4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Radiation syndromes: Early effects of whole-body irradiation

A hierarchy of health effects that appear sequentially after high doses of whole-body irradiation consists of the haematopoietic, gastrointestinal and central nervous syndromes, which are collectively referred to as the 'acute radiation syndromes' and have been extensively reviewed (Bond *et al*., 1965; Young, 1987; UNSCEAR, 1988). The dose range over which these syndromes occur is shown in Table 35. The cutaneous radiation syndrome and the chronic radiation syndrome are now considered sufficiently distinct to be included in the list of radiