IR (UNSCEAR, 1993) these may represent the extreme end of a spectrum of developmental deletions associated with leukaemia. Bearing in mind that microdeletions are rarely detected cytogenetically, their role in leukaemia requires further investigation. A recent review indicates that therapy-related myelodysplasia is often accompanied by somatic deletions involving chromosome 5 and 7 material (Pedersen-Bjergaard and Rowley, 1994). This raises the possibility that preleukaemic microdeletions might be induced by IR in the germ line.

9.11
Inherited Diseases associated with Predisposition to Leukaemia

There are four major groups of inherited single-gene disorders which have a causative association with leukaemia. These are the inherited bone-marrow-failure disorders, the genetic instability syndromes, the primary immunodeficiencies and the inherited cancer-predisposing syndromes (Taylor and Birch, 1995). The first three involve defective haemopoiesis, and in some cases there is a clear increase in the sensitivity of lymphoid cells to radiation or chemicals. Studies on the radiation sensitivity of the fourth group, the inherited cancer syndromes, are less complete.

9.11.1
Bone Marrow Failure, Genetic Instability and Primary Immunodeficiency

Inherited disorders involving bone marrow failure, genetic instability or primary immunodeficiency associated with an increase in leukaemia are shown in Table 9.2. Where this is known, their radiation sensitivity is also indicated. It is largely through the study of disorders such as ataxia telangiectasia that the relationship between IR sensitivity and leukaemia has been established. However, without direct evidence of AT mutations in leukaemia cells, it would be a mistake to assume that

Table 9.2 Inherited disorders associated with bone marrow failure, chromosome instability and immunodeficiency

Disorder/syndrome	Type[a]	Genetics[b]	Chromosome	IR sensitive?	Leukaemia/ lymphoma?
Fanconi anaemia	BM	AR	9 (+)	yes/no[c]	yes
Familial aplastic anaemia	BM	AR/AD	?	?	?
Dyskeratosis congenita	BM	XLR	Xq	yes	rare
Schwachman syndrome	BM	AR	?	?	yes

Disorder/syndrome	Type[a]	Genetics[b]	Chromosome	IR sensitive?	Leukaemia/ lymphoma?
Amegakaryocytic thrombocytopenia	BM	XLR	?	?	yes
Blackfan Diamond	BM	AD/AR	?	?	yes
Kostmann's agranulocytosis	BM	AR	?	?	?
Ataxia telangiectasia	GIS	AR	11q23	yes	yes
Nijmegan breakage syndrome	GIS	AR	?	yes	yes
Bloom's syndrome	GIS	AR	15		yes
A-SCID (Swiss)	PID	AR	8	yes	yes
XL-SCID (Bruton)	PID	XLR	Xq13	?	yes
XL-HIM	PID	XLR	Xq26	?	yes
PNP deficiency	PID	AR	14q	?	?
XLP	PID	XLR	Xq25	?	yes
Wiskott-Aldrich	PID	XLR	Xp11	?	yes

[a] BM—bone marrow failure; GIS—genetic instability syndrome; PID—primary immunodeficiency disorder.
[b] AR—autosomal recessive; AD—autosomal dominant; XLR—X-linked recessive.
[c] Evidence of IR sensitivity in some cases.

the radiation-sensitive defect in AT underlies IR-induced leukaemia in the general population. It is worth bearing in mind that many of these disorders have complex multisystem defects and that some of these may also contribute to leukaemia. One of the most commonly inherited disorders is Fanconi anaemia (FA), an autosomal recessive aplastic anaemia generally occurring in childhood and associated with the different degrees of congenital abnormality (Schroeder *et al.,* 1976; Alter, 1987). Fanconi anaemia lymphoid and bone marrow cells exhibit spontaneous chromosome breaks which are increased in frequency by clastogenic agents such as mitomycin C and diepoxybutane. The leukaemia risk in FA is about 9 per cent, a rate which is about 15000 times normal and the leukaemias are generally myeloid in type (Auerbach and Allen, 1991; Alter, 1992). The FA gene has at least four complementation groups (Buchwald *et al.,* 1989) of which the C group has been cloned and mapped to chromosome 9q22 (Strathdee *et al.,* 1992; Gibson *et al.,* 1993; Verlander *et al.,* 1994). There is evidence to suggest that some FA patients are hypersensitive to radiation and the condition is often considered to be one of the chromosome instability disorders. Some of the interest in FA derives from the high estimated heterozygote frequency, about 1 in 300 of the population. Although early suggestions that carriers might be at risk of cancer or leukaemia (Swift, 1971) confirmatory evidence of this has yet to be obtained (Swift *et al.,* 1980).

There are also a number of FA-like disorders including WT syndrome, IVIC syndrome, as well as reports of familial monosomy 7 and ataxia pancytopenia, and most likely a number of unrecognised disorders, all with bone marrow dysfunction and, in some cases, with a genetic background (Alter, 1987). Dyskeratosis congenita is a rare ectodermal dysplasia with predominantly X-linked inheritance (Connor *et al.,* 1986), and some evidence of bleomycin and X-ray sensitivity (DeBauche *et al.,* 1990), in which AML has been identified in affected families (Marsh *et al.,* 1992), Shwachman-Diamond syndrome is a childhood pancreatic dysfunctional disorder (Shwachman *et al.,* 1964) with progressive bone marrow failure due to an autosomal recessive gene (Agget *et al.,* 1980). SDS can be regarded as a preleukaemia syndrome (Stevens *et al.,* 1978; Woods *et al.,* 1981) though there is some doubt about whether chromosomal instability is involved (Koiffmann *et al.,* 1991). A number of other bone marrow disorders include amegakaryocytic thrombocytopenia, Blackfan Diamond syndrome, Kostmann's agranulocytosis and hypoplastic

preleukaemia (Alter, 1987; Young and Alter, 1994). Although these conditions are very rare, their genetic basis may provide clues to genes involved in radiation sensitivity.

The genetic instability disorders include two main, though very rare, conditions-ataxia telangiectasia, and Bloom's syndrome. AT is an autosomal recessive radiation-hypersensitivity disorder (Boder, 1975; Tadjoedin and Fraser, 1965) characterised by defective DNA repair and a high risk of cancer, of which leukaemia and lymphoma are the most common (Taylor, 1992). AT cells exhibit a high rate of spontaneous clonal chromosome rearrangement of which translocations involving chromosomes 7 and 14 are frequently encountered (Scheres *et al.,* 1980; Butterworth and Taylor, 1986). The frequency of AT in the population is very low, about 1 in $(3–5)\times10^5$, but gene carriers are thought to be between 1 in 100 and 1 in 300 (Swift *et al.,* 1986). This high rate is significant since it has been predicted from family studies that carriers are at an increased risk of cancer, specifically breast cancer (Swift *et al.,* 1976; Morrell *et al.,* 1990). Precise confirmation of this will require cloning of the AT gene which has been mapped to 11q23. Like FA, there are several AT complementation groups (at least four) and AT-like variants of which AT(Fresno) and Nijmegen breakage syndrome are the most well known (Weemaes *et al.,* 1994). Bloom's syndrome is also an autosomal recessive chromosomal instability disorder which is found particularly among the Ashenazi Jewish population (German, 1992). There is a high risk of leukaemia in BS and quite a wide spectrum of tumours in general (German and Passarge, 1989). The gene for BS is thought to be on chromosome 15 and there are no complementation groups (McDaniel and Schultz, 1992). At present there is no evidence that BS gene carriers are at increased risk from cancer, though this conclusion may have to be revised when population-carrier testing and associated epidemiological studies are carried out once the BS gene has been cloned.

The primary immunodeficiency disorders are a heterogeneous group of autosomal and X-linked diseases mainly of childhood, often with severe outcomes reflecting an inability to mount an effective immune response to viruses or bacteria (Filipovich *et al.,* 1994). They include the autosomal and X-linked forms of severe combined immunodeficiency (scid), X-linked agammaglobulinaemia, X-linked hyper-IgM, X-linked lymphoproliferative disease and purine nucleotide phophorylase deficiency. In most types of primary immunodeficiency disorder there is an increased risk of Hodgkin or non-Hodgkin lymphoma, with, in some cases, such as XLP, the involvement of Epstein-Barr virus. With the exception of the autosomal scid gene on chromosome 8, which may be involved in radiation sensitivity (Komatsu *et al.,* 1993), there is relatively little information on the effects of radiation on cells from primary immunodeficiencies. However, the association between DNA repair and immunoglobulin gene recombination (Fulop and Phillips, 1990; Cavazzana-Calvo *et al.,* 1993) suggests that many of these disorders might show an increased level of radiation sensitivity.

9.11.2 Inherited Cancer

The fourth category of disorder includes the cancer-predisposing syndromes. These include retinoblastoma, Wilm's tumour, von Hippel Lindau disease, familial polyposis coli, types 1 and 2 neurofibromatosis (NF), Gorlin's syndrome, Li-Fraumeni syndrome and others (Table 9.3). Most, if not all, involve a *de novo* or inherited germ-line mutation in a TS-like gene in a proportion of cases, with autosomal dominant inheritance. In the inherited forms, the presence of a predisposing mutation in the germ line leads to the appearance of cancer at an earlier age than in the sporadic forms (Li and Bader, 1987). Although the genetics appear to be dominant, expression of the tumour phenotype requires inactivation of the remaining TS allele at the somatic cell level. Tumorigenesis is therefore recessive at the cellular level. The relationship

between radiation sensitivity and leukaemia risk in inherited cancer has not been explored in detail. In retinoblastoma, where there is clearly increased sensitivity to radiation therapy, there is little to suggest that this increases the risk of leukaemia. In NF1, on the other hand, there is little evidence of radiation sensitivity but a clearly increased risk of leukaemia (Bader and Miller, 1978). Recent studies have suggested an increased risk of leukaemia in Wilm's tumour families (Hartley *et al.* 1994). The effect that loss of TS gene function in general has on the bone marrow and haemopoiesis requires further study. The best-documented association involves the p53 gene and the familial cancer condition called Li-Fraumeni syndrome (LFS). One of the tumours occurring in LFS is leukaemia in childhood. The role of the p53 gene as a cell-cycle checkpoint control which protects cells from external damage including that mediated by IR suggests that this may be one of the genes crucial in protecting bone marrow.

Table 9.3 Inherited cancer-predisposing syndromes

Tumour/syndrome	Genetics[a]	Chromosome	Gene	IR sensitivity?	Leukaemia/ lymphoma?
Retinoblastoma	AD	13q14	Rb1	yes	no
Wilm's	AD	11p	WT1	?	yes
Neurofibromatosis 1	AD	17q11	NF1	?	yes
Neurofibromatosis 2	AD	22q	NF2	?	?
Von Hippel Lindau	AD	3	VHL	?	?
FAP[b]	AD	5q	APC	?	?
HNPCC[c]	AD	2		?	?
Gorlins/BCNS	AD	9q	?	yes	?
Li-Fraumeni	AD	17	p53	?	yes

[a] AD—autosomal dominant.
[b] FAP—familial adenomatous polyposis.
[c] HNPCC—hereditary non-polyposis colon cancer.

9.12 Conclusions

The bone marrow is one of the most sensitive sites for IR-induced DNA damage and this sensitivity is associated with an increased risk, albeit indirectly, of developing leukaemia. The apparently variable distribution of this risk among individuals exposed to IR could just be chance, but it may be due to hereditary differences. It seems probable that these heritable differences become more important as radiation doses approach background levels. Paradoxically, it is not only difficult to determine the risk of leukaemia from low-dose IR exposure but it is also difficult to measure the IR response. Whatever the quantitative level of hereditary contribution, it will probably involve the sum and interaction of several susceptibility genes, not all of which may be involved in the repair of radiation-induced DNA damage. These interacting genes may determine that the liability of leukaemia following IR exposure has a threshold effect. Most people will possess sufficient genetic buffering capacity to withstand the long-term effects of IR, such as leukaemia, but there may be individuals who are at genetically higher risk. The extreme examples of this are inherited single-gene disorders which cause bone marrow and immunological disorders.

These can dramatically increase the risk of leukaemia and though hypersensitivity to low doses of IR may be a crucial factor, the role of the inherited gene mutations is likely to be complex.

The ability of IR to induce pre- and postzygotic genetic damage may be an indirect cause of leukaemia, through constitutive effects on bone marrow function. Formal proof that germ cell mutations, other than those causing well-defined inherited disorders, can lead to leukaemia is lacking but the scope of the investigations carried out has been limited. Future studies of radiation risk in relation to leukaemia will require careful ascertainment of radiation exposure and leukaemia rates in different population groups, in association with detailed molecular epidemiological analysis. There is much to commend a careful search for molecular signatures associated with IR damage. A clearer picture of the contribution of and interaction between IR and heredity in the development of bone marrow disease and leukaemia may then begin to emerge.

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References

ABE, K., KAJII, T. & NIIKAWA, N. (1989) Disomic homozygosity in 21-trisomic cells: a mechanism responsible for transient myeloproliferative syndrome, *Human Genetics,* **82**, 313–16.

ABRAHAMSON, S. (1990) Childhood leukaemia at Sellafield, *Radiation Research,* **123,** 237–8.

ADAMS, J.M., HARRIS, A.W., PINKERT, C.A. *et al.* (1985) The c-*myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice, *Nature,* **318,** 533–8.

AGGETT, P.J., CAVANAGH, N.P.C., MATTHEW, D.J., PINCOTT, J.R., SUTCLIFFE, J. & HARRIES, J.T. (1980) Shwachman's syndrome. *Arch. Dis. Child,* **55**, 331–47.

ALFI, O.S., CHANG, R. & AZEN, S.P. (1980) Evidence for genetic control of nondisjunction in man, *Am. J. Hum. Genet.,* **32**, 477–83.

ALTER, B.P. (1987) The bone marrow failure syndromes. In D.G.Nathan & F.A.Oski (eds), *Hematology in Childhood and Infancy* (3rd edn)., Philadelphia: W.B.Saunders, Chapter 7, pp. 159–241.

(1992) Leukemia and preleukemia in Fanconi's anemia, *Cancer Genet. Cytogenet.,* **58**, 206–8.

ANDERSON, R.C. (1951) Familial leukemia, *Am. J. Dis. Child,* **81**, 313–22.

AUERBACH, A.D. & ALLEN, R.G. (1991) Leukemia and preleukemia in Fanconi anemia patients: a review of the literature and report of the international Fanconi anemia registry, *Cancer Genet. Cytogenet.,* **51**, 1–12.

AUERBACH, A.D. GHOSH, R., POLLIO, P.C. & ZHANG, M. (1989) Diepoxybutane test for prenatal and postnatal diagnosis of Fanconi Anemia. In T.M.Schroeder-Kurth & A.D.Auerbach (eds) *Fanconi Anemia: Clinical Cytogenetic and Experimental Aspects.* Berlin: Springer-Verlag, pp. 71–82.

BADER, J.L. & MILLER, R.W. (1978) Neurofibromatosis and childhood leukaemia, *J. Pediatr,* **92**, 925–9.

BAIRD, P.A., ANDERSON, T.W., NEWCOMBE, H.B. & LOWRY, R.B. (1988) Genetic disorders in children and young adults: a population study, *Am. J. Hum. Genet.,* **42**, 677–93.

BALARAJAN, R. & RALEIGH, V.S. (1992) The ethnic populations of England and Wales: the 1991 census, *Health Trends,* **24**, 113–16.

BEIR V (1990) *Health Effects of Exposure to Low Levels of Ionizing Radiation.* (Committee on the Biological Effects of Ionizing Radiations) Washington, DC: National Academy Press.

BERAL, V. (1990) Childhood leukemia near nuclear plants in the United Kingdom: the evolution of a systematic approach to studying rare disease in small geographic areas, *Am. J. Epidemiol.,* **132**, S63–S68.

BERAL, V., ROMAN, E. & BOBROW, M. (1993) *Childhood Cancer and Nuclear Installations.* London: BMJ.

BITHELL, J.F., DUTTON, S.J., DRAPER, G.J. & NEARY, N.M. (1994) Distribution of childhood leukaemias and non-Hodgkin's lymphomas near nuclear installations in England and Wales, *Br. Med. J.,* **309**, 501–5.

BITTLES, A.H. (1990) *Consanguineous Marriage; Current Global Incidence and its Relevance to Demographic Research.* Research report 90–186, Population Studies Center, University of Michigan, Ann Arbor, USA.

BITTLES, A.H., MASON, W.M., GREENE, J. & RAO, N.A. (1991) Reproductive behaviour and health in consanguineous marriages, *Science,* **252**, 789–94.

BODER, E. (1975) Ataxia-telangiectasia: some historic, clinical and pathologic observations, *Birth Defects: Original Article Series,* **11**, 255–70.

BROSS, I.D.J. & NATARAJAN, N. (1972) Leukemia from low level radiation: identification of susceptible children, *N. Engl. J. Med.,* **287**, 107–10.

BUCHWALD, M., CLARKE, C., NG, J., DUCKWORTH-RYSIECKI, G. & WEKSBERG, R. (1989) Complementation and gene transfer studies in Fanconi Anemia. In T.M. SCHROEDER-KURTH & A.D.AUERBACH (eds) *Fanconi Anemia: Clinical Cytogenetic and Experimental Aspects,* Berlin: Springer-Verlag, pp. 226–35.

BUNDEY, S., ALAM, H., KAUR, A., MIR, S. & LANCASHIRE, R.J. (1990) Race, consanguinity and social features in Birmingham babies: a basis for a prospective study, *J. Epidemiol. Community Health,* **44**, 130–5.

BUTTERWORTH, S.V. & TAYLOR, A.M.R. (1986) A subpopulation of t(2; 14)(p11; q32) cells in ataxia-telangiectasia B lymphocytes, *Human Genetics,* **73**, 346–9.

CARDIS, E., GILBERT, E.S., CARPENTER, L. *et al.,* (1994) Direct estimates of cancer mortality due to low doses of ionising radiation: an international study, *Lancet,* **344**, 1039–43.

CARTWRIGHT, R.A., ALEXANDER, F.E., MCKINNEY, P.A. & RICKETTS, T.J. (1990) *Leukemia and Lymphoma: An Atlas of Distribution within Areas of England and Wales 1984–88.* London: Leukaemia Research Fund.

CAVAZZANA-CALVO, M., LE DEIST, F., DE SAINT BASILE, G., PAPADOPOULO, D., DE VILLARTAY, J.P. & FISCHER, A. (1993) Increased radiosensitivity of granulocyte macrophage colony forming units and skin fibroblasts in human autosomal recessive severe combined immunodeficiency, *J. Clin. Invest.,* **91,** 1214–18.

CHAGANTI, R.S.K., MILLER, D.R., MEYERS, P.A. & GERMAN, J. (1979) Cytogenetic evidence of the intrauterine origin of acute leukemia in monozygotic twins, *N. Engl. J. Med.,* **300,** 1032–4.

CLARK, R.E., GEDDES, D., WHITAKER, K. & JACOBS, A. (1988) Myelodysplastic syndrome in a kindred with ins (16) (p 11.2), *Clinical Genetics,* **33**, 418–23.

COHEN, M.M. & LEVY, H.P. (1989) Chromosome instability syndromes, *Adv. Hum. Genet.,* **18,** 43–149.

CONNOR, J.M. GATHERER, D., GRAY, F.C., PIRRIT, L.A. & AFFARA, N.A. (1986) Assignment of the gene for dyskeratosis congenita to Xq28, *Human Genetics,* **72**, 348–51.

COOK-MOZAFFARI, P.J., DARBY, S.C., DOLL, R. *et al.* (1989a) Geographical variation in mortality from leukaemia and other cancers in England and Wales in relation to proximity to nuclear installations, 1969–78, *Br. J. Cancer,* **59**, 476–85.

COOK-MOZAFFARI, P., DARBY, S. & DOLL, R. (1989b) Cancer near potential sites of nuclear installations, *Lancet,* ii, 1145–7.

COOPER, D.N. & KRAWCZAK, M. (1993) *Human Gene Mutation,* Oxford: Bios Scientific Publishers.

COURT BROWN, W.M. & DOLL, R. (1965) Mortality from cancer and other causes after radiotherapy for ankylosing spondylitis, *Br. Med. J.,* **2**, 1327.

COWELL, J.K. & HOGG, A. (1992) Genetics and cytogenetics of retinoblastoma, *Cancer Genet. Cytogenet.,* **64,** 1–11.

CUTTNER, J. (1992) Increased incidence of hematologic malignancies in first-degree relatives of patients with chronic lymphocytic leukemia, *Cancer Invest.,* **10,** 103–9.

DAVIES, K. (1991) Breaking the fragile X, *Nature,* **351,** 439–40.

DE AMBROSIS, A., CASCIANO, I., QUERZOLA, F., VIDALI, G. & ROMANI, M. (1992) Chromatin structure, DNA methylation, and gene expression at sites of viral integration in human fibroblasts: implications for chromosomal fragility, *Cancer Genet. Cytogenet,* **60,** 1–7.

DEBAUCHE, D.M., PAI, G.S. & STANLEY, W.S. (1990) Enhanced G2 chromatid radiosensitivity in dyskeratosis congenita. *Am. J. Hum. Genet.,* **46,** 350–7.

DE GREEF, I. GERAEDTS, J.P.M. & LEEKSMA, C.H.W. (1982) Malignant hematologic disorders in two Robertsonian 13; 14 translocation carriers , *Cancer Genet. Cytogenet,* **7***,* 181–4.

DOLL, R., EVANS, H.J. & DARBY, S.C. (1994) Paternal exposure not to blame, *Nature,* **367,** 678–80.

DRAPER, G.J., HEAF, M.M. & WILSON, L.M.K. (1977) Occurrence of childhood cancers among sibs and estimation of familial risks. *J. Med. Genet.* **14**, 81–90.

EDWARDS, J.H. (1969) Familial predisposition in man, *Br. Med. Bull.,* **25**, 58–64.

EHLING, U.H. (1991) Genetic risk assessment, *Am. Rev. Genet.,* **25**, 255–80.

EPSTEIN, C.J. (1986) *The Consequences of Chromosome Imbalance: Principles, Mechanisms and Models.* Cambridge: Cambridge University Press.

ERIKSSON, M. & BERGSTROM, I. (1987) Familial malignant blood disease in the country of Jamtland, Sweden, *Eur. J. Haematol.,* **38,** 241–5.

EVANS, H.J. (1990a) Leukaemia and radiation, *Nature,* **345**, 16–17.

(1990b) Ionising radiations from nuclear establishments and childhood leukaemias—an enigma, *Bioessays,* **12**, 541–9.

FALCONER, D.S. (1990) *An Introduction to Quantitative Genetics* (3rd edn). Harlow: Longman.

FILIPOVICH, A.H., MATHUR, A., KAMAT, D., KERSEY, J.H. & SHAPIRO, R.S. (1994) Lymphoproliferative disorders and other tumours complicating immunodeficiencies, *Immunodeficiency,* **5**, 91–112.

FIORETOS, T., HEISTERKAMP, N. & GROFFEN, J. (1994) No evidence for genomic imprinting of the human *BCR* gene, *Blood,* **83**, 3441–4.

FONG, C. & BRODEUR, G.M. (1987) Down's syndrome and leukemia: epidemiology, genetics, cytogenetics and mechanisms of leukemogenesis, *Cancer Genet. Cytogenet.* **28**, 55–76.

FOON, K.A. & TODD, R.F. (1986) Immunologic classification of leukemia and lymphoma, *Blood,* **68**, 1–31.

FORD, A.M., RIDGE, S.A., CABRERA, M.E. *et al.* (1993) *In utero* rearrangements in the trithorax-related oncogene in infant leukaemias, *Nature,* **363**, 358–60.

FULOP, G.M. & PHILLIPS, R.A. (1990) The *scid* mutation in mice causes a general defect in DNA repair, *Nature,* **347**, 479–82.

GARDNER, M.J. & WINTER, P.D. (1984) Mortality in Cumberland during 1959–78 with reference to cancer in young people around Windscale, *Lancet,* i, 216–17.

GARDNER, M.J., SNEE, M.P., HALL, A.J. *et al.* (1990) Results of case-control study of leukaemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria, *Bri. Med. J.,* **300**, 423–9.

GELEHRTER, T.D. & COLLINS, F.S. (1990) *Principles of Medical Genetics,* Baltimore: Williams & Wilkins.

GERMAN, J. (1992) Bloom's syndrome: incidence, age of onset, and types of leukemia in the Bloom's Syndrome Registry. In C.S.BARTSOCAS & D.LOUKOPOULOS (eds), *Genetics of Hematological Disorders,* Washington, DC: Hemisphere Publishers, pp. 241–58.

GERMAN, J. & PASSARGE, E. (1989) Bloom's syndrome. XII: Report from the registry for 1987 . *Clin. Genet.,* **35**, 57–69.

GIBSON, R.A., BUCHWALD, M., ROBERTS, R.G. & MATHEW, C.G. (1993) Characterisation of the exon structure of the Fanconi anaemia group C gene by vectorette PCR, *Hum. Molec. Genet.,* **2**, 35–8.

GOH, K-O. & SWISHER, S.N. (1965) Identical twins with chronic myelocytic leukemia, *Arch. Int. Med.,* **115**, 475–8.

GOH, K-O., SWISHER, S.N. & HERMAN, E.C. (1967) Chronic myelocytic leukemia and identical twins: additional evidence of the Philadelphia chromosome as postzygotic abnormality, *Arch. Int. Med.,* **120**, 214–19.

GRAMATOVICI, M., BENNETT, J.M., HISCOCK, J.G. & GREWAL, K.S. (1993)Three cases of familial hairy cell leukemia, *Am. J. Hematol,* **42**, 337–9.

GREAVES, M.F. (1988) Speculations on the cause of childhood acute lymphoblastic leukemia, *Leukemia,* **2**, 120–5.

(1990) The Sellafield childhood leukemia cluster: are germline mutations responsible? *Leukemia,* **4***,* 391–6.

GREAVES, M.F. & ALEXANDER, F.E. (1993) An infectious etiology for common acute lymphoblastic leukemia in childhood? *Leukemia,* **7***,* 349–60.

GREEN, D.M. (1986) Childhood cancer in siblings, *Pediatr. Hematol. Oncol.,* **3**, 229–39.

GREENBERG, D.A. (1993) Linkage analysis of 'necessary' disease loci versus 'susceptibility' loci, *Am. J. Hum. Genet.,* **52***,* 135–43.

GUNZ, F.W. (1977) The epidemiology and genetics of the chronic leukemias, *Clin. Haematol.,* **6**, 3–20.

GUNZ, F.W. & ATKINSON, H.R. (1964) Medical radiations and leukaemia: retrospective study, *Br. Med. J.,* **1**, 389–93.

GUNZ, F.W., GUNZ, J.P., VEALE, A.M.O. *et al.* (1975) Familial leukaemia: a study of 909 families, *Scand. J. Haematol.,* **15**, 117–31.

HAAS, O.A., ARGYRIOU-TIRITA, A. & LION, T. (1992) Parental origin of chromosomes involved in the translocation t(9; 22), *Nature,* **359**, 414–16.

HALL, J.G. (1990) Genomic imprinting; review and relevance to human disease, *Am. J. Hum. Genet.,* **46**, 857–73.

HARTLEY, A.L., BIRCH, J.M., HARRIS, M. *et al.* (1994) Leukemia, lymphoma and related disorders in families of children diagnosed with Wilms' tumor, *Cancer Genet. Cytogenet.,* **77**, 129–33.

HAYASHI, Y., EGUCHI, M., SUGITA, K. *et al.* (1988) Cytogenetic findings and clinical features in acute leukemia and transient myeloproliferative disorder in Down's syndrome, *Blood,* **72**, 15–23.

HEATH, C.W. & HASTERLIK, R.J. (1963) Leukemia among children in a suburban community, *Am. J. Med.,* **34**, 796.

HEATH, C.W. & MOLONEY, W.C. (1965) Familial leukaemia: five cases of acute leukemia in three generations, *N. Engl. J. Med.,* **272**, 882–7.

HENSHAW, P.S. & HAWKINS, J.W. (1944) Incidence of leukemia in physicians, *J. Nat. Cancer Institute,* **4**, 339–46.

JONAS, D.M., HEILBRON, D.C & ABLIN, A R. (1978) Rubinstein-Taybi syndrome and acute leukemia, *J. Pediatr.,* **92**, 851–2.

KEITH, L. & BROWN, E. (1971) Epidemiologic study of leukemia in twins (1928–1969), *Acta Genet. Med. Gemellol.,* **20**, 9–22.

KEITH, L., BROWN, E.R., AMES, B. & STOTSKY, M. (1975) Possible obstetric factors effecting leukemia in twins. In J.CLEMMESEN & D.S.YOHN (eds), *Bibl Haemat,* vol. 43. Comparative Leukaemia Research, Basel: Karger, pp. 221–3.

KINLEN L. (1988) Evidence for an infective cause of childhood leukaemia: comparison of a Scottish new town with nuclear reprocessing sites in Britain, *Lancet,* ii, 1323–7.

(1993) Can paternal preconceptional radiation account for the increase of leukaemia and non-Hodgkin's lymphoma in Seascale? *Br. Med. J.,* **306**, 1718–21.

KLUG, W.S. & CUMMINGS, M.R. (1994) *Concepts of genetics* (4th edn). New York: Macmillan.

KNUDSON, A.G. (1985) Hereditary cancer, oncogenes, and antioncogenes, *Cancer Research,* **45**, 1437–43.

KOHN, H.I. & FRY, R.J.M. (1984) Radiation carcinogenesis, *N. Engl. J. Med.,* **310**, 504–11.

KOIFFMANN, C.P., GOZALEZ, C.H., SOUZA, D.H., ROMANI, E.G., KIM, C.A. & WAJNTAL, A. (1991) Is Shwachman syndrome (McKusick 26040) a chromosome breakage syndrome? *Human Genetics,* **87**, 106–7.

KOMATSU, K, OHTA, T., JINNO, Y., NIIKAWA, N. & OKUMURA, Y. (1993) Functional complementation in mouse-human radiations hybrids assigns the putative murine *scid* gene to the pericentric region of human chromosome 8, *Hum. Mol. Genet.,* **2***,* 1031–4.

KROISEL, P.M., ROSENKRANZ, W. & URBAN, C. (1992) Balanced familial (2; 8) translocation in a young girl with pH-positive chronic myelogenous leukaemia. In C.S.Barsocas & D.Loukopoulos (eds), *Genetics of Hematological Disorders.* New York: Hemisphere, pp. 151–4.

LEBEAU, M.M. & ROWLEY, J.D. (1984) Heritable fragile sites in cancer, *Nature,* **308**, 607–8.

LEE, E.J., SCHIFFER, C.A., MISAWA, S. & TESTA, J.R. (1987) Clinical and cytogenetic features of familial erythroleukaemia, *Br. J. Haematol.,* **65**, 313–20.

LEVITAN, M. (1988) *Textbook of Human Genetics* (3rd edn). Oxford: Oxford University Press.

LI, F.P. (1993) Molecular epidemiology studies of cancer in families, *Br. J. Cancer,* **68**, 217–19.

LI, F.P. & BADER, J.L. (1987) Epidemiology of cancer in childhood. In D.G. Nathan & F.A.Oski (eds), *Hematology of Infancy and Childhood* (3rd edn). Philadelphia: W.B. Saunders, pp. 918–41.

LI, F.P., POTTER, N.U., BUCHANAN, G.R. *et al.* (1978) A family with acute leukemia, hypoplastic anemia and cerebellar ataxia: association with bone marrow C-monosomy, *Am. J. Med.,* **65**, 933–40.

LILLY, F. & DURAN-REYNALS, M. (1985) Genetic regulation of viral and chemical lym-phomagenesis in the mouse. In *Genetic Control of Host Resistance to Infection and Malignancy*. New York: Alan R.Liss, pp. 631–8.

LINDAHL, T. (1993) Instability and decay of the primary structure of DNA, *Nature,* **362**, 709–15.

LINET, M.S. (1985) *The Leukaemias. Epidemiological Aspects*. Oxford: Oxford University Press.

LINET, M.S., VAN NATTA, M.L., BROOKMEYER, R. *et al.* (1989) Familial cancer history and chronic lymphocytic leukemia, *Am. J. Epidemiol,* **130**, 655–64.

LIPTON, J.M. & NATHAN, D.G. (1987) The anatomy and physiology of hematopoiesis. In D.G.Nathan & F.A.Oski (eds), *Haematology of Infancy and Childhood* (3rd edn). Philadelphia: W.B.Saunders, chapter 6.

MANN, J.R., DODD, H.E., DRAPER, G.J. *et al.* (1993) Congenital abnormalities in children with cancer and their relatives: results for a case-control study (IRESCC), *Br. J. Cancer,* **68**, 357–63.

MARKKANEN, A., RUUTU, T., RASI, V. *et al.* (1987) Constitutional translocation t(3; 6) (p14; p11) in a family with hematologic malignancies , *Cancer Genet. Cytogenet.,* **25**, 87–95.

MARCH, H.C. (1944) Leukemia in radiologists, *Radiology,* **43**, 275–8.

MARSH, J.C.W., WILL, A.J., HOWS, J.M. et al. (1992) 'Stem cell' origin of the hematopoietic defect in dyskeratosis congenita, *Blood,* **79**, 3138–44.

MCDANIEL, L.D. & SCHULTZ, R.A. (1992) Elevated sister chromatid exchange phenotype of Bloom syndrome cells is complemented by human chromosome 15, *Proceedings of the National Academy of Sciences,* **89**, 7968–72.

MCLAUGHLIN, J.R., CLARKE, E.A., NISHRI, E.D. & ANDERSON, T.W. (1993a) Childhood leukemia in the vicinity of Canadian nuclear facilities, *Cancer Causes and Control,* **4**, 51–8.

MCLAUGHLIN, J.R., KING, W.D., ANDERSON, T.W., CLARKE, E.A. & ASMORE, J.P. (1993b) Paternal radiation exposure and leukaemia in offspring: the Ontario case-control study, *Br. Med. J.,* **307**, 959–66.

MILLER, R.W. (1964) Radiation, chromosomes and viruses in the aetiology of leukemia, *N. Engl. J. Med.,* **271**, 30–6.

MIRO, R., CLEMENTE, I.C., FUSTER, C. & EGOZCUE, J. (1987) Fragile sites, chromosome evolution, and human neoplasia, *Human Genetics,* **75**, 345–9.

MITLEMAN, F. (1991) *Catalog of Chromosome Aberration in Cancer* (4th edn). New York: Wiley/Liss.

MIYASHITA, T., ASADA, M., FUJIMOTO, J. *et al.* (1991) Clonal analysis of transient myeloproliferative in Down's syndrome, *Leukemia,* **5**, 56–9.

MODAN, B. & LUBIN, E. (1974) Radiation induced leukemia in man, *Ser. Haemat.,* **7**, 192–210.

MORRELL, D., CHASE, C.L. & SWIFT, M. (1990) Cancers in 44 families with ataxia-telangiectasia, *Cancer Genet. Cytogenet.,* **50**, 119–23.

MULVIHILL, J.J. (1975) Congenital and genetic diseases. In J.F.Fraumeni (ed.) *Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control*. New York: Academic Press , pp. 3–35.

NAROD, S.A. (1990) Radiation, genetics and childhood leukaemia, *Eur. J. Cancer,* **26**, 661–4.

NIIKAWA, N., DENG, H-X, ABE, K. *et al.* (1991) Possible mapping of the gene for transient myeloproliferative syndrome at 21q11.2, *Human Genetics,* **87**, 561–6.

NRPB (1993) *Documents of the NRPB,* **4** (4). National Radiological Protection Board, Didcot, Oxon.

OGAWA, S., HIRANO, N., SATO, N. *et al.* (1994) Homozygous loss of the cyclin-dependent kinase 4-inhibitor (p16) gene in human leukemias, *Blood,* **84**, 2431–5.

ONODERA, N., MCCABE, N.R. & RUBIN, C.M. (1992) Formation of a hyperdiploid karyotype in childhood acute lymphoblastic leukemia, *Blood,* **80**, 203–8.

PARKER, L., CRAFT, A.W., SMITH, J. *et al.* (1993) Geographical distribution of preconceptional radiation doses to fathers employed at the Sellafield nuclear installation, West Cumbria, *Br. Med. J.,* **307**, 966–71.

PAUL, B., REID, M.M., DAVISON, E.V., ABELA, M. & HAMILTON, P.J. (1987) Familial myelodysplasia: progressive disease associated with emergence of monosomy 7, *Br. J. Haematol.,* **65**, 321–3.

PEDERSEN-BJERGAARD, J. & ROWLEY, J.D. (1994) The balanced and the unbalanced chromosome aberrations of acute myeloid leukemia may develop in different ways and may contribute differently to malignant transformation, *Blood,* **83**, 2780–6.

PETERSON, K. & SAPIENZA, C. (1993) Imprinting the genome: imprinted genes, imprinting genes, and a hypothesis for their interaction, *Ann. Rev. Genet.,* **27**, 7–31.

PONDER, B.A.J. (1992), Molecular genetics of cancer, *Br. Med. J.,* **304**, 1234–6.

PUI, C-H., CRIST, W.M. & LOOK, A.T. (1990) Biology and clinical significance of cytogenetic abnormalities in childhood acute lymphoblastic leukemia, *Blood,* **76**, 1449– 63.

RABBITTS, T.H. (1994) Chromosomal translocations in human cancer, *Nature,* **372**, 143–9.

RAIMONDI, S.C. (1993) Current status of cytogenetic research in childhood acute lymphoblastic leukaemia, *Blood,* **81**, 2237–51.

REILLY, E.B., RAPAPORT, S.I., KARR, N.W., MILLS, H. & CARPENTER, G.E. (1952) Familial chronic lymphatic leukemia, *Arch. Int. Med.,* **90**, 87–9.

RICHARDS, R.I. & SUTHERLAND, G.R. (1992) Dynamic mutations: a new class of mutations causing human disease, *Cell,* **70**, 709–12.

ROSNER, F. & LEE, S.L. (1972) Down's syndrome and acute leukemia: myeloblastic or lymphoblastic? *Am. J. Med.,* **53**, 203–18.

ROWLEY, J.D. (1982) Identification of the constant chromosome regions involved in human hematologic malignant disease, *Science,* **216**, 749–51.

SANKARANARYANAN, K. (1988) Prevalance of genetic and partially genetic diseases in man and the estimation of genetic risks of exposure to ionizing radiation, *Am. J. Genet.,* **42**, 651–62.

SCHERES, J.M.J.C., HUSTINX, T.W.J., WEEMAES, C.M.R. (1980) Chromosome 7 in ataxia-telangiectasia, *J. Pediatr.,* **97**, 440–1.

SCHROEDER, T.M., TILGEN, D., KRUGER, J. & VOGEL, F. (1976) Formal genetics of Fanconi's Anemia, *Human Genetics,* **32**, 257–88.

SCHULL, W.J., ISHIMARU, T., KATO, H. & WAKABAYASHI, T. (1980) Radiation carcinogenesis: the Hiroshima and Nagasaki experiences. In H.V.Gelboim *et al.* (eds) *Genetic and Environmental Factors in Experimental and Human Cancer.* Tokyo: Japanese Science Society, pp. 313–26.

SHABTAI, F., KLAR, D., HART, J. & HALBRECHT, I. (1985) On the meaning of fragile sites in cancer risk and development, *Cancer Genet. Cytogenet.,* **18**, 81–5.

SHANNON, K. (1992) Genetic alterations in leukemia: events on a grand scale, *Blood,* **80**, 1–2.

SHANNON, K.M., TURHAN, A.G., CHANG, S.S.Y. *et al.* (1989) Familial bone marrow monosomy 7: evidence that the predisposing locus is not on the long arm of chromosome 7, *J. Clin. Invest.,* **84**, 984–9.

SHWACHMAN, H., DIAMOND, L.K., OSKI, F.A. & KHAW, K.T. (1964) The syndrome of pancreatic insufficiency and bone marrow dysfunction, *J. Pediatrics,* **65**, 645–63.

STANBRIDGE, E.J. (1990) Human tumor suppressor genes, *Ann. Rev. Gene.,* **24**, 615–17.

STEVENS, M.J., LILLEYMAN, J.S. & WILLIAMS, R.B. (1978) Shwachman's syndrome and acute lymphoblastic leukaemia, *Br. Med. J.,* ii, 18.

STEWART, A., WEBB, J. & HEWITT, D. (1958) A survey of childhood malignancies, *Br.Med. J.,* 1495–1508.

STILLER, C.A., MCKINNEY, P.A., BUNCH, K.J., BAILEY, C.C. & LEWIS, I.J. (1991) Childhood cancer and ethnic group in Britain: a United Kingdom children's cancer study group (CCSG) study, *Br. J. Cancer,* **64**, 543–8.

STRATHDEE, C.A., GAVISH, H., SHANNON, W.R. & BUCHWALD, M. (1992) Cloning of cDNAs for Fanconi's anaemia by functional complementation, *Nature,* **356**, 763–7.

SUTHERLAND, G.R. (1977) Fragile sites on human chromosomes: demonstration of their dependence on the type of tissue culture medium, *Science,* **197**, 265–6.

SWIFT, M. (1971) Fanconi's anaemia in the genetics of neoplasia, *Nature,* **230**, 370–3.

SWIFT, M., SHOLMAN, L., PERRY, M. & CHASE, C. (1976) Malignant neoplasms in the families of patients with ataxia-telangiectasia, *Cancer Research,* **36**, 209–15.

SWIFT, M., CALDWELL, R.J. & CHASE, C. (1980) Reassessment of cancer predisposition of Fanconi anemia heterozygotes, *J. Nat. Cancer. Inst.,* **65**, 863–7.

SWIFT, M., MORRELL, D., CROMARTIE, E., CHAMBERLIN, A.R., SKOLNICK, M.H. & BISHOP, D.T. (1986) The incidence and gene frequency of ataxia-telangiectasia in the United States, *Am. J. Hum. Genet.,* **39**, 573–83.

TADJOEDIN, M.K. & FRASER, F.C. (1965) Heredity of ataxia-telangiectasia (Louis-Bar syndrome), *Am. J. Dis Child,* **110**, 64–8.

TAYLOR, A.M.R. (1992) Ataxia telangiectasia genes and predisposition to leukaemia, lymphoma and breast cancer, *Br. J. Cancer,* **65**, 5–9.

TAYLOR, G.M. & BIRCH, J.M. (1995) The hereditary basis of human leukaemia. In E.S. Henderson, T.A.Lister & M.F.Greaves (eds), *Leukemia* (6th edn) Philadelphia: W.B. Saunders (in press).

UNSCEAR (1982) *Sources and Effects of Ionizing Radiation.* New York: United Nations.

(1993) *Sources and Effects of Ionizing Radiation.* New York: United Nations.

URQUHART, J.D., BLACK, R.J., MUIRHEAD, M.J. *et al.* (1991) Case-control study of leukaemia and non-Hodgkin's lymphoma in children in Caithness near the Dounreay nuclear installation, *Br. Med. J.,* **302**, 687–92.

VAN DONGEN, J.J.M., ADRIAANSEN, H.J. & HOOIJKAAS, H. (1987) Immunological marker analysis of cells in the various hematopoietic differentiation stages and their malignant counterparts. In D.J.Ruiter, G.J.Fleuren & S.O.Warnaar (eds), *Application of Monoclonal Antibodies in Tumor Pathology.* Dordrecht: M.Nijhoff, pp. 87–116.

VERLANDER, P.C., LIN, J.D., UDONO, M.U. *et al.* (1994) Mutation analysis of the Fanconi Anemia gene *FACC, Am. J. Hum. Genet.,* **54**, 595–601.

VOGEL, F. (1992) Risk calculations for hereditary effects of ionizing radiation. *Human Genetics,* **89**, 127–46.

VOGEL, F. & MOTULSKY, A.G. (1986) *Human Genetics: Problems and Approaches.* Berlin: Springer-Verlag.

WAKEFORD, R., TAWN, E.J., MCELVENNY, D.M., BINKS, K., SCOTT, L.E. & PARKER, L. (1994) The Seascale childhood leukaemia cases: the mutation rates implied by paternal preconceptional radiation doses, *J. Radiol. Prot.,* **14**, 117–24.

WATSON, M.S., CARROLL, A.J., SHUSTER, J.J. *et al.* (1993) Trisomy 21 in childhood acute lymphoblastic leukemia: a pediatric oncology study group study, *Blood,* **82**, 3098– 102.

WEEMAES, C.M.R., SMEETS, D.F.C.M. & VAN DER BURGT, C.J.A.M. (1994) Nijmegen breakage syndrome: a progress report. In: Ataxia Telengiectasia: the effect of a pleiotropic gene, *Proceedings of the 6th International Workshop on Ataxia Telengiectasia, Birmingham, UK., Int. J. Radiation Biol.,* **66**, 5185–8.

WILLIAMS, D.L., LOOK, A.T., MELVIN, S.L. *et al.* (1984) New chromosomal translocations correlate with specific immunophenotypes of childhood acute lymphoblastic leukaemia, *Cell,* **36**, 101–9.

WILLMAN, C.L., SEVER, C.E., PALAVICINI, M.G. *et al.* (1993) Deletion of IRF-1, mapping to chromosome 5q31. 1, in human leukemia and pieleukemic myclodysplasia, *Science,* **259,** 968–71.

WOODS, W.G., ROLOFF, J.S., LUKENS, J.N. & KRIVIT, W. (1981) The occurrence of leukemia in patients with the Shwachman syndrome, *J. Pediatrics,* **99,** 425–8.

YOKORO, K., SEYAMA, T. & YANAGIHARA, K. (1986) Experimental radiation carcinogenesis in rodents, *Gann Monograph on Cancer Research,* **32,** 89–122.

YOUNG, N.S. & ALTER, B.P. (1994) *Aplastic Anemia: Acquired and Inherited.* Philadelphia: W.B.Saunders.

YUNIS, J.J. (1984) Fragile sites and predisposition to leukemia and lymphoma, *Cancer Genet. Cytogenet.* **12,** 85–8.

10
Experimental Radiation Leukaemogenesis

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10
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10.1
Early Studies on Radiation Leukaemogenesis

Only sporadic reports of induction of leukaemias by radiation appeared in the literature until the 1950s. Systematic studies were then undertaken by Kaplan and Brown (1952), Upton *et al.* (1958, 1960b) and Mole (1958) in their pioneering studies, in mice, of radiation-induced thymic lymphosarcoma, lymphoid leukaemias and myeloid leukaemias. These were painstaking experiments involving large numbers of animals which were screened for long periods, in many cases requiring lifetime studies. Several different types of leukaemia can be induced in animals following exposure to ionising radiations (Table 10.1).

Table 10.1 Early papers describing experimental radiation-induced leukaemogenesis

Leukaemia type	Authors
Myeloid/myelomonocytic	Upton *et al.,* 1958
	Upton *et al.,* 1960b
	Major, 1979
	Mole *et al.,* 1983
	Ullrich and Storer, 1979a
Thymic lymphoma	Kaplan and Brown, 1952
	Ullrich *et al.,* 1976
	Ullrich and Storer, 1979a
Lymphoid tumours and leukaemias	Mole, 1958
	Loutit and Carr, 1978
Erythroblastic	Yoshida *et al.,* 1986
	Seed *et al.,* 1985
Megakaryoblastic	Inoue *et al.,* 1983
	Seed *et al.,* 1985

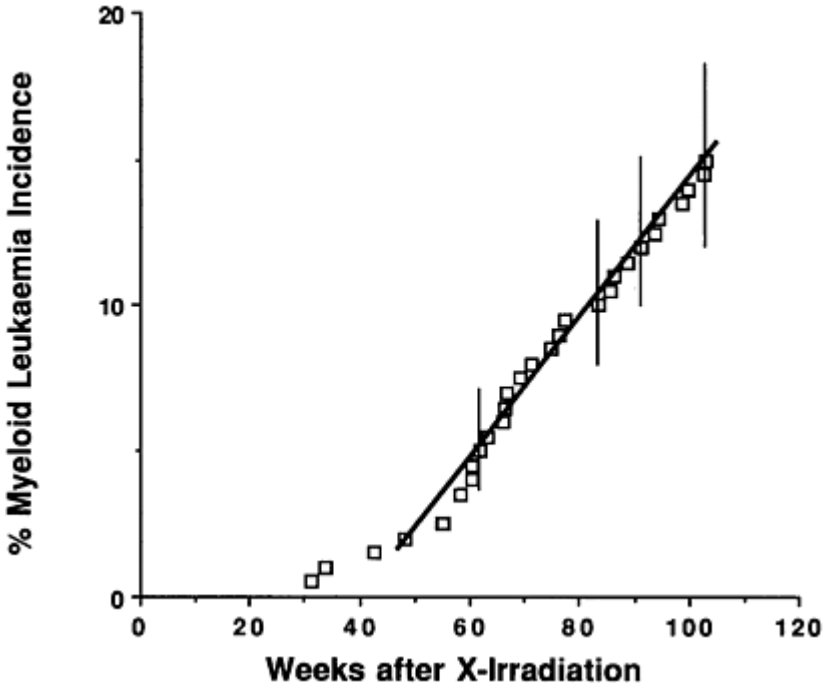

Figure 10.1 The incidence of myeloid leukaemia with time in CBA/H male mice exposed to 3 Gy whole-body X-irradiation

10.2 Morphological Appearances and Properties of Experimental Radiation-induced Leukaemias

Following exposure to whole-body ionising radiation, leukaemias and lymphomas develop in a variety of mouse strains. Myeloid leukaemia is the main type of leukaemia induced by radiation exposure. Thymic lymphomas and non-thymic lymphomas are also induced by irradiation and may involve the spread of malignant cells into the blood (leukaemia).

In general, the latent period is long. For myeloid leukaemias in CBA/H male mice, the first leukaemias appear about 225 days after irradiation and there is a linear increase in incidence over the lifespan of the animal (Figure 10.1). The latent period for appearance of non-lymphocytic leukaemias in dogs was in the range 300–2000 days (Seed *et al.,* 1985). In contrast, thymic lymphomas appear from about 100 days after irradiation and reach a peak incidence after 200–250 days. The non-thymic lymphomas increase in incidence with dose but appear late after irradiation.

The appearance of the myeloid leukaemias is associated with infiltration of many internal body organs. In particular, the spleen is almost always involved, as is the liver. Histological examination of the liver reveals extensive infiltration of leukaemic cells in the portal areas with extension into the sinusoids. The pattern of haemopoiesis in the bone marrow is also markedly changed. Histological examination of the sternum often reveals bone erosion and infiltration into the surrounding muscle. In normal bone marrow, the broad spectrum of different haemopoietic lineages can be seen, with cells of the erythroid, myeloid and lymphoid lineages prominent (Figure 10.2). In the mouse, the ring or doughnut shaped nucleus of metamyelocytes is characteristic. The leukaemic bone marrow consists predominantly of early blast cells with varying

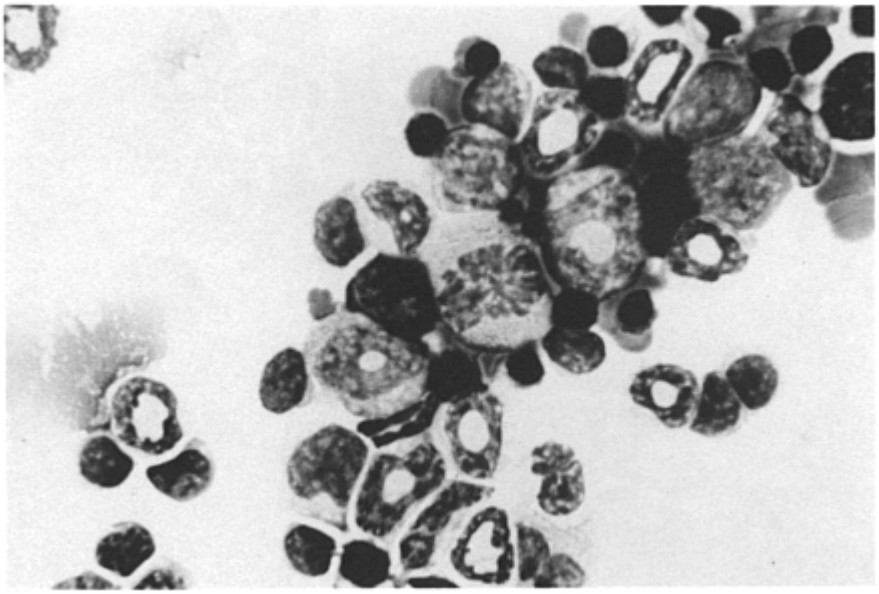

Figure 10.2 Cytospin preparation from the bone marrow of an unirradiated control CBA/H mouse. Note the range of different cell lineages present and the prominent ring or 'doughnut' metamyelocytes. (×1000)

proportions of promyelocytes, myelocytes and metamyelocytes (Figure 10.3). Similarly large numbers of these cells can be found in the blood (Figure 10.4). In the bone marrow, there is a marked shift in the differential count towards an increase in the immature granulocytes (Figure 10.5). Similarly in the peripheral blood, immature granulocytes, which are not seen in the normal unirradiated animal, now appear (Figure 10.5). Diagnosis of radiation-induced myeloid leukaemia often requires histological examination of tissues as the disease frequently remains asymptomatic. The radiation-induced myeloid leukaemias are positive for myeloperoxidase, napthol-ASD chloroacetate and -glucuronidase but negative for acid phosphatase and esterase (Resnitzky *et al.,* 1985).

Two lymphocytic forms of leukaemia are observed (Figure 10.6). The radiation-induced tumours induced following repeated doses of X-irradiation in the C57BL strain are thymic lymphomas. These exhibit a lymphoblastic form with metastatic spread to the lympho-myeloid complex often with malignant cells in the blood. These lymphoblasts from lymphomas have leptochromatic nuclei usually with two or three nucleoli and scanty deep blue cytoplasm (Figure 10.6a). A typical chronic lymphocytic leukaemia pattern is usually associated with chemically-induced carcinogenesis and is not usually observed following radiation (Figure 10.6b). A more detailed description of leukaemias and tumours of the lympho-myeloid complex in the mouse can be found in Dunn (1954).

If the leukaemias are induced in inbred strains, then the leukaemogenicity of the cells can be tested by transplantation into secondary syngeneic recipients. Meldrum and Mole (1982) have shown that the growth of transplanted myeloid leukaemic cells are not modified by prior sublethal irradiation exposure of the recipient or by attempts to immunise the recipient with radiation-inactivated leukaemic cells. As the number of leukaemic cells injected is reduced, a cell concentration is eventually reached where there is an average of one clonogenic cell per injection volume. At this concentration (a Poisson mean of one clonogenic leukaemic cell per innoculum) 63 per cent of recipients develop leukaemia while 37 per cent receive no clonogenic cells and thus are leukaemia-free. Typical values for primary myeloid leukaemias are in the

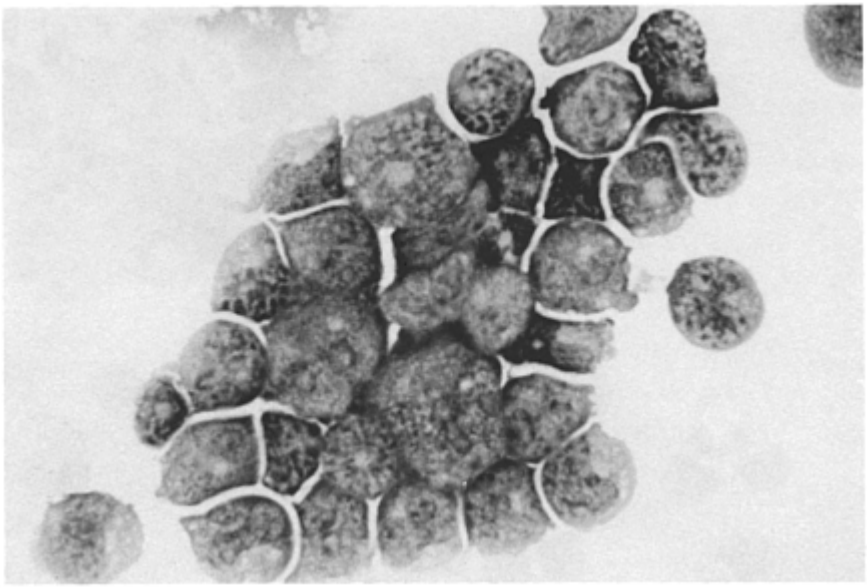

Figure 10.3 Cytospin preparation from the bone marrow of an irradiated CBA/H male mouse containing numerous leukaemic cells. This is an example of a primary radiation-induced myeloid leukaemia. The bone marrow contains an increased proportion of immature granulocyte precursors. (×1000)

range 1 clonogenic cell per 10^2 to 10^4 cells, i.e. a clonogenic fraction of 10^{-2} to 10^{-4} (Meldrum and Mole, 1982). If the leukaemias are passaged at a high cell dose, then the number of cells required for 'a take' decreases markedly after several passages (Hepburn *et al.,* 1987). Typically for many murine leukaemias, this can be as low as 1–10 cells. This is associated with morphological changes in the leukaemic population with a more primitive phenotype emerging. In contrast, a much larger number of cells is required to passage a primary leukaemia (10^3 to 10^4 cells). This pattern can be retained if at each passage the cells are injected at this minimum cell dose (Hepburn *et al.,* 1987). Thus the primary leukaemias, like most solid tumours, have a small stem cell fraction capable of maintaining the malignant clone.

10.3
Radiation Dose-Response Relationships for Different Leukaemias

Radiation dose response relationships and comparisons with other radiation-induced malignancies are reviewed in the UNSCEAR report (1986) and by Fry (1981, 1984) and Upton (1984, 1985).

Gray (1965) and Mole (1975) addressed the problem of dose-response relationships in radiation carcinogenesis. They concluded that the induction of tumours by low LET radiation conforms to the hypothesis that the induction process is dependent on a power function of the radiation dose (D^2) but modified by the fact that a dose-dependent sterilisation effect reduces the tumour yield. Careful analysis of the dose-response relationships of myeloid leukaemia induction in CBA/H mice reflected this effect. These mice have virtually a zero incidence of the disease in unirradiated controls. Following exposure to low but increasing doses of X-irradiation, the incidence of myeloid leukaemia in CBA/H male mice increased to reach a maximum of 20 per cent following exposure to 3 Gy whole-body X-irradiation (Major and Mole,

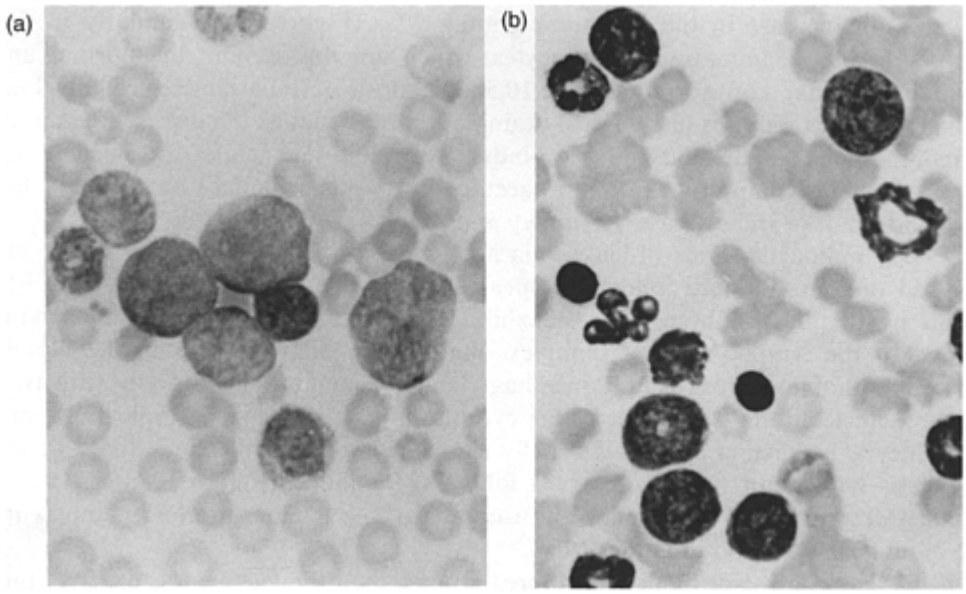

Figure 10.4 Blood films from CBA/H male mice with primary radiation-induced myeloid leukaemia. The nucleated cell count is markedly increased and examples of immature granulocyte precursors are found in the peripheral blood. (×1000)

1978; Major, 1979; Mole *et al.,* 1983). Increasing the dose further reduced the incidence of leukaemia until by 6 Gy this was reduced to <2%. This gave a relationship of the form:

$$Y=aD^2e^{-bD}$$

where Y is the yield of myeloid leukaemias, D is the radiation dose and a and b are constants.

This was interpreted as a leukaemia induction process proportional to D^2, implying the interaction of two targets damaged by ionising radiation. Subsequent survival of these transformed or potential leukaemic cells would be dose-dependent and proportional to e^{-bD}. Thus the balance of leukaemia production is an interaction between malignant transformation increasing with dose against cellular inactivation markedly increasing with dose. A similar dose-response relationship was reported by Di Majo *et al.* (1986).

Comparisons between the induction of different types of leukaemia have been undertaken by several laboratories. Upton *et al.* (1958, 1960b) compared the incidence of thymic lymphoma and myeloid leukaemia in RF and C57BlxA mice. These data were analysed more fully taking into account competing risks (Robinson and Upton, 1978) and comparing the incidence of thymic lymphoma, myeloid leukaemia and reticulum cell sarcoma (Figure 10.7). This early work was followed up by Ullrich and Storer (1979a), who also included reticulum cell sarcoma. Mole (1986a) has also compared the incidence of myeloid leukaemia and thymic lymphoma in the CBA/H strain. The conclusions of these analyses were broadly similar. Thymic lymphoma incidence increases with radiation dose:

$$Y=aD^2+c$$

where Y is the yield of lymphomas, D is the radiation dose and a and c are constants (Mole, 1986a). As we have seen, the incidence of myeloid leukaemia reaches a peak and then decreases with higher doses above 2–3 Gy. The incidence of lymphosarcoma and reticulum cell sarcoma decreases with increasing radiation dose (Figure 10.7). However, the incidence of these tumours is relatively high in unirradiated control mice of this strain.

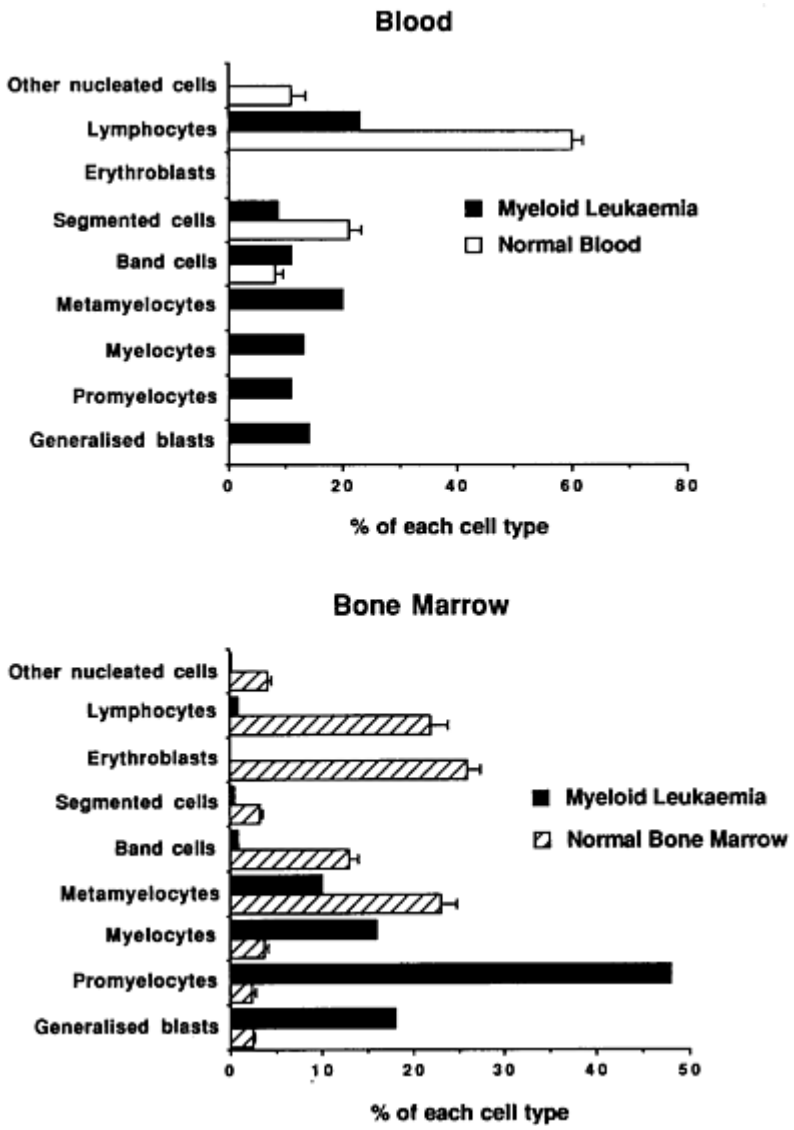

Figure 10.5 Differential cell counts of peripheral blood and bone marrow comparing normal unirradiated controls (CBA/H mice) with a typical example of a radiation-induced myeloid leukaemia in this strain

Fractionated exposure of C57Bl/Ka mice (4×1.75 Gy at weekly intervals) results in a very high incidence of thymic lymphoblastic lymphomas (Kaplan and Brown, 1952). Different time and dose schedules were used in these studies, however, to define an optimal protocol for induction.

In a case control study of 150 000 women treated with radiation for invasive cancer of the uterine cervix, an increase in acute myeloid and chronic myeloid leukaemia was observed with no increase in chronic lymphocytic leukaemia (Boice *et al.,* 1987). The relative risk increased with radiation dose to the bone

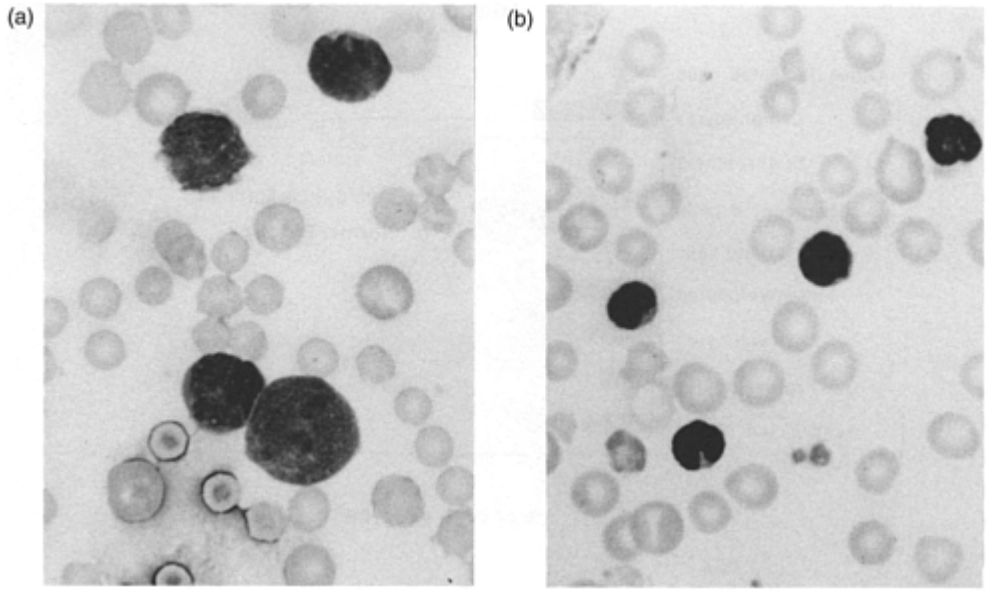

Figure 10.6 (a) Blood film from a CBA mouse with a primary lymphoid leukaemia. Note the lymphoblast and atypical lymphocytes present in the peripheral blood (×1000); (b) blood film from a CBA mouse with a primary leukaemia. Note the presence of large numbers of small lymphocytes. This chronic lymphocytic leukaemic pattern is associated with chemically-induced leukaemias and is not usually observed following irradiation (×1000)

marrow but decreased at higher doses and thus was of a similar pattern to that observed in mice with a quadratic-exponential relationship with dose (see Chapter 11).

10.4
Influence of Age at Exposure on Leukaemia Incidence

The age and the sex of the animal both influence the incidence of radiation-induced leukaemia. Age at exposure has a marked effect on the leukaemia incidence. Irradiation of newborn animals produces a low incidence of myeloid leukaemia (6 per cent) compared with adults (45 per cent) but has less effect on thymic lymphoma incidence (Upton *et al.,* 1958). Irradiation at 6 months of age gave the opposite effect. Young animals in general were more refractory to both myeloid leukaemia and thymic lymphoma induction, the susceptibility increasing rapidly in 14–28-day-old animals (Upton *et al.,* 1960a). Males were more susceptible to myeloid leukaemia induction and females to thymic lymphoma induction. Later experiments indicated a difference in the age response between RF mice exposed to single doses of X-irradiation at 5–6 or 9–10 weeks of age. The young mice were less sensitive than the older mice to myeloid leukaemia induction (Robinson and Upton, 1978).

Irradiation at low dose rates reduced the incidence of lymphatic leukaemia in the NMRI mouse strain (Schmahl, 1988). Foetal irradiation in beagles had no effect on leukaemia incidence provided irradiation was restricted to the gestation period (Seed *et al.,* 1987). Leukaemia was not observed in 71 irradiated offspring. If, however, the radiation exposure was continued into the postnatal period then a very high incidence of leukaemia was observed.

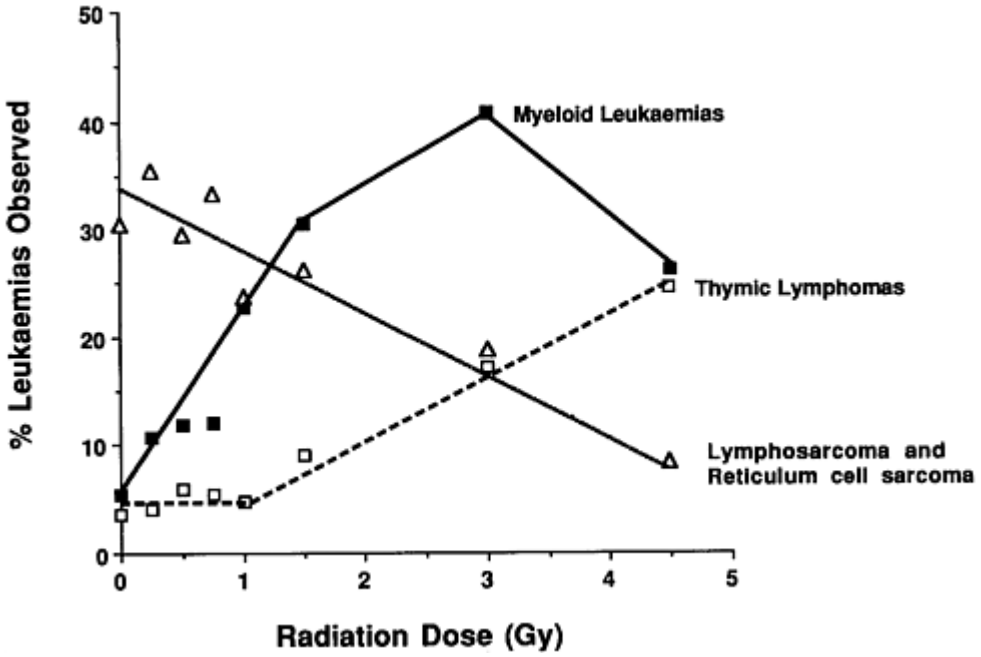

Figure 10.7 Dose-response relationships for induction of myeloid leukaemia, thymic lymphomas and lymphosarcomas and reticulum cell sarcoma following exposure of RF/Un mice to X-irradiation. (Plotted from Robinson and Upton, 1978)

10.5
Radiation-induced Leukaemia following Exposure to High LET Radiations

Like irradiation with low LET radiations, exposure to high LET radiations is also leukaemogenic. There was an increased incidence of myeloid leukaemia in male RF mice following exposure to doses of less than 2 Gy of neutron irradiation (Upton *et al.,* 1970). Fractionating the dose daily gave a similar increase in incidence. With thymic lymphomas, daily doses of neutron irradiation were more effective than the single acute doses in female mice. Ullrich *et al.* (1976) investigated the effects of dose and dose rate on leukaemia incidence in RF mice exposed to neutrons. In the lower dose range, there was little effect on myeloid leukaemia incidence. The incidence of reticulum cell sarcoma, which is high in unirradiated mice (63 per cent), decreased with increasing neutron dose. For thymic lymphoma, the incidence increased up to 40 per cent after 1 Gy acute dose of neutrons while the incidence following -irradiation was lower over this dose range. There was little difference between the incidence in the low dose rate and acute-dose groups following neutron irradiation at doses up to 1 Gy. Above this dose, there was a higher incidence in the low dose rate group (1 cGy per day) than in the acute-dose group.

Extensive studies in CBA/H male mice using fission neutrons demonstrated that the incidence of myeloid leukaemia increased in the range 2–200 cGy reaching a peak incidence of 22 per cent (Mole and Davids, 1982; Mole, 1984). The yield fitted the following equation:

$$Y=aD\mathrm{e}^{-bD}$$

where Y is the yield of myeloid leukaemias, D is the neutron dose and a and b are constants. This contrasts with low LET radiation where the first term of this equation is dependent on the square of the dose.

Myeloid leukaemias have also been induced following exposure to monoenergetic fast neutrons although the detailed dose-response relationships have not been defined (Rithidech *et al.,* 1993).

10.6
Radionuclide Induction of Leukaemias

Concern over the release of radionuclides into the environment has prompted research into the long-term effects of radionuclides in the body. Many of these radionuclides are bone-seeking radioisotopes and can induce osteosarcoma. However, there are reports of leukaemia induction in experimental studies.

Following a single injection of either strontium-90 or plutonium-239, at appropriate doses, generalised lymphomas in CBA/H mice can be induced (Loutit and Carr, 1978). Ingested -emitters, at levels which induce osteosarcoma, appear very rarely to cause leukaemia in man. This low incidence could be explained by the relatively high radiation doses received resulting in death of exposed target cells rather than their mutation (Thorne and Vennart, 1976). It was thus postulated that lower levels of radionuclide might be leukaemogenic.

Early reports of the induction of myeloid leukaemia by plutonium-239 administration were difficult to interpret as the incidence of myeloid leukaemia was high in the control animals (Svoboda *et al.,* 1981). Muller *et al.* (1988) demonstrated that protraction of a relatively small dose from radium-224 (3.6 day half-life) resulted in the early induction of lymphomas (13 per cent) occurring within one year. Administration of the same activity in a single injection did not cause any cases of leukaemia in this early observation period. Again there was a high incidence of spontaneous occurrence of lymphomas in the mouse strain used (NMRI), reaching an incidence of 45 per cent in some cases. These spontaneous tumours, however, appeared late in the mouse's lifespan.

Myeloid leukaemias were detected in CBA/H mice which received single or multiple injections of radium-224 (Humphreys *et al.,* 1985). It was not possible to apply rigorous statistical analysis to the data in view of the relatively small number of leukaemias induced (eleven myeloid leukaemias). Like fission neutrons, bone-seeking -emitters emit high LET irradiation and it is not surprising, therefore, that the relationship for myeloid leukaemia incidence *(Y)* and dose *(D)* was of a similar form, namely:

$$Y = aD\mathrm{e}^{-bD}$$

where a and b are constants.

In a larger follow-up series, the results of the earlier experiments and the form of the dose relationship were confirmed (Humphreys *et al.,* 1993). In all, 62 myeloid leukaemias in 1600 mice were detected in the radium-224 treated groups. Therefore there was a dose range for which increased radiation exposure resulted in an increased incidence of leukaemia. In the same experiment, the incidence of osteosarcoma increased linearly with radiation dose. At the lower injected doses of this radionuclide, myeloid leukaemia presented a greater risk than osteosarcoma.

The effect of administration of plutonium-239 in the range 1.85–18.5 kBq kg^{-1} as a single injection or as multiple injections over an eight-week period were reported by Humphreys *et al.* (1987). Myeloid leukaemias were observed in these CBA/H female mice in both the single (1/117 mice) and multiple (4/133 mice) injected groups. The numbers were too small to make any meaningful predictions of the dose-response relationship. Small numbers of lymphatic leukaemias, reticulum cell sarcomas and thymic lymphosarcomas were also induced.

In the ICR mouse strain, Svoboda and Bubenikova (1990) demonstrated that plutonium-239 caused the late developing spontaneous leukaemias to occur earlier. However, they could not detect any increase in incidence. The CBA/H mouse strain has a virtually zero incidence of myeloid leukaemia (1 case reported in

more than 1400 mice) and so has major advantages in detecting radiation-induced leukaemias compared with other strains with a high background incidence.

10.7
Effects of Fractionation and Dose Rate of Exposure

The leukaemia incidence in patients with ankylosing spondylitis, who received fractionated doses of X-irradiation, did not increase with increasing bone marrow dose. Mole and Major (1983) used the CBA/H myeloid leukaemia model to investigate the dose-response relationships following exposure to ionising radiation protracted over several weeks. The incidence of myeloid leukaemia increased from 0 to 25 per cent in mice receiving 0–4.5 Gy whole-body -irradiation at a dose rate of 0.25 Gy min^{-1}. When, however, the same dose was given either as a continuous dose at a dose rate of 0.04–0.11 mGy min^{-1} or as five brief daily exposures for four weeks in total (twenty exposures), then the incidence of myeloid leukaemia was 5 per cent. Thus the incidence was reduced compared with a single exposure and was also dose-independent for both treatments. The shape of the dose-response relationship for protracted irradiation below 1.5 Gy has still to be established.

This type of response is also consistent with the results from an extensive study on women with invasive cancer of the uterine corpus, the majority of whom were treated at older ages (Curtis *et al.,* 1994). Treatment consisted of external beam therapy delivered either as a fractionated relatively high dose rate treatment (10–40 cGy min^{-1}) or brachytherapy with continuous low dose rate treatment (0.04 cGy min^{-1}). The increased relative risk of leukaemia was similar for both types of treatment and indicated a largely constant, increased risk across the entire dose range. The risk from protracted exposure was lower than that for a single instantaneous whole-body exposure (see Chapter 11).

Dose rate effects have been described in several studies. Upton *et al.* (1970) investigated the incidence of myeloid leukaemia in RF mice following -irradiation at high (80 cGy min^{-1}) and low (0.06 cGy min^{-1}) dose rates. The chronic exposures resulted in a low incidence of leukaemia with a flat dose-response relationship. A similar effect was observed in female RF mice (Ullrich and Storer, 1979b). In contrast, the incidence of thymic lymphomas was slightly lower at low doses but similar at higher doses following protraction of the dose in female RF mice. Ullrich and Storer (1979b) found the converse in male RF mice where thymic lymphoma incidence was much less following protracted irradiation than after a single exposure and was also dose-independent. At high dose rates (550 cGy min^{-1}), the leukaemia incidence was increased in CBA/H mice compared with that after dose rates of 50 and 4 cGy min−1 (Mole, 1986a).

Early experiments indicated that fractionating the exposure had a small effect on leukaemia incidence (Upton *et al.,* 1958). The time interval between doses is the critical factor. Three fractions of 1.5 Gy separated by 2 days slightly lowered the incidence of both myeloid leukaemia and thymic lymphoma compared with 4.5 Gy as a single exposure, whereas three fractions separated by 5 days had no effect on myeloid leukaemia incidence but slightly increased thymic lymphoma incidence. Resnitzky *et al.* (1985) found similar responses in relation to myeloid leukaemia where two 1.5 Gy fractions separated by 2 days decreased the leukaemia incidence relative to 3 Gy in a single dose but three 1.5 Gy fractions separated by 3 days increased the incidence slightly.

A more detailed study by Mole (1986a) investigated the relationship between myeloid leukaemia incidence after two equal doses of X-irradiation separated by different times. Little change in incidence occurred following two equal doses of 1.25 Gy separated by intervals of 1–48 hours. At longer time intervals of 3–7 days, the incidence decreased quite markedly from 24–18 per cent to 6.6 per cent. It would

seem that fixation of an initial event is required for induction of acute myeloid leukaemia and that this process may require several days to be completed.

Continuous -exposure for 22 hours to CBA/H male mice gave a similar form to the dose-response curve for myeloid leukaemia, namely:

$$Y=aD^2e^{-bD}$$

when compared with single -exposure. However, the incidence was lower at the lower doses but increased at higher doses. Compared with the shape of the dose-response for single brief exposure, the bell-shaped curve was flatter and was shifted to higher doses for the continuous irradiation data.

10.8
Treatments which Modify Leukaemia Induction

Following exposure to acute whole-body doses of X-irradiation, defects in the bone marrow can be detected months after the initial exposure. For example, in the rat it has been demonstrated that following an acute exposure of 1.7 Gy, the ability of the bone marrow to respond to an anaemic stress is compromised in the irradiated animals. This was interpreted as an imbalance in cell output from the stem cell compartment into the myeloid compartment at the expense of the erythroid compartment. This suggested the idea that prolonged anaemic stress in irradiated animals might result in abnormally severe and uncontrolled granulocytosis. Following anaemic stress induced by two bleeds of 1/3 blood volume each time, the irradiated animals all developed leukaemia. The timing of the anaemic stress was not critical and occurred 1–3 months after the initial radiation exposure. Thus 100 per cent of irradiated and bled animals developed leukaemia by 16 months compared with between 5 and 40 per cent of irradiated-only animals, depending on the radiation dose (Gong, 1971).

The RF mouse strain is also susceptible to anaemic stress. The spontaneous incidence in this strain is about 4 per cent but this can be increased to virtually 100 per cent following anaemic stress only (Gong *et al.,* 1972). Therefore the RF strain seems particularly susceptible to myeloid leukaemia induction. However, other groups have not been able to confirm these studies (Coggle and Gordon, 1976).

The co-leukaemogenic effect of combining radiation with other agents has also been reported by several groups. The responses of steroids indicated that the incidence of myeloid leukaemia, which does not develop spontaneously in SJL/J mice, could be increased from about 20 per cent incidence following 3 Gy treatment to 50 per cent when combined with steroid treatment (Resnitzky *et al.,* 1985). Both hydrocortisone acetate administered before and after irradiation or before only with prednisone administered after irradiation increased the incidence of myeloid leukaemia. These corticoid treatments reduced the spontaneous incidence of reticulum cell neoplasms in these mice. An increased incidence of myeloid leukaemia was observed only when metyraprone was administered in the drinking water after irradiation. ACTH had no co-leukaemogenic effect. Prednisone combined with thymectomy and irradiation also further increased the incidence of myeloid leukaemia. Combining prednisone and cytophosphan with radiation exposure increased the incidence of myeloid leukaemia even further to 80 per cent (Resnitzky *et al.,* 1987; Haran-Ghera *et al.,* 1992).

Splenectomy has a marked effect on the incidence of myeloid leukaemia in RF mice decreasing from an incidence of 45 per cent (3 Gy) to 16 per cent (Upton *et al.,* 1958). Similarly, partial body shielding also decreased the incidence from 29 per cent (4.5 Gy) to 11 per cent. In both cases the decrease is much greater than might be expected in relation to the number of potential target cells in the haemopoietic tissues that were shielded. In contrast, splenectomy has no effect on thymic lymphoma induction whereas partial body

shielding reduces the lymphoma incidence. As might be predicted, thymectomy abolishes the production of lymphomas but has no effect on myeloid leukaemia incidence (Upton *et al.,* 1958).

Acute myeloid leukaemia is induced also as a secondary neoplasm in Hodgkin's disease patients in remission. Those patients who did not receive alkylating agents or corticosteroid agents in their therapy had a lower incidence of secondary tumours (Valagussa *et al.,* 1982).

The incidence of radiation-induced myeloid leukaemia can also be influenced by the administration of haemopoietic growth factors. Administration of these growth factors or cytokines can both promote and prevent AML development depending on the time of administration of these factors relative to the radiation exposure. The incidence of myeloid leukaemia in irradiated and dexamethasone treated SJL/J mice is increased following injection of CSF-1, 14 days after irradiation, whereas rGM-CSF has no effect unless administered 140 days after irradiation. Conversely, rIL-6 inhibits AML production (Haran-Ghera *et al.,* 1992).

Following 3 Gy whole-body X-irradiation, cytokines were differentially induced in different tissues and the kinetics of production and levels induced also varied with the mouse strain studied (Tartakovsky *et al.,* 1993). Thymus and spleen were poor producers of CSF-1 and IL-6. By contrast, there was a marked increase in CSF-1 production in the bone marrow a few days after irradiation in SJL/J mice and also, though less dramatic, in BL/6 mice. The converse was true in relation to IL-6 production in the lung. Exposure of BL/6 mice to split-dose irradiation induces thymic lymphomas. Using an interval of 14 days (4×1.7 Gy) between exposure results in 20 per cent incidence of lymphoma. This can be increased to 62 per cent by injection of CSF-1 and IL-6. Similarly, the incidence of myeloid leukaemia in SJL/J mice can be increased from 20 per cent (3 Gy only) to 53 per cent when CSF-1 is also administered. Dexamethasone also increases both CSF-1 production and myeloid leukaemia incidence in these irradiated SJL/J mice (Tartakovsky *et al.,* 1993).

Injection of syngeneic bone marrow cells after the initial irradiation exposure modified the pattern of AML appearance but has no effect on overall incidence (Mole, 1986a, 1988). Administering 3×10^7 cells 3 days after irradiation shortens the latent period of appearance of leukaemia. Virtually all the leukaemias had developed by 400 days after irradiation and bone marrow transplantation whereas to reach the same incidence following irradiation only took 550 days. It is difficult to see why addition of unirradiated cells should influence the latent period. The timing of the repopulation is also critical as injection 1 day after irradiation has no effect. It is interesting to note that the latent period of AML appearance in ankylosing spondylitis, after localised and fractionated X-irradiation, is shorter than that observed in Japanese bomb survivors who received brief whole-body exposure (Mole, 1986b).

It has also been argued that administration of normal bone marrow cells to irradiated mice might reduce the proliferation pressure on irradiated target cells and thus delay the incidence of radiation-induced leukaemia. Following 3 Gy whole-body X-irradiation to CBA/Ca female mice, the incidence of myeloid leukaemia observed was 16 per cent with a latency of 450 days. If these mice received a bone marrow transplant of 2×10^7 cells at 1, 7 and 14 days after irradiation, the leukaemia incidence remained at 19 per cent and thus was not significantly increased but the onset of leukaemia was delayed from 450 days to 830 days (Cronkite *et al.,* 1987). This was interpreted as implying that a certain number of divisions were required in the potentially leukaemic cells before overt leukaemia develops.

The leukaemia incidence has also been investigated after *in utero* irradiation followed by treatment with a chemical carcinogen (ethylnitrosourea). Radiation alone lowers the incidence of leukaemias observed. These were mainly lymphatic leukaemias with some myeloid leukaemias and reticulosarcomas also observed. Addition of the chemical carcinogen (ethylnitrosourea) administered 3 weeks postnatally resulted in a synergistic induction of leukaemia (Schmahl, 1988).

10.9
Radiation-induced Leukaemia in Dogs

Continuous irradiation of young adult beagles with -irradiation results in the induction of leukaemia (Seed *et al.,* 1985). The majority of leukaemias induced are myeloid, monocytic or myelomonocytic (78 per cent) with some erythroleukaemias (20 per cent) and megakaryocytic leukaemias (2 per cent). Two patterns of response were observed. A radiosensitive group had a short lifespan associated with aplasia in the bone marrow while a radioresistant group was relatively longer-lived and leukaemia prone. The transition between one pattern and the other was dose rate dependent and occurred at 90 per cent survival following 3.8 cGy day^{-1}, at 40 per cent survival following 7.5 cGy day^{-1} and at 30 per cent survival following 12.8 cGy day^{-1}.

Plotting dose rate against myeloid leukaemia incidence gave a peak incidence of >30 per cent at 3.8 cGy day^{-1} with decreasing incidences at both higher and lower dose rates. Continuous exposure throughout life was much more leukaemogenic than continuous irradiation given for a limited period up to a fixed accumulated dose.

The leukaemic responses were associated with an initial phase of haemopoietic suppression followed by the emergence of a phase of acquired radioresistance of the haemopoietic progenitors (Seed *et al.,* 1987).

Myelogenous leukaemias were induced in beagles following exposure to -irradiation. Examination of the DNA isolated from these leukaemic cells revealed that transforming sequences could be detected using the NIH 3T3 mouse fibroblast assay in three of the four leukaemias examined (Frazier *et al.,* 1987).

10.10
Leukaemia Induction *in vitro*

Haemopoiesis can be maintained *in vitro* provided an appropriate adherent stromal layer which supports the proliferation and differentiation of haemopoietic stem cells is established (Dexter, 1979; Dexter *et al.,* 1984). Leukaemic cells from the radiation-induced myeloid leukaemias can be maintained *in vitro*. However, in the majority of cases, the leukaemic cells do not survive well in culture and persist in small numbers, exhibiting a growth pattern like that of minimal residual disease (Riches *et al.,* 1991). The production of various growth factors from these long-term bone marrow cultures is increased following exposure to X-irradiation (Gualtieri *et al.,* 1984; Naparstek *et al.,* 1985). A similar effect is observed following X-irradiation of a cloned bone marrow stromal cell line (Naparstek *et al.,* 1986b).

Haemopoietic cell lines have previously been established from long-term bone marrow cultures. They depend for continuous growth in liquid culture on the addition of specific growth factors and thus are termed factor-dependent cell lines (Dexter *et al.,* 1980; Greenberger, 1980). Co-cultivation of some of these factor-dependent cell lines with an irradiated stromal cell line, for longer than five weeks, resulted in the establishment of factor-independent clones (Naparstek *et al.,* 1986a). The stromal cells were exposed to 50–100 Gy X-irradiation. The factor-independent clones were tumorigenic in syngeneic recipients and produced leukaemic involvement of the spleen, lymph node and bone marrow. The local tumours consisted of primitive blast cells with a myeloid or myeloid/monocytic form (Naparstek *et al.,* 1986a).

The process of transformation was not coupled with autocrine production or expression of mRNA for the growth factors which are normally required for their growth. It was proposed that irradiation of these stromal cells at high doses (>50 Gy) resulted in the production of a new growth factor or blocking of the existing growth factor pathway (Naparstek *et al.,* 1986a, b). Evidence is now accumulating that chronic radiation exposure of stromal cells also results in the establishment of factor-independent clones (Seed *et al.,* 1994) (see also Chapter 5).

10.11
Potential Leukaemic Cells in Experimental Leukaemias

The presence of potential leukaemic cells can be detected in animals exposed to carcinogens. Test cells are usually injected into a secondary host, which may have been also irradiated, to allow expression of their leukaemic potential.

Haran-Ghera (1973) demonstrated the presence of latent potential leukaemic cells following DMBA treatment. Bone marrow from carcinogen treated animals was transplanted to irradiated recipients. These secondary recipients then developed a high incidence of lymphatic leukaemia. Later studies using AKR mice, which spontaneously develop leukaemia, demonstrated potential leukaemic cells in the bone marrow (Haran-Ghera, 1980). Boniver *et al.* (1981) also detected potentially neoplastic cells in the thymus of mice following fractionated irradiation. Later work demonstrated the continuous accumulation of preleukaemic cells in the thymus after detecting low numbers initially after irradiation (Kotler *et al.,* 1994). From this input, a clone was selected which expanded further to develop into a frank lymphoma. Potential leukaemic cells have also been demonstrated in the spleen of irradiated RFM mice (Bessho and Hirashima, 1982).

Several studies, using *in vitro* and *in vitro/in vivo* systems, have tried to address the problem of the importance of initial versus late events in radiation carcinogenesis. There is evidence from these experiments that the initial radiation event is common and that subsequent further events required for transformation are rare. Kennedy *et al.* (1980) demonstrated that the number of transformed foci produced following exposure of C3H 10T1/2 cells to irradiation, is independent of the number of cells replated after an initial 2-week period. Cells could be diluted and repassaged at 1 in 10 to 1 in 10000 dilution and produce virtually the same number of transformed clones. Similarly, Gould (1984) showed, using an *in vitro/in vivo* transplantation assay, that the number of radiation-induced thyroid tumours produced from rat thyroid cells irradiated *in vitro* was independent of the cell number injected. Thus in both cases this was interpreted as strong support for a common radiation-induced initial event followed by selection of a rare secondary event to give rise to the transformed state.

We have found the incidence of myeloid leukaemias in CBA/H male mice following exposure to 3 Gy whole-body X-irradiation reached a cumulative incidence of 15 per cent (20–11 per cent, 80 per cent binomial confidence limits) by 2 years postirradiation (Figure 10.1). Leukaemias were observed from 32 weeks onwards and diagnosis was confirmed from blood smears and histological examination. The presence of potential leukaemic cells was investigated by transplanting the bone marrow 40 weeks following exposure to 3 Gy whole-body X-irradiation. The bone marrow from two femora was transferred from one donor into one sublethally irradiated syngeneic recipient (4.5 Gy), a one-to-one transfer each time.

The incidence of myeloid leukaemia in two such transplantation experiments was markedly increased compared to the incidence *de novo* (Figure 10.8). The cumulative incidence was 40 per cent (50–30 per cent; 80 per cent binomial confidence limits) and 61 per cent (71–50 per cent; 80 per cent binomial confidence limits) in these two experiments. Mice receiving spleen cells from donors irradiated with 3 Gy 40 weeks previously, also developed a high incidence of myeloid leukaemia (28 per cent). The leukaemias were observed between 9 and 19 weeks post-bone-marrow transplantation and between 12 and 40 weeks post-transplantation of spleen cells. The presence of the leukaemias was confirmed from blood smears and histological examination in all animals and twenty of the leukaemias were retransplanted and grew progressively.

Control mice that were sublethally irradiated (4.5 Gy) and transplanted with normal bone marrow cells did not develop leukaemia during the period of 36 weeks post-transplantation.

The effects of time after the initial radiation exposure on leukaemia incidence were also studied using this transplantation assay. Sublethally irradiated recipients received bone marrow cells from 3 Gy irradiated

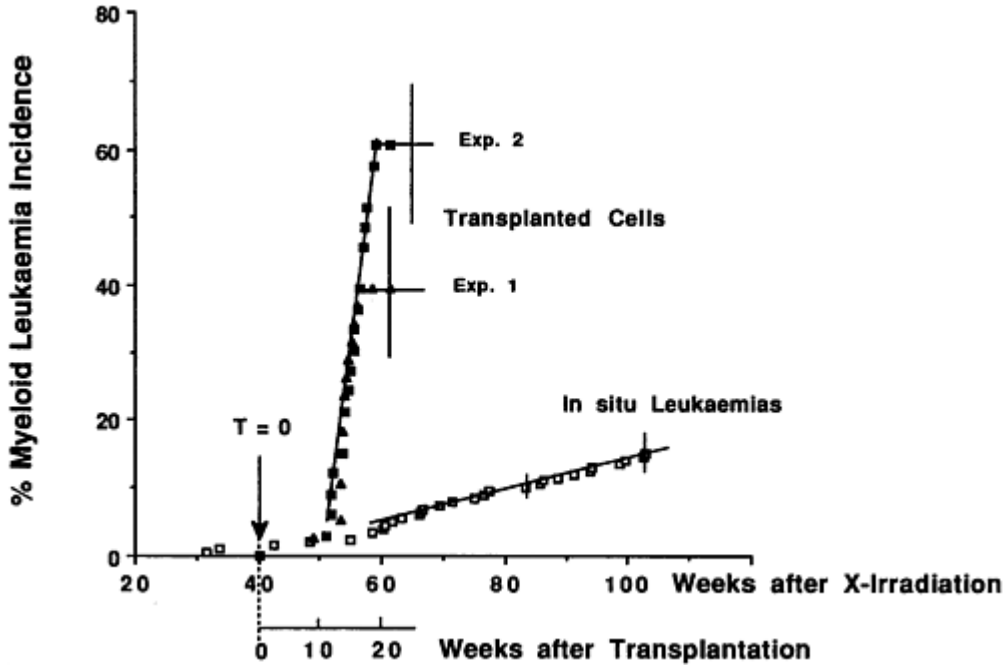

Figure 10.8 Myeloid leukaemia incidence with time in secondary 4.5 Gy irradiated CBA recipients, who received bone marrow (two femora to one recipient) from donors irradiated with 3 Gy 40 weeks previously. The arrow indicates the time of transplantation

donors at 1, 4, 7 or 10 months after the 3 Gy initial exposure. The incidence of leukaemia was extremely low (0–3 per cent) in mice receiving transplants from 0 to 7 months after irradiation (Table 10.2). However, the pooled incidence in recipients receiving bone marrow 10 months after irradiation was 49 per cent.

Compared with this, the incidence of *in situ* myeloid leukaemia in 3 Gy whole-body X-irradiated CBA/H male mice was only 15 per cent. Thus as the transfers were one-to-one, not using pooled cells, it might have been predicted that the leukaemia incidence could at most be 15 per cent, but more likely to be less as the femoral marrow represents only a fraction of the total marrow irradiated. At least a further 34 per cent of the donor irradiated mice that would fail to develop leukae

Table 10.2 Myeloid leukaemia incidence in CBA/H male mice following exposure to 3 Gy X-irradiation or following transplantation of bone marrow cells (two femora to one recipient) to sublethally irradiated recipients (4.5 Gy) at different times after 3 Gy X-irradiation of the donors

Time after 3 Gy irradiation (months)	No. mice irradiated or transplanted	Number of leukaemias	% Leukaemias (80% binomial limits)
1	38	0	0.0 (4.0–0.0)
4	43	1	2.3 (7.5–0.7)
7	33	1	3.0 (9.6–0.9)

Time after 3 Gy irradiation (months)	No. mice irradiated or transplanted	Number of leukaemias	% Leukaemias (80% binomial limits)
10	71	35	49.3 (56.8–41.8)
Normal bone marrow	45	0	0.0 (3.5–0.0)
3 Gy only	200	30	15.0 (18.5–12.0)

mia in their lifespan, when the bone marrow was left *in situ,* were capable of expressing the leukaemia on transplantation of their irradiated bone marrow cells. Potential myeloid leukaemic cells are thus detected in this transplantation assay and clearly demonstrate that these cells can be present for the lifespan of quite a large number of animals without progressing to a frank leukaemia.

Single exposure to 3 Gy X-irradiation results in the production of 20 per cent myeloid leukaemia incidence in CBA/H male mice. At higher doses as we have seen, the incidence decreases and is similar to unirradiated controls at 6 Gy (Mole, 1988). If a further 6 Gy is administered anything from a few hours to 4 or 8 months after the initial 3 Gy exposure, the incidence of myeloid leukaemia is markedly reduced. Only if this second dose is administered 12 to 16 months after the initial 3 Gy exposure is there little modifying effect on myeloid leukaemia incidence. Myeloid leukaemias appear after about 8 months in this strain of mice. Thus presumably this second dose of radiation, when administered at the appropriate time, is sufficient to kill or drastically reduce the number of potential leukaemic cells accumulating secondary events and undergoing clonal expansion in order subsequently to produce a frank leukaemia.

10.12 Summary

Careful studies by a number of groups have provided a wealth of data on radiation-induced leukaemia in experimental animals. The dose-response relationships of different LET radiations and for different types of leukaemia have been defined:

For myeloid leukaemia

following low LET irradiation	$Y=aD^2e^{-bD}$
following high LET irradiation	$Y=aDe^{-bD}$

For thymic lymphoma

following low LET irradiation	$Y=aD^2+c$

Further studies have investigated the effects of dose rate and dose fractionation and have relevance to radiation protection. These experimental models have also provided a means of investigating the treatment of leukaemias and the regulation of leukaemic cells. More fundamental investigations of the mechanisms of radiation-induced carcinogenesis are also in progress. The role of cytokines, the detection of potential leukaemic cells and the significance of stromal cells promises to be of importance in our understanding of the mechanisms of radiation-induced leukaemogenesis.

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References

BESSHO, M. & HIRASHIMA, K. (1982) Experimental studies on the mechanism of leukaemogenesis, following the hemopoietic stem cell kinetics, *Acta Haematologica Japonica,* **45**, 1296–306.

BOICE, J.D., BLETTNER, M., KLEINERMAN, R.A. *et al.* (1987) Radiation dose and leukemia risk in patients treated for cancer of the cervix, *J. National Cancer Institute,* **79,** 1295–311.

BONIVER, J., DECIEVE, A., LIEBERMAN, M., HONSIK, C., TRAVIS, M. & KAPLAN, H. S. (1981) Marrow-thymus interactions during radiation leukemogenesis in C57BL/Ka mice, *Cancer Research,* **41**, 390–2.

COGGLE, J.E. & GORDON, M.Y. (1976) Anaemia and the induction of myeloid leukaemia in RFM mice, *Experimental Haematology,* **4**, 48–55.

CRONKITE, E.P., INOUE, T. & BULLIS, J.E. (1987) Radiation leukemogenesis: bone marrow transfusion induces delayed appearance of leukaemia, *Experimental Hematology,* **15,** 590.

CURTIS, R.E., BOICE, J.D., STOVALL, M. *et al.* (1994) Relationship of leukemia risk to radiation dose following cancer of the uterine corpus, *J. National Cancer Institute,* **86,** 1315–24.

DEXTER, T.M. (1979) Cell interactions *in vitro.* In L.G.Lajtha (ed.), *Cellular Dynamics of Haemopoiesis,* Clinics in Haematology, vol. 10. London: Saunders, pp. 453–68.

DEXTER, T.M., GARLAND, J., SCOTT, D., SCOLNICK, E. & METCALF, D. (1980) Growth of factor-dependent hematopoietic precursor lines, *J. Experimental Medicine,* **152,** 1036–47.

DEXTER, T.M., SPOONCER, E., SIMMONS, P. & ALLEN, T.D. (1984) Long-term bone marrow culture: an overview of techniques and experience. In D.G.Wright & J.S. Greenberger (eds), *Long Term Bone Marrow Culture.* New York: Alan Liss, pp. 57–96.

DI MAJO, V., COPPOLA, M., REBESSI, S. *et al.* (1986) Dose-response relationship of radiation-induced harderian gland tumors and myeloid leukemia of the CBA/Cne mouse, *J. National Cancer Institute,* **76**, 955–63.

DUNN, T.B. (1954) Normal and pathologic anatomy of the reticular tissue in laboratory mice, with a classification and discussion of neoplasms, *J. National Cancer Institute,* **14**, 1281–433.

FRAZIER, M.E., SEED, T.M., SCOTT, L.L. & STIEGLER, G.L. (1987) Radiation-induced carcinogenesis in dogs. In E.M.Fielden, J.F.Fowler, J.H.Hendry & D.Scott (eds), *Radiation Research, Proceedings of the 8th International Congress of Radiation Research.* London: Taylor & Francis, pp. 488–93.

FRY, R.J.M. (1981) Experimental radiation carcinogenesis: what have we learned? *Radiation Research,* **87**, 224–39.

(1984) Relevance of animal studies to the human experience. In J.D.Boice & J.F.Fraumeni (eds), *Progress in Cancer Research and Therapy,* vol. 26. New York: Raven Press, pp. 337–46.

GONG, J.K. (1971) Anemic stress as a trigger of myelogenous leukemia in rats rendered leukemia-prone by X-rays, *Science,* **174,** 833–5.

GONG, J.K., BRAUNSCHWEIGER, P.G. & GLOMSKI, C.A. (1972) Anemic stress as a trigger of myelogenous leukaemia in the unirradiated RF mouse, *Science,* **177**, 274–6.

GOULD, M.N. (1984) Radiation initiation of carcinogenesis *in vivo:* a rare or common cellular event. In J.D.Boice & J.F.Fraumeni (eds), *Progress in Cancer Research and Therapy,* vol. 26. New York: Raven Press, pp. 403–20.

GRAY, L.H. (1965) Radiation biology and cancer. In R.J.Shalet *et al.* (eds), *Cellular Radiation Biology.* Baltimore: Williams & Wilkins, pp. 7–25.

GREENBERGER, J.S. (1980) Self-renewal of factor-dependent hematopoietic progenitor cell lines derived from long-term bone marrow cultures demonstrates significant mouse strain genotypic variation, *J. Supramolecular Structure,* **13,** 501–11.

GUALTIERI, R.J., SHADDUCK, R.K., BAKER, D.G. & QUESENBERRY, P.J. (1984) Haemopoietic regulatory factors produced in long term murine bone marrow cultures and effect of *in vitro* irradiation, *Blood,* **64,** 516–25.

HARAN-GHERA, N. (1973) Relationship between tumour cell and host in chemical leukaemogenesis, *Nature New Biology,* **246**, 84–6.

(1980) Potential leukaemic cells among bone marrow cells of young AKR/J mice, *Proceedings of the National Academy of Sciences,* **77**, 2923–6.

HARAN-GHERA, N., PELED, A., KRAUTGHAMER, R. & RESNITZKY, P. (1992) Initiation and promotion in radiation-induced myeloid leukaemia, *Leukaemia,* **6**, 689–95.

HEPBURN, M., DOHERTY, I., BRISCOE, C. & RICHES, A. (1987) Transplantation and morphological studies of primary and passaged murine radiation-induced myeloid leukaemias, *Leukaemia Research,* **11,** 1001–9.

HUMPHREYS, E.R., LOUTIT, J.F., MAJOR, I.R. & STONES, V.A. (1985) The induction by ^{224}Ra of myeloid leukaemia and osteosarcoma in male CBA mice, *Int. J. Radiation Biology* **47**, 239–47.

HUMPHREYS, E.R., LOUTIT, J.F. & STONES, V.A. (1987) The induction by ^{239}Pu of myeloid leukaemia and osteosarcoma in female CBA mice, *Int. J. Radiation Biology* **51,** 331–9.

HUMPHREYS, E.R., ISAACS, K.R., RAINE, T.A., SAUNDERS, J., STONES, V.A. & WOOD, D.L. (1993) Myeloid leukaemia and osteosarcoma in CBA/H mice given ^{224}Ra, *Int. J. Radiation Biology,* **64**, 231–5.

INOUE, T., YOSHIDA, K. & SEKI, M. (1983) Megakaryoblastic leukaemia cell line (MG-8057) induced by X-irradiation in C3H/He mice, *Experimental Hematology,* **11** (suppl. 14), 156.

KAPLAN, H.S. & BROWN, M. (1952) A quantitative dose-response study of lymphoid-tumor development in irradiated C57 black mice, *J. National Cancer Institute,* **13,** 185– 208.

KENNEDY, A.R., FOX, M., MURPHY, G. & LITTLE, J.B. (1980) Relationship between X-ray exposure and malignant transformation in C3H10T1/2 cells, *Proceedings of the National Academy of Sciences,* **77**, 7262–6.

KOTLER, M., RUCHLEMER, R., AVNI, O. & YEFENOF, E. (1994) Radiation leukemogenesis: quantitative relationship between pre-leukemic cells in the thymus and lymphoma induction, *Int. J. Cancer,* **56,** 761–5.

LOUTIT, J.F. & CARR, T.E.F. (1978) Lymphoid tumours and leukaemia induced in mice by bone-seeking radionuclides, *Int. J. Radiation Biology,* **33**, 245–63.

MAJOR, I. (1979) Induction of myeloid leukaemia by whole-body single exposure of CBA male mice to X-rays, *Br. J. Cancer,* **40,** 903–13.

MAJOR, I & MOLE, R.H. (1978) Myeloid leukaemia in X-ray irradiated CBA mice, *Nature,* **272**, 455–6.

MELDRUM, R.A. & MOLE, R.H. (1982) Radiation-induced myeloid leukaemia in CBA/H mice: a non-immunogenic malignant disease in syngeneic mice, *British Journal of Cancer,* **45**, 403–12.

MOLE, R.H. (1958) Lymphoid tumours and leukaemia induced in mice by bone-seeking radionuclides, *Br. Med. Bull,* **14**, 174–7.

(1975) Ionizing radiation as a carcinogen: practical questions and academic pursuits, *Br. J. Radiol ,* **48**, 157–69.

(1984) Dose-response relationships. In J.D.Boice & J.F.Fraumeni (eds), *Progress in Cancer Research and Therapy,* vol. 26. New York: Raven Press, pp. 403–20.

(1986a) Radiation-induced acute myeloid leukaemia in the mouse: experimental observations *in vivo* with implications for hypotheses about the basis of carcinogenesis, *Leukaemia Research,* **10,** 859–65.

(1986b) Leukaemia induction in man by radionuclides and some relevant experimental and human observations. In W.Gossner, G.B.Gerber, U.Hagen & A.Luz (eds), *The Radiobiology of Radium and Thorotrast.* Munich: Urban & Schwarzenberg, p. 1.

(1988) Radiation-induced acute myeloid leukaemia: an unusually valuable experimental model for testing basic assumptions about the process of carcinogenesis. In O.H.Iversen (ed.), *Theories of Carcinogenesis.* Washington: Hemisphere, pp. 133–41.

MOLE, R.H. & DAVIDS, J.A.G. (1982) Induction of myeloid leukaemia and other tumours in mice by irradiation with fission neutrons. In J.J.Broerse & G.B.Gerber (eds), *Neutron Carcinogenesis.* Luxembourg: Commission of the European Communities, pp. 31–42.

MOLE, R.H. & MAJOR, I.R. (1983) Myeloid leukaemia frequency after protracted exposure to ionizing radiation: experimental confirmation of the flat dose-response found in ankylosing spondylitis after a single treatment course with X-rays, *Leukaemia Research,* 7, 295–300.

MOLE, R.H., PAPWORTH, D.G. & CORP, M.J. (1983) The dose-response for X-ray induction of myeloid leukaemia in male CBA/H mice, *Br. J. Cancer,* **47**, 285–91.

MULLER, W.A., LINZNER, U. & LUZ, A. (1988) Early induction of leukaemia (malignant lymphoma) in mice by protracted low -doses, *Health Physics,* **54,** 461–3.

NAPARSTEK E., DONNELLY, J., KASE, K. & GREENBERGER, J.S. (1985) Biologic effects of X-irradiation of murine long-term bone marrow cultures on the production of granulocyte-macrophage colony stimulating factors. *Experimental Hematology,* **13**, 701–8.

NAPARSTEK, E., FITZGERALD, T.J., SAKAKEENY, M.A. *et al.* (1986a) Induction of malignant transformation of cocultivated hematopoietic stem cells by X-irradiation of murine bone marrow stromal cells *in vitro, Cancer Research,* **46,** 4677–84.

NAPARSTEK, E., PIERCE, J., METCALF, D. *et al.* (1986b) Induction of growth alterations in factor-dependent hematopoietic progenitor cell lines by cocultivation with irradiated bone marrow stromal cell lines, *Blood,* **67,** 1395–403.

RESNITZKY, P., ESTROV, Z. & HARAN-GHERA, N. (1985) High incidence of acute myeloid leukemia in SJL/J mice after X-irradiation and corticosteroids, *Leukemia Research,* **9***,* 1519–28.

RESNITZKY, P., TRAKHTENBROT, L., PELED, A., ZIPORI, D. & HARAN-GHERA, N. (1987) Further studies on radiation-induced myeloid leukaemia in SJL/J mice at high incidence rates, *Experimental Haematology,* **15,** 589.

RICHES, A.C., HEPBURN, M., MELVILLE, J. & BRISCOE, C.V. (1991) Persistence of murine myeloid leukaemic cells in long-term bone marrow cultures, *Bone Marrow Transplantation,* **7**, 329–33.

RITHIDECH, K.N., BOND, V.P., CRONKITE, E. & THOMPSON, M.H. (1993) A specific chromosomal deletion in murine leukemic cells induced by radiation with different qualities, *Experimental Hematology,* **21**, 427–31.

ROBINSON, C.V. & UPTON, A.C. (1978) Competing-risk analysis of leukemia and non-leukemia mortality in X-irradiated, male RF mice, *J. National Cancer Institute,* **60**, 995– 1007.

SCHMAHL, W. (1988) Synergistic induction of tumours in NMRI mice by combined foetal X-irradiation with low doses and ethylnitrosourea administered to juvenile offspring, *Carcinogenesis,* **9**, 1493–8.

SEED, T.M., KASPAR, L.V., FRITZ, T.E. & TOLLE, D. (1985) Cellular responses in chronic radiation leukemogenesis. In E.Huberman & S.H.Barr (eds), *Carcinogenesis,* vol. 10. New York: Raven Press, pp. 363–79.

SEED, T.M., KASPAR, L.V., TOLLE, D.V. & FRITZ, T.E. (1987) Chronic radiation leukemogenesis: postnatal hematopathologic effects resulting from *in-utero* irradiation, *Leukemia Research,* **11**, 171–9.

SEED, T.M., GOLTRY, K. & GREENBERGER, J.S. (1994) Leukaemic cell transformation under chronic radiation *in vitro, Experimental Hematology,* **22**, 762.

SVOBODA, V. & BUBENIKOVA, D. (1990) Haemoblastoses in mice contaminated with low activities of ^{239}Pu, *Neoplasma,* **37**, 639–46.

SVOBODA, V., BUBENIKOVA, D. & KOTASKOVA, Z. (1981) Myeloid leukemia in ^{239}Pu-treated mice, *J. Cancer Research and Clinical Oncology,* **100**, 255–62.

TARTAKOVSKY, B., GOLDSTEIN, O., KRAUTGHAMER, R. & HARAN-GHERA, N. (1993) Low doses of radiation induce systemic production of cytokines: possible contribution to leukemogenesis. *Int. J. Cancer,* **55**, 269–74.

THORNE, M.C. & VENNART, J. (1976) The toxicity of ^{90}Sr, ^{226}Ra and ^{239}Pu, *Nature,* **263**, 555–8.

ULLRICH, R.L. & STORER, J.B. (1979a) Influence of gamma irradiation on the development of neoplastic disease in mice. I: Reticular tissue tumors, *Radiation Research,* **80**, 303–16.

ULLRICH, R.L. & STORER, J.B. (1979b) Influence of gamma irradiation on the development of neoplastic disease in mice: III: Dose rate effects, *Radiation Research,* **80**, 325–42.

ULLRICH, R.I., JERNIGAN, M.C., COSGROVE, G.E., SATTERFIELD, I.C., BOWLES, N.D. & STORER, J.B. (1976) The influence of dose and dose-rate on the incidence of neoplastic disease in RFm mice after neutron irradiation, *Radiation Research,* **68**, 115– 31 .

UNSCEAR (1986) (United Nations Scientific Committee on the Effects of Atomic Radiation) *Genetic and Somatic Effects of Ionizing Radiation.* New York: United Nations.

UPTON, A.C. (1984) Biological aspects of radiation carcinogenesis. In J.D.Boice & J.F. Fraumeni (eds), *Progress in Cancer Research and Therapy,* vol. 26. New York: Raven Press, pp. 9–19.

(1985) Biological basis for assessing carcinogenic risks of low-level radiation. In E.Huberman & S.H.Barr (eds), *Carcinogenesis,* vol. 10. New York: Raven Press, pp. 381–401.

UPTON, A.C., WOLFF, F.F., FURTH, J. & KIMBALL, A.W. (1958) A comparison of the induction of myeloid and lymphoid leukemias in X-irradiated RF mice, *Cancer Research,* **18**, 842–8.

UPTON, A.C., ODELL, T.T. & SNIFFEN, E.P. (1960a) Influence of age at time of irradiation on induction of leukemia and ovarian tumors in RF mice, *Proceedings of the Society of Experimental Biology and Medicine,* **104**, 769–72.

UPTON, A.C., KIMBALL, A.W., FURTH, J., CHRISTENBERRY, K.W. & BENEDICT, W.H. (1960b), Some delayed effects of atom-bomb radiations, in mice, *Cancer Research,* **20**, (8, part 2), 1–60.

UPTON, A.C., RANDOLPH, M.L. & CONKLIN, J.W. (1970) Late effects of fast neutrons and gamma rays in mice as influenced by the dose rate of irradiation: induction of neoplasia, *Radiation Research,* **41**, 467–91.

VALAGUSSA, P., SANTORO, A., FOSSATI BELLANI, F., FRANCHI, F., BADFI, A. & BONADONNA, G. (1982) Absence of treatment-induced second neoplasms after ABVD in Hodgkin's disease, *Blood,* **59,** 488–94.

YOSHIDA, K., NEMOTO, K., NISHIMURA, M., HAYATA, I., INOUE, T. & SEKI, M. (1986) Nature of leukemic stem cells in murine myelogenous leukemia, *Int. J. Cell Cloning,* **4**, 91–102.

11
Human Studies in Radiation Leukaemogenesis

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11
Human Studies in Radiation Leukaemogenesis

11.1
Introduction

There have been many epidemiological studies of leukaemia following exposure to ionising radiation and estimates of radiation-induced risk in humans rely heavily on their results. Studies have been made of a wide range of irradiated populations, including medical and occupational exposures, as well as of the survivors of the atomic bombings of Hiroshima and Nagasaki. These studies have provided quantitative estimates of the risk of leukaemia following exposure to radiation and of the way it varies with sex, age at exposure, time since exposure and dose (National Research Council, 1990). Current interest in radiation leukaemogenesis focuses on the shape of the leukaemia dose-response curve and the variation in risk with age at irradiation and with dose rate.

This chapter discusses results from the main studies for which individual dose estimates have been made, including estimates of risk per unit dose. The doses received by study populations vary from the relatively low doses of recent workers in the nuclear industry, in countries such as the UK, USA and Canada (mean cumulative total body dose of around 0.05 Sv), to the much higher doses received by cancer patients during radiotherapy (up to 60 Sv to the tumour site). The main high dose studies which have provided quantitative estimates of leukaemogenic risk are the Life Span Study of atomic bomb survivors and several cohorts of medically irradiated patients. Studies of populations exposed to low doses of radiation (occupational exposure, diagnostic X-rays and fallout from nuclear weapons testing or nuclear accidents) have also been carried out. However, the small risks at these low doses and the measurement errors involved in their dosimetry mean that these studies do not usually provide precise estimates of risk, and their main role is to check that extrapolations from high dose studies do not substantially underestimate risk.

11.2
The Life Span Study of Atomic Bomb Survivors

The Life Span Study (LSS) of 120 000 survivors of the atomic bombings in Hiroshima and Nagasaki in 1945 is unique in radiation epidemiology because of the number of people included and the long follow-up period. Unlike many other studies, the population contains persons of both sexes and all ages at exposure, and covers a wide range of exposure levels. About half the cohort received a total bone marrow dose of less than 0.01 Gy, and are used as a control group.

Within a few years of the bombings, an excess incidence of leukaemia was observed among survivors (Folley *et al.,* 1952). A leukaemia registry was established which included data on all known cases of leukaemia and related disorders diagnosed among survivors resident in Hiroshima, Nagasaki and the surrounding areas, whether or not they were included in the LSS cohort. For each case, clinical records and histological materials were reviewed by two or more experienced leukaemia registry haematologists. In the late 1980s, those cases for whom adequate bone marrow slides or diagnostic peripheral blood were available (about 60 per cent) were reclassified using modern diagnostic criteria and disease classification systems, including the French-American-British (FAB) system for acute leukaemias. As a result of this, diseases such as myelodysplastic syndrome and acute T-cell leukaemia can be identified and studied.

Leukaemia is a rare form of cancer accounting for only about 3 per cent of the total cancer cases in the cohort up to 1987. However, if attention is restricted to the numbers of excess cancer cases attributable to the irradiation, about 15 per cent of them were leukaemia (Preston *et al.,* 1994), demonstrating that it is more easily induced by radiation than most other cancers. There are two common ways of describing this excess risk, on either a relative (multiplicative) or absolute (additive) scale. The summary measures of risk are defined as follows.

If R(exp) is the absolute rate at which leukaemia cases occur per person year (PY) of observation in the exposed population, and R(unexp) is the rate at which they would have occurred in the absence of exposure, the relative risk (RR) is defined as the ratio of the two rates:

$$\mathrm{RR}=R(\mathrm{exp})/R(\mathrm{unexp})$$

and the excess relative risk (ERR) is defined as:

$$\mathrm{ERR}=[R(\mathrm{exp})-R(\mathrm{unexp})]/R(\mathrm{unexp})$$

Similarly, the excess absolute risk (EAR) is defined as the absolute difference between the rates in the exposed and unexposed populations:

$$\mathrm{EAR}=R(\mathrm{exp})-R(\mathrm{unexp})$$

Usually the appropriate value for R(unexp) is estimated by considering data from a separate group which may have a different composition from that of the exposed group with respect to factors that influence the disease rate such as sex, attained age or calender period. If this is the case, adjustments must be made to account for these differences. Average values of the ERR or EAR for an entire population, or values specific to a particular subgroup (e.g. those who received doses in a specific range, or who were exposed when they were in a certain age range) can be calculated. Alternatively, models involving the ERR or EAR as parameters can be postulated and their values estimated.

For a given excess absolute risk, the magnitude of the excess relative risk will vary depending on the size of the background risk. For example, the excess absolute risk per 10^4 PY per sievert is greater for stomach cancer than for leukaemia ($\mathrm{EAR}_{\mathrm{stomach}}=4.8$, $\mathrm{EAR}_{\mathrm{leukaemia}}=2.7$). However, leukaemia is a rare disease in Japan compared with stomach cancer and this is reflected in the fact that the excess relative risk at 1 Sv is much greater for leukaemia than for stomach cancer ($\mathrm{ERR}_{\mathrm{stomach}}=0.32$, $\mathrm{ERR}_{\mathrm{leukaemia}}=3.9$).

The relationship between absorbed bone marrow dose and leukaemia risk is central in the study of radiation leukaemogenesis. The atomic bombs emitted both - and neutron radiation, and doses to individual survivors have been reconstructed, based on the precise location and shielding of study members, and taking into account the penetration of the - and neutron radiation through the weapons materials and the air. The dosimetry system used (DS86) (Roesch, 1987) has provided estimates for 92 per cent of survivors, and the weighted bone marrow doses in sieverts were computed as the sum of the -ray dose and ten times the neutron dose to the bone marrow. This is equivalent to assuming a constant relative biological effectiveness (RBE) of ten for neutrons.

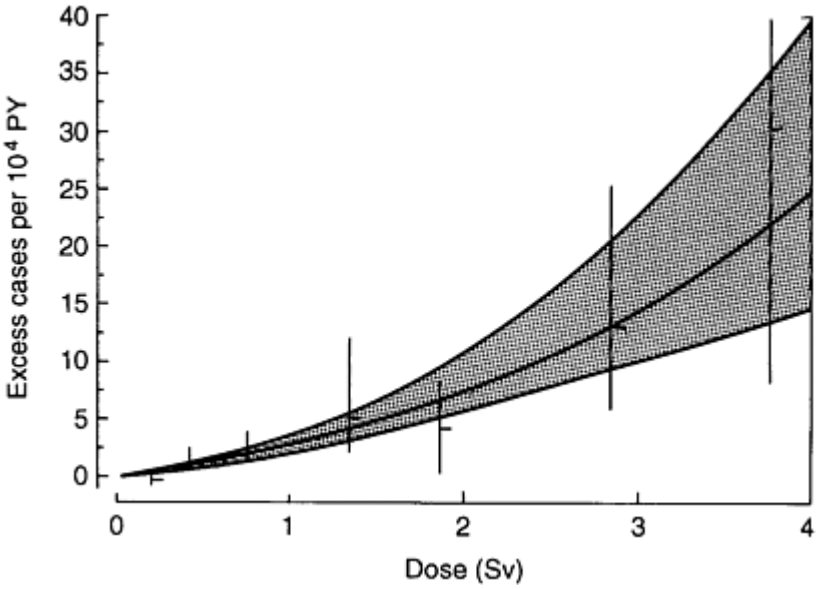

Figure 11.1 Dose-response curve for leukaemia incidence in the Life Span Study of Japanese atomic bomb survivors. The solid curve is based on a quadratic excess absolute risk (EAR) model without effect modification. The shaded area is a 95 per cent confidence region for the fitted curve. The vertical lines are 95 per cent confidence intervals for the EAR in dose categories. Point estimates of the risk for each category are indicated by short horizontal lines. (Reproduced from Preston *et al.,* 1994. © *Radiation Research*)

The most recent analyses of leukaemia incidence in the cohort are based on the 231 cases of primary leukaemia diagnosed in the period 1 October 1950 to the end of 1987 (Preston *et al.,* 1994). The study population presented in these analyses consisted of a subset of about 86 000 persons who were Hiroshima or Nagasaki residents at the time of the bombs and who had DS86 dose estimates of less than 4 Gy. The study population includes survivors of all ages at the time of the bombs (ATB), and well over half the cohort (58.4 per cent) are female, reflecting the fact that a large number of young men were away on military service at the time.

Initial analyses were carried out on all leukaemias which were considered as a single group. The average excess absolute risk was 2.7 cases per 10^4 PY per Sv, and there was strong evidence of a curvilinear dose-response relationship (Figure 11.1). The EAR did not depend on city, but varied with time since exposure. Furthermore, patterns of risk also differed between males and females and varied with age at exposure. Figure 11.2 shows the estimated pattern of excess absolute risk following a dose of 1 Sv for males and females exposed at various ages. For males, especially those exposed early in life, the EAR is greatest within the first 10 years of exposure and then decreases. Some increase in risk remains up to 40 years following exposure, however, especially among those who were older at exposure. Among females, there was some tendency for high EARs in the first 10 years after exposure, especially among those exposed early in life, but much less so than in males. At longer times after exposure, EARs in males and females were similar. When radiation-related risks were examined on a relative scale, ERRs were also at a maximum during the first 10 years after exposure. The average excess relative risk following a dose of 1 Sv, at 3.9, was larger than the corresponding estimate for any of the solid tumours, which indicates that a large proportion of the leukaemias that have occurred in the exposed members of the cohort are likely to have been radiation-induced.

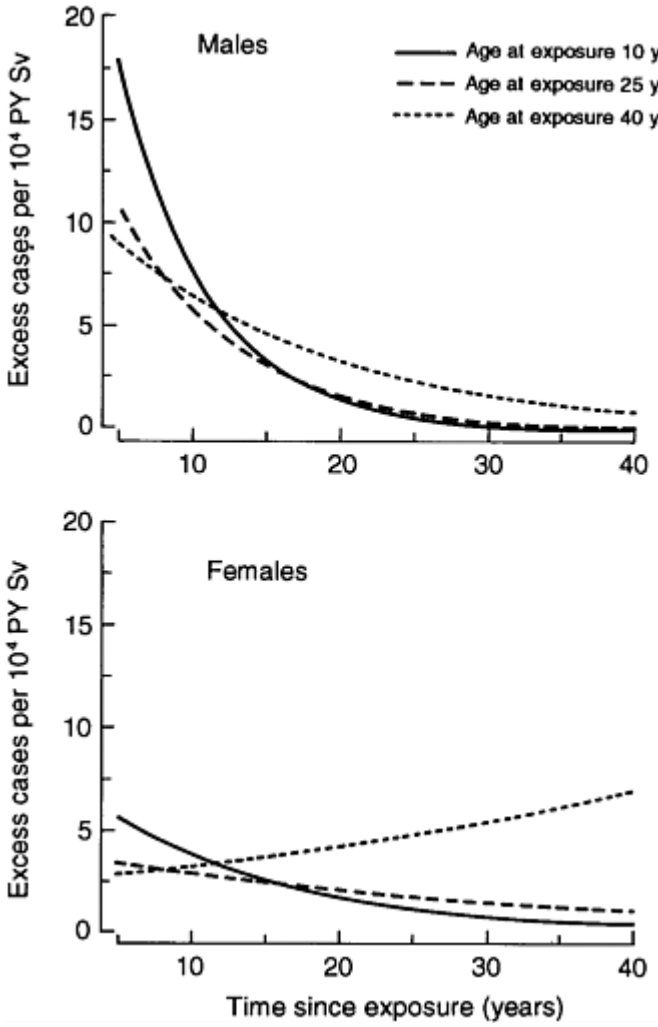

Figure 11.2 Smoothed patterns of excess absolute risk of leukaemia in the Life Span Study of Japanese atomic bomb survivors following a dose of 1 Sv to the bone marrow. (Reproduced from Preston *et al.* 1994. © *Radiation Research*)

One of the major advantages of a study of this size is that analyses can be made on subtypes of leukaemia. Of the 231 leukaemia cases with known doses between 0 and 4 Gy, 32 were acute lymphocytic leukaemia (ALL), 103 were acute myelogenous leukaemia (AML), 57 were chronic myelogenous leukaemia (CML), and 23 were acute T-cell leukaemia (ATL). Analyses of the remaining cases were limited to the 15 Hiroshima cases with DS86 dose estimates, of whom 7 were unspecified acute leukaemias, 4 chronic lymphocytic leukaemias (CLL), 2 myelodysplastic syndromes and 2 hairy-cell leukaemias.

Both the magnitude and temporal pattern of radiation-induced risk varied significantly among the various types of leukaemia. There was strong evidence of a dose-response relationship for ALL, AML and CML. When considered on an absolute scale, the dose-response relationships for ALL and CML were adequately described by a linear model, but for AML there was evidence that the dose-response relationship was non-

linear, with a significant improvement in fit when either a quadratic model or a 'linear-spline' model, which allowed the increase per unit dose to differ above and below 0.5 Sv, was fitted to the data. When averaged over the follow-up period, the estimated mean EARs per 10^4 PY per Sv were 1.1 for AML, 0.9 for CML and 0.6 for ALL. In contrast, when measured on a relative scale, the greatest excess relative risk at 1 Sv was for ALL (9.1), with lower risks for CML (6.2) and AML (3.3), the differences being due to the different background rates of the three subtypes. Chronic lymphocytic leukaemia (CLL) is rare in Japan and so conclusions about its radiosensitivity cannot be drawn from this study. There was no evidence of a dose-response relationship for adult T-cell leukaemia in Nagasaki, where it is endemic and known to be caused by the human T-cell leukaemia virus type-1. The variation of risk with time since exposure was also examined for subtypes of leukaemia. For ALL, following an initial increase in risk, there was a significant decrease in EAR with increasing time since exposure of about 14 per cent per year, while for CML the decrease was highly significant for men (21 per cent per year), but not for women (3 per cent per year). For AML, EAR was significantly greater in the early period after exposure than subsequently among those aged under 20 at exposure, but not among those older at exposure.

The Life Span Study (LSS) is the most important study in radiation epidemiology and is used to set radiation protection standards. However, as in any observational study, the conclusions that can be drawn from it are limited. In addition, risk estimates from the LSS may not be directly transferable to other populations with different baseline risks (Muirhead *et al.,* 1993). Further, the exposure received by atomic bomb survivors was instantaneous and uniform throughout the body. Other studies of irradiated populations are therefore necessary to provide estimates of risks following protracted and partial-body irradiation as well as to support evidence from the LSS.

11.3 Studies of Medically Irradiated Populations

The main sources of information on the effects of high doses of radiation, apart from the LSS, are studies of several medically irradiated populations, including ankylosing spondylitis patients (Weiss *et al.,* 1995), and women irradiated for cervical

Table 11.1 Characteristics of the major studies of leukaemia in medically irradiated populations

Study	Type of study	Study population	Follow-up period	Number of leukaemia cases	Relative risk (RR) of non-CLL	Variation of RR with time since exposure
Ankylosing spondylitis study (Weiss *et al.* 1995)	Cohort Mortality	14 556 exposed 1021 unexposed 16.5% female Age at exposure 16+	Up to 50 years	53[a]	3.1 (2.4–4.1)[b]	Maximum 1–4 years after first exposure (RR=11.0)
Cervical cancer patients (*Boice et al.* 1987; Thomas *et al.,* 1992)	Nested case-control Incidence	143 cases 545 matched controls Age at exposure 30–70	Up to 30 years	143[a]	2.0 (1.0–4.2)[c]	Maximum 1–4 years after exposure (RR=8.9)

Study	Type of study	Study population	Follow-up period	Number of leukaemia cases	Relative risk (RR) of non-CLL	Variation of RR with time since exposure
Uterine corpus cancer study (Curtis *et al.,* 1994)	Nested case-control Incidence	161 cases 775 matched controls Mean age at exposure 62	Up to 50 years	161[a]	1.9 (1.3–2.9)[b]	Maximum 5–9 years after exposure (RR=2.3)
US benign gynaecological diseases (Inskip *et al.,* 1993)	Cohort Mortality	4153 exposed Age at exposure 13–88	Up to 60 years	40[a]	1.7 (1.3–2.3)[c]	Maximum 2–4 years after exposure (RR=4.4)
Scottish benign gynaecological diseases (Darby *et al.,* 1994)	Cohort Mortality	2067 exposed Age at exposure 23–65	Up to 50 years	12	2.1 (1.1–3.6)[b]	Maximum 5–9 years after exposure (RR=6.3)

[a] Excluding chronic lymphocytic leukaemia.
[b] 95% confidence interval.
[c] 90% confidence interval.

cancer (Boice *et al.,* 1987), cancer of the uterine corpus (Curtis *et al.,* 1994) and benign gynaecological diseases (BGD) (Inskip *et al.,* 1993; Darby *et al.,* 1994).

Details of these studies are shown in Table 11.1. The two case-control studies were carried out among international cohorts of 150 000 women with invasive cancer of the uterine cervix (143 non-CLL cases), and 110 000 women with invasive cancer of the uterine corpus (161 non-CLL cases) respectively. In both studies, four controls were individually matched to each case, based on age, calendar year of primary cancer diagnosis, and survival time. The remaining studies shown in Table 11.1 are cohort studies of leukaemia mortality, where patients were followed from the time of irradiation (or diagnosis for unirradiated patients) until death, emigration or the end of follow-up. For all the studies, risks were examined using a relative scale. In the case-control studies, relative risk estimates were calculated by comparing the odds of radiation exposure among cases with those of the matched controls using conditional logistic regression methods (Breslow and Day, 1980), while in the cohort studies relative risks were calculated as the ratios of observed deaths to those expected from national mortality rates. The cohort studies included fewer leukaemia cases than the case-control studies, but confidence intervals of relative risks were of similar size since the expected number of deaths are assumed to be known without error.

In each study, the risk of leukaemia other than CLL was increased by a factor of about 2–3 (Table 11.1). In contrast, no significant excess risk of CLL has been observed in any of the studies, providing considerable evidence that CLL is not associated with ionizing radiation (National Research Council, 1990). In most of the studies further analyses, including the variation in risk with time since exposure and dose, have been based on all leukaemias other than CLL considered as a single group, as the numbers of leukaemias of any specific subtype tend to be small.

The variation in ERR with time since exposure among medically irradiated populations is broadly similar to that seen in the LSS. In each study except the uterine corpus cancer study the RR of non-CLL leukaemia

followed a wave-like pattern, with the maximum ERR occurring within 10 years of exposure, followed by a significantly decreasing RR with increasing time since exposure. The maximum RR in the early period following exposure ranged from 4.4 in the American BGD study to 11.0 among irradiated ankylosing spondylitis patients. All studies except the cervical cancer study provided evidence that the increased risk persisted for at least 15 years after exposure. In the LSS the variation of risk with time since exposure was less marked among those older at exposure, especially among women. Therefore the fact that there was no significant variation in risk with time since exposure in the uterine corpus cancer study, where the majority of women were irradiated at age 55+, was not unexpected. In the two studies which analysed trends in subtypes of leukaemia (the ankylosing spondylitis study and the American BGD study), a significant decrease in RR with time since exposure was seen for CML, but not for acute leukaemia. After adjusting for time since exposure and radiation dose, the trend in the RR of non-CLL leukaemia with age at exposure was not significant in any of the studies.

The type of radiotherapy received by patients in these studies consisted of either external X-ray therapy, internal brachytherapy (radium implants) or a combination of the two modalities, and it is important to consider these differences when interpreting results. Table 11.2 shows the types of irradiation received in each study. In the studies which included both modalities, the mean total marrow dose from exter

Table 11.2 Leukaemia dose-response analyses of medically irradiated populations

Study	Mean bone marrow dose (Gy)	Type of radiotherapy and dose rate	Preferred dose-response model	Linear component of ERR per Gy	Reduction in ERR at l Gy due to cell sterilisation	Predicted ERR at a uniform dose of 1 Gy
Ankylosing spondylitis study (Weiss *et al.*, 1995)	Total marrow 4.38 Lower spine 8.62	External X-ray therapy only. Most patients (93%) received 1 or 2 courses, each consisting of daily fractions for a two-week period	Compartmental, linear with cell killing	12.4[a] (2.3–52.1)[b]	47% (17%, 79%)[b]	6.00[a]
Cervical cancer study (*Boice et al.* 1987; Thomas *et al.*, 1992)	Total marrow 7.1 Lower spine 25.1	5.6% external beam only, mean dose 9.6 Gy, given in daily fractions over several weeks 17.5% brachytherapy only, mean dose 2.7 Gy, continuous for 36 hours or more 70% both modalities	Compartmental, linear with cell killing	0.3[a] (0.1–0.5)[b]	7% (–3%, 16%)[b]	0.2[a]

Study	Mean bone marrow dose (Gy)	Type of radiotherapy and dose rate	Preferred dose-response model	Linear component of ERR per Gy	Reduction in ERR at 1 Gy due to cell sterilisation	Predicted ERR at a uniform dose of 1 Gy
Uterine corpus cancer study (Curtis *et al.,* 1994)	Total marrow 5.21 Sacrum 20.7	50% any external beam therapy, mean dose 9.9 Gy, Daily factions for 4–6 weeks (10–40 cGy min^{-1}) 49.2% brachytherapy only, mean dose 1.7 Gy, continuous dose for 3 days (0.04 cGy min^{-1}) 30% both modalities	Linear with cell killing	External: 0.05 (<0–0.55)[b] Internal: 4.7 (1.1–13.4)[b]	–4% (–40%, 23%)[b] 59% (30%, 75%)[b]	0.1[c] 1.3
US benign gynaecological diseases (Inskip *et al.,* 1993)	Total marrow 1.19 Lower spine 5.2	10% external beam only, mean dose 2.3 Gy, fractionated over several days 67.5% brachytherapy only, mean dose 0.62 Gy, continuous dose for 10–24 hours 22.5% both modalities	Linear[d]	External: 0.5 (<0,3.3)[e] Internal: 3.7 (<0,15)[e]	–	0.5 3.7
Scottish benign gynaecological diseases (Darby *et al.,* 1994)	Total marrow 1.3 Sacral marrow 5.5	External X-rays, several fractions spread over about a week	Linear[d]	1.2 (<0,2.5)[b]	–	1.2

[a] Final model also allowed for reduction in ERR with increasing time since exposure. Value given is for ten years after exposure.

[b] 95% confidence interval.

[c] The subgroup of women who received substantial external irradiation to the bone marrow outside the pelvic area had a particularly high leukaemia risk (RR=5.5).

[d] No model including a cell sterilisation term was fitted.

[e] 90% confidence interval.

nal irradiation was several times greater than that from brachytherapy. There was also a difference in dose rate, with external irradiation usually given in daily fractions spread over several weeks at a relatively high dose rate, and brachytherapy given as a continuous, relatively low dose rate for 36 hours or more.

In contrast to the Japanese A-bomb survivors who received uniform marrow doses, exposure in the medically irradiated patients was usually highly non-uniform. Among women irradiated for cervical or corpus uterine cancer, the mean total marrow dose was 5–7 Gy, but the mean dose to the marrow in the pelvic area was over 20 Gy. A similar pattern of exposure was given to women irradiated for BGD, where the mean total marrow dose was about 1.2 Gy, and the dose to the marrow in the pelvic area was 5 Gy. However, the ankylosing spondylitis patients tended to receive a slightly more uniform dose distribution, with mean total marrow doses of 4.4 Gy, and mean dose of the lower spine of 8.6 Gy.

Radiobiological theory suggests that leukaemia dose-response models should include a term to allow for cell sterilisation following high doses of irradiation to parts of the bone marrow (see Chapter 10). The dose-response relationship is thus predicted to include an increasing linear-quadratic term and a decreasing exponential term to allow for cell sterilisation. This suggests the following form for the excess relative risk of leukaemia following marrow dose *D:*

$$\mathrm{ERR}(D)=(\alpha_1 D+\alpha_2 D^2)\exp(-\beta D)$$

A non-linear model such as this cannot take into account the non-uniformity of the dose distribution if it is based simply on total marrow dose. However, local cell sterilisation in heavily exposed parts of the marrow can be accommodated by considering the marrow as a number of separate compartments and defining the total risk for each individual as the sum of risks for different marrow sites:

$$\mathrm{ERR}(D)=\Sigma\, w_i(\alpha_1 D_i+\alpha_2 D^2{}_i)\exp(-\beta D_i)$$

where D_i indicates the marrow dose delivered to the *i*th marrow compartment and w_i the weighting given to the *i*th compartment (Weiss *et al.,* 1995). Both the ankylosing spondylitis and cervical cancer studies used models of this type, thereby taking into account simultaneously the non-uniformity of dose and the cell sterilisation effect at high doses. In both studies, the final preferred model was one which included a linear induction term and a negative exponential cell sterilisation term, as well as an adjustment for time since exposure. In neither study was there evidence that a quadratic term in dose gave an improved fit. Although the results of the two studies agreed qualitatively, the best-fitting models differed considerably in quantitative terms. Fitted risks are summarised in Table 11.2. The linear component of ERR per gray was greater in the spondylitics than in the cervical cancer study by more than an order of magnitude, and at 1 Gy a 47 per cent reduction in ERR due to cell sterilisation was indicated for the spondylitis study, compared with only 7 per cent for the cervical cancer patients. The predicted ERR following a uniform dose of 1 Gy was considerably greater for the spondylitis study than for the cervical study. Comparison with the LSS shows that the predicted risk at 1 Gy among Japanese atomic bomb survivors in the period 1–25 years after exposure is similar to that in the spondylitis study (ERR=5.48). Fitted risks in the cervical cancer patients are much lower, although cell sterilisation is estimated to have only a small effect at a dose of 1 Gy in that study.

It is not known which features of the studies are responsible for the quantitative differences in risk estimates. The effect of dose rate on leukaemia risk is not yet well understood. One possibility is that the lower dose rate, received by the cervical cancer patients from the radium implants, resulted in a lower leukaemia risk per unit dose because of repair of sublethal cell damage and, possibly, also in a weaker cell sterilisation effect. However, this hypothesis is not supported by the uterine corpus cancer study, in which the effects of brachytherapy alone and external irradiation (often in conjunction with brachytherapy) have been compared. Using a linear-exponential model based on total marrow dose, the linear component of ERR per gray for patients receiving brachytherapy alone was 4.7 (95 per cent CI 1.1– 13.4), consistent with the

risk seen in the spondylitics, whereas the risk among patients receiving external irradiation was substantially lower (linear component of ERR per gray=0.05, 95 per cent CI<0.0–0.55). In addition, the evidence for a reduction in risk due to cell sterilisation was stronger for the brachytherapy patients than for other patients. In the uterine cancer study the estimated linear component of ERR per gray for externally irradiated patients was consistent with that of the cervical cancer patients. It is interesting to note, however, that a subgroup of the externally irradiated uterine cancer patients who received substantial doses to the bone marrow in the central trunk region of the body as well as to the pelvic marrow (i.e. those with similar dose distributions to those in the ankylosing spondylitis patients) had a greater excess relative risk (ERR=4.5, 95 per cent CI 1.0–14.1) than women with more non-uniform exposures (ERR=0.90, 95 per cent CI 0.1–2.2).

Women irradiated for BGD received lower doses than cancer patients (mean total marrow dose about 1.2 Gy). Both studies of these women have fitted linear models based on total marrow dose, although doses to parts of the bone marrow may have been sufficient to induce cell sterilisation (mean dose to the pelvis from external irradiation was 5.7 Gy in the American study, while for the Scottish study the mean dose to the sacral marrow was 5.5 Gy). Based on total marrow dose, the predicted ERR at 1 Gy in these studies using a linear model was consistent with estimates from the irradiated cancer patients. In neither study were there enough data to allow the fitting of compartmental models with cell killing, such as were used in the analysis of the spondylitis and cervical cancer studies.

11.4 Occupational Exposures

The LSS and studies of medically irradiated patients who have received high doses are currently used to estimate risks following exposure to the much lower doses of ionising radiation usually received by the general public and those who work with radiation. Clearly the extrapolation of effects from high dose, short-term exposures to low dose, protracted exposure involves several assumptions. It is important, therefore, to examine the leukaemia risk directly from studies of people who have protracted exposures to radiation, delivered at low dose rates. One of the main sources of exposure to man-made ionising radiation is the nuclear industry and an international study of nearly 96 000 workers in the nuclear industry has recently been published (IARC, 1994). The study analysed leukaemia mortality in seven cohorts of nuclear workers in the UK, USA and Canada, who were exposed to low-level, predominantly -radiation for long periods, and who were monitored by personal dosimeters. The distribution of cumulative dose was skewed, with 11 per cent having zero dose, about 60 per cent having doses below 10 mSv and 80 per cent below 50 mSv (Figure 11.3).

By the end of the follow-up period for the study, nearly 16 000 deaths had occurred, including 119 from leukaemia other than CLL. Analyses were based on a linear relative risk model in which the relative risk was assumed to be of the form 1+ *D*, where *D* is the cumulative dose in sieverts and the excess relative risk per sievert. There was a significant linear dose-response relationship (ERR=2.2 per Sv, 90 per cent CI 0.1–5.7). This estimate was consistent with estimates from the Japanese atomic bomb survivors, lying between the value obtained from a linear model (ERR=3.7 per Sv) and from the linear term of a model that included both linear and quadratic terms in dose (ERR=1.4 per Sv); see Table 11.3.

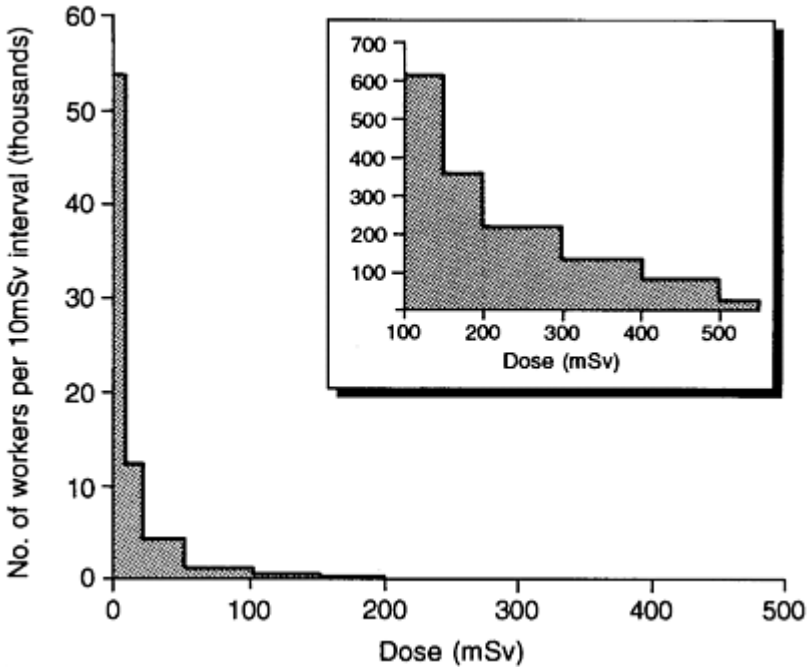

Figure 11.3 Distribution of final cumulative radiation doses among 96 000 workers in the nuclear industry from seven cohorts included the study of mortality associated with low doses of ionising radiation. (Reproduced from IARC Study Group on Cancer Risk among Nuclear Industry Workers, © *The Lancet Ltd* 1994)

11.5 Studies of Diagnostic X-rays

On the basis of extrapolation from high dose studies it has been estimated that diagnostic X-rays account for about 11 per cent of radiation exposure to the US population (National Research Council, 1990). At present there is no conclusive direct evidence on the effect of diagnostic X-rays in adults, although four out of five case-control studies of myeloid leukaemia following exposure to diagnostic X-rays in adults have shown an increasing risk with increasing number of X-rays to the trunk (Preston-Martin *et al.,* 1989). These studies can have only limited interpretation because there was often a potential for recall bias and accurate dosimetry infor

Table 11.3 Estimates of the excess relative risk per Sv for leukaemia other than chronic lymphocytic leukaemia from studies of nuclear workers and survivors of the Japanese atomic bombings (based on IARC, 1994)

Study population	Dose estimate	Adjustments included in model	Model for radiation risk	ERR per Sv (90% CI)
Nuclear workers	Whole-body dose	Age, sex, calendar period, facility and socio-economic status	Linear	2.2 (0.1–5.7)
Japanese atomic bomb survivors	Bone marrow dose	Age, city and calendar period. Analysis restricted to men	Linear	3.7 (2.0–5.7)

Study population	Dose estimate	Adjustments included in model	Model for radiation risk	ERR per Sv (90% CI)
		exposed between the ages of 20 and 60		
			Linear-quadratic	1.4 (<0–6.5)[a]

[a] Estimate based on linear term in linear-quadratic model.

mation was lacking: dose estimates were in most cases based on the number of X-ray visits, using published dosimetry surveys of average dose per X-ray. However, there was no suggestion that X-rays were given for a preleukaemic condition, or of an association with the conditions for which the X-rays were given.

Studies of the effect of exposure to diagnostic X-rays *in utero* are important, as the foetus may be more susceptible to the carcinogenic effects of ionising radiation than an adult or young child. Numerous case-control studies of medical exposure to diagnostic X-rays during pregnancy have been carried out for both singletons and twins, and these have consistently found the relative risk of both leukaemia and other childhood cancers to be around 1.4 following maternal obstetric X-ray examination (Bithell, 1989). Accurate estimation of the dose to the foetus or embryo from these studies is difficult, but if the dose is assumed to be about 10 mSv, then the estimated ERR per Sv derived from the various case-control studies is around 40. So far this estimate has not been confirmed by the half dozen or so cohort studies of the effects of prenatal irradiation that have been carried out, notably that of 1263 children exposed *in utero* to the Japanese atomic bombings, in whom no cases of childhood leukaemia were observed following a mean uterine dose of 0.18 Gy (Yoshimoto *et al.,* 1988). The estimate of 40 is also somewhat higher than the ERR per sievert of leukaemia mortality observed in children exposed to the Japanese atomic bombings at ages less than 10, which was around 17. For these reasons some have doubted the causal nature of the relationship seen in the case-control studies. However, there is no biological reason to expect the foetus to be resistant to the effects of ionising radiation and some risk following exposure is therefore to be expected. For a further discussion of this topic see UNSCEAR (1994).

11.6
Leukaemia Risks following Exposure to High LET Radiation

Small excesses of leukaemia have been demonstrated following injection with substantial doses of radium-224 in the form of radium chloride which was given in Germany as a treatment for tuberculosis, ankylosing spondylitis and other diseases (Spiess *et al.,* 1989), and also in patients injected with thorotrast, a 25 per cent colloidal solution of thorium dioxide, which was a commercially prepared contrast agent used in medical radiography in several countries from about 1930 until after the Second World War (Andersson and Storm, 1992). In both these instances, the risk of leukaemia was small compared with the risk of other cancers: osteosarcoma in the case of radium-224 and liver cancer in the case of thorotrast. Thorotrast is no longer used, and radium-224 is no longer given in its original high dose form, although it is thought to be efficacious in treating ankylosing spondylitis and treatment of this disease with much lower amounts of radium-224 continues to be used in Germany.

It is well established that working in an atmosphere with a high concentration of radon and its progeny leads to an increased risk of lung cancer. It has also been suggested, on the basis of geographical studies, that radon might also be a cause of leukaemia (Henshaw *et al.,* 1990; and see Chapter 3). In order to investigate this possibility further, a collaborative analysis of eleven studies of radon-exposed miners has recently been carried out (Darby *et al.,* 1995). A total of 64000 miners, who had been followed for an

average of seventeen years, were included in the study, and over 1000 radon-related lung cancer deaths had occurred in these men. There was no overall excess of mortality from leukaemia (RR=1.16, 95 per cent CI 0. 90–1.47, based on 69 deaths), but there was a significant excess in the first ten-year period since starting work (RR=1.93, 95 per cent CI 1.19–2.95, based on 21 deaths). As the men were employed in the mines for an average of only 6.4 years, an excess of leukaemia during this early period would be consistent with the short induction period for radiation-induced leukaemia observed in the survivors of the Japanese atomic bombings and in studies of medically exposed populations. However, there was no significant association between risk of leukaemia and cumulative radon exposure, and the possibility of a chance finding cannot be ruled out.

11.7
Effects of Environmental Exposures

Exposure to ionising radiation is widespread in the environment and over 80 per cent of the average annual effective dose to adults comes from natural sources (National Research Council, 1990). The average total bone marrow dose to a member of the US population is about 1.1 mSv, and this exposure is estimated to cause about 11 per cent of all leukaemia deaths (Darby, 1991).

Higher levels of environmental radiation have occurred following contamination by various artificial sources, including the atmospheric nuclear weapons test in the 1950s and 1960s. A study of childhood leukaemia in the Nordic countries showed some evidence of an increased risk following exposure to fallout from these weapons tests (Darby *et al.,* 1992). During the high fallout period, the estimated bone marrow dose was about 1.5 mSv, and the rates of leukaemia were slightly higher than in the surrounding medium fallout period (relative risk for ages 0–14:1.07, 95 per cent CI 1.00–1.14). Such an increase is consistent with a relative risk of 1.03 predicted on the basis of the experience of the survivors of the Japanese atomic bombings. A case-control study of leukaemia in Utah following fallout from nuclear weapons tests at the Nevada test-site found an association between bone marrow dose and non-CLL mortality when all ages and all time periods were combined (Stevens *et al.,* 1990). The risk was greatest among those aged less than 20 at exposure, and in the period of highest exposure. The observed risks were about double those predicted by the Japanese data, but the difference was not statistically significant.

Within the area of the former Soviet Union, a series of releases and accidents occurred at the Mayak nuclear weapons facility in the southern Urals in the 1950s. As a result, several hundred thousand people received both internal and external radiation exposures. Until recently little information was available about the exposure or their effects. However, some data have recently been published (Kossenko and Degteva, 1994), and although of variable quality, they indicate that a considerable number of leukaemias, and possibly other cancers also, have been caused by the releases. Several reports presenting data on the effects of the accident at the Chernobyl nuclear power station in 1986 are now available (Prisyazhiuk *et al.,* 1991; Parkin *et al.,* 1993). These find no evidence that leukaemias have occurred in excess as a result of the accident, although there is now evidence that some excess of thyroid cancers has occurred (Prisyazhiuk *et al.,* 1991; Kazakov *et al.,* 1992).

A report in the mid-1980s of an unexpectedly large number of cases of leukaemia in young people in Seascale, a small town 3 km south of the Sellafield nuclear reprocessing plant in northwest England (Black, 1984), gave rise to much concern about the possible effects of both local pollution from radioactive waste and of paternal occupational irradiation on any subsequent children. After much subsequent research neither of these two explanations seems likely to account for the excess, and the most probable explanation for it is

either chance or some factor unconnected with radiation, such as an infectious agent (Doll *et al.,* 1994; Kinlen, 1993).

11.8 Conclusions

As a result of theoretical, experimental and epidemiological work in recent years, there has been a substantial increase in knowledge about the leukaemogenic effects of radiation. Radiobiological theory predicts the shape of the dose-response curve to be linear-quadratic with a decreasing exponential term due to cell sterilisation, and there is now epidemiological support for these models. Epidemiological studies also provide estimates of the risk following exposure to low doses of ionising radiation. Such exposure is widespread, and there is much public concern about the possible risk involved.

The study of Japanese atomic bomb survivors has been central in estimating radiation-induced risks, but even in this large study the number of excess cancer deaths is small, and analyses of other irradiated populations are necessary to support and add to the results from this study and for setting limits of radiation protection for occupational, medical and public exposures.

Recent interest has focused on the variation of leukaemia risk with bone marrow dose and dose rate. The Life Span Study indicated that a linear-quadratic or linear-spline model provided a good fit to the data for all leukaemias as a group, with an excess relative risk (ERR) per sievert of 3.9. Dose-response relationships in high-dose studies are, at present, used to estimate the dose-response curve at lower doses and dose rates. This is partly due to the small excess risk at these lower doses, and partly to the lack of accurate dosimetry, particularly for environmental exposures. However, there is no evidence from low-dose studies that risk estimates differ from those extrapolated from high-dose studies, and the recent study of nuclear workers estimated that the ERR per sievert was 2.2 (90 per cent CI 0.1–5.7). Finally, one topic about which little is known at present, and about which additional information would be desirable, is the joint effect of radiation and other agents.

References

ANDERSSON, M. & STORM, H.H. (1992) Cancer incidence among Danish thorotrast-exposed patients, *J. Nat. Cancer Inst.,* **84,** 1318–25.

BITHELL, J.F. (1989), Epidemiological studies of children irradiated *in utero*. In K.Baverstock & J.W.Stather, (eds) *Low Dose Radiation: Biological Bases of Risk Assessment*. London: Taylor & Francis, pp. 77–87.

BLACK, D. (1984) *Investigation of the Possible Increased Incidence of Cancer in Western Cumbria*. London: HMSO.

BOICE, J.D., BLETTNER, M., KLEINERMAN, R.A. *et al.* (1987) Radiation dose and leukaemia risk in patients treated for cancer of the cervix, *J. Nat. Cancer Inst.* **79,** 1295– 311.

BRESLOW, N.E. & DAY, N.E. (1980). *The Design and Analysis of Case-control Studies,* Statistical methods in cancer research, vol. I. Oxford University Press: Lyon.

CURTIS, R.E., BOICE, J.D., STOVALL, M. *et al.* (1994) Relationship of leukaemia risk to radiation dose following cancer of the uterine corpus. *J. Nat. Cancer Inst.,* **86**, 1315–324.

DARBY, S.C. (1991); The contribution of natural ionizing radiation to cancer mortality in the United States. In J.Brugge *et al.* (eds) *The Origins of Human Cancer*. New York: Cold Spring Harbor Laboratory Press, pp. 183–90.

DARBY, S.C., OLSEN, J.H., DOLL, R. *et al.* (1992). Trends in childhood leukaemia in the Nordic countries in relation to fallout from atmospheric nuclear weapons testing, *Br. Med. J.,* **304**, 1005–9.

DARBY, S.C., REEVES, G.K., KEY, T., DOLL, R. & STOVALL, M. (1994) Mortality in a cohort of women given X-ray therapy for metropathia haemorrhagica, *Int. J. Cancer,* **56**, 793–801.

DARBY, S.C., WHITLEY, E., HOWE, G.R. *et al.* (1995). Radon and cancers other than lung cancer in underground miners: a collaborative analysis of 11 studies. *J. Nat. Cancer Inst.* **87** (5), 378–84.

DOLL, R., EVANS, H.J. & DARBY, S.C. (1994) Paternal exposure not to blame. *Nature,* **367**, 678–80.

FOLLEY, J.H., BORGES, W. & YAMAWAKI, T. (1952) Incidence of leukaemia in survivors of the atomic bomb in Hiroshima and Nagasaki, Japan, *Am. J. Med.* **13**, 311–21.

HENSHAW, D.L., EATOUGH, J.P. & RICHARDSON R.B. (1990). Radon as a causative factor in induction of myeloid leukaemia and other cancers, *Lancet,* **335**, 1008–12.

IARC (1994) (Study Group On Cancer Risk Among Nuclear Industry Workers). Direct estimates of cancer mortality due to low doses of ionising radiation: an international study, *Lancet,* **344**, 1039–43.

INSKIP, P.D., KLEINERMANN, R.A., STOVALL, M. *et al.* (1993) Leukaemia, lymphoma, and multiple myeloma after pelvic radiotherapy for benign disease, *Radiat. Res.* **135**, 108–24.

KAZAKOV, V.S., DEMIDCHIK, E.P. & ASTAKHOVA, L.N. (1992) Thyroid cancer after Chernobyl, *Nature,* **359**, 21.

KINLEN, L.J. (1993) Can paternal preconceptional radiation account for the increase of leukaemia and non-Hodgkin's lymphoma at Seascale? *Br. Med. J.* **306**, 1718–21.

KOSSENKO, M.M. & DEGTEVA, M.O. (1994) Cancer mortality and radiation risk evaluation for the Techa river population *Sci. Total Environ.* **142**, 73–89.

MUIRHEAD, C.R., COX, R., STATHER, J.W., MACGIBBON, B.H., EDWARDS, A.A. & HAYLOCK, R.G.E. (1993). *Estimates of Late Radiation Risks to the UK population.* National Radiological Protection Board.

NATIONAL RESEARCH COUNCIL (1990) *Health Effects of Exposure to Low Levels of Ionizing Radiation* (BEIR V). Washington, DC: National Academy Press.

PARKIN, D.M., CARDIS, E., MASUYER, E. *et al.* (1993) Childhood leukaemia following the Chernobyl accident: the European Childhood Leukaemia-Lymphoma Incidence Study (ECLIS), *Eur. J. Cancer,* **29A**, 87–95.

PRESTON, D.L., KUSUMI, S., TOMONAGA, M. *et al.* (1994) Cancer incidence in atomic bomb survivors. Part III: Leukaemia, lymphoma and multiple myeloma, 1950–1987, *Radiat. Res.* **137**, S68–S97.

PRESTON-MARTIN, S., THOMAS, D.C., YU, M.C. & HENDERSON, B.E. (1989) Diagnostic radiography as a risk factor for chronic myeloid and monocytic leukaemia, *Br. J. Cancer,* **59**, 639–44.

PRISYAZHIUK, A., PJATAK, O.A., BUZANOV, V.A., REEVES, G.K. & BERAL, V. (1991) Cancer in the Ukraine, post-Chernobyl, *Lancet,* **338**, 1334–5.

ROESCH, W. (ed.) (1987) *Re-assessment of Atomic Bomb Radiation Dosimetry in Hiroshima and Nagasaki.* Find Report. RERF.

SPIESS, H., MAYS, C.W. & CHMELEVSKY D. (1989) Malignancies in patients injected with ^{224}Ra. In D.J.TAYLOR *et al.,* (eds), *Risks from Radium and Thorotrast,* BIR Report 21. London British Institute of Radiology, pp. 7–12.

STEVENS, W., THOMAS, D.C., LYON, J.L. *et al.* (1990) Leukaemia in Utah and radioactive fallout from the Nevada Test Site, *J.A.M.A.,* **264**(5), 585–91.

THOMAS, D.C., BLETTNER, M. & DAY, N.E. (1992) Use of external rates in nested case-control studies with application to the International Radiation Study of Cervical Cancer Patients, *Biometrics,* **48,** 781–94.

UNSCEAR (1994) *Sources and effects of ionizing radiation*. Report of the United Nations Scientific Committee on the effects of atomic radiation to the General Assembly, with scientific annexes. New York; United Nations.

WEISS, H.A., DARBY, S.C. & DOLL, R. (1994) Cancer mortality following X-ray treatment for ankylosing spondylitis, *Int. J. Cancer,* **59, 3**27–38.

WEISS, H.A., DARBY, S.C., FEARN, T. & DOLL R. (1995). Leukaemia mortality after X-ray treatment for ankylosing spondylitis. *Radiat. Res.,* **142**, 1–11.

YOSHIMOTO, Y., KATO H. & SCHULL, W.J. (1988) Risk of cancer among children exposed *in utero* to A-bomb radiations, 1950–84. *Lancet,* ii, 665–9.

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Plate 1 Smear of normal bone marrow cells stained with May Grünwald and Giesma. (1200×)

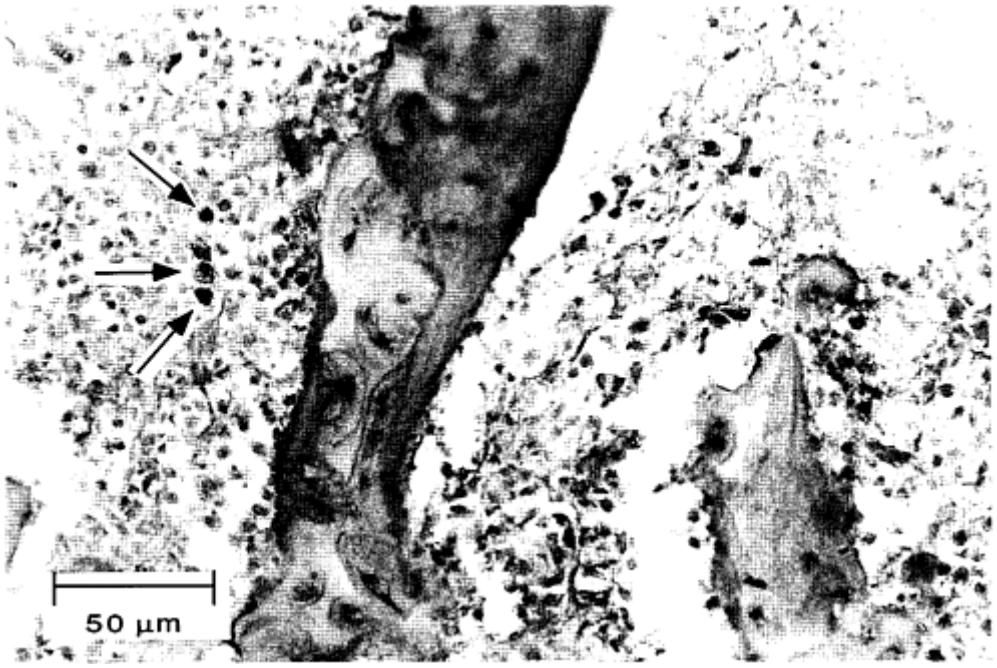

Plate 2 CD38+cells (brown) within α-particle range of a trabecular bone surface of a lumbar vertebra at 40 weeks gestation

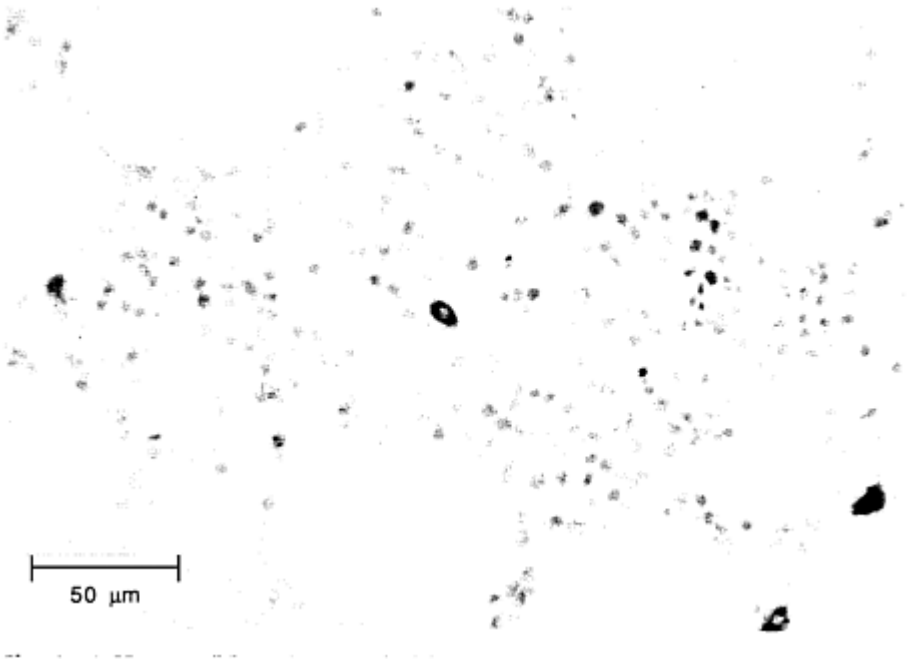

Plate 3 A CD34+cell (brown) in normal adult bone marrow, in the centre of the haemopoietic tissue and close to several fat cells

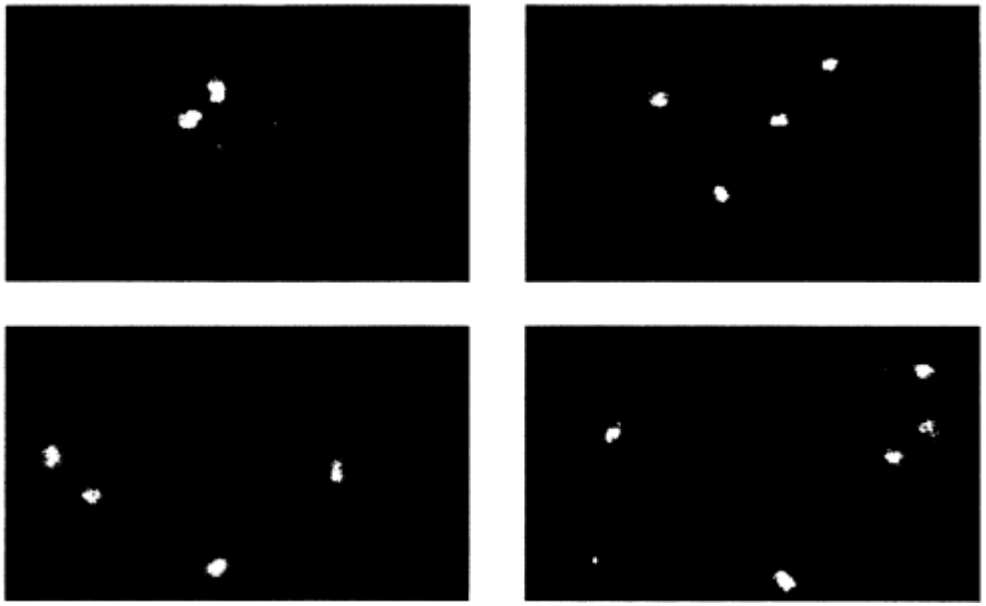

Plate 4 Fluorescence *in situ* hybridisation analysis reveals the instability of the Y-chromosome in a primary, radiation-induced acute myeloid leukaemia in a CBA/H mouse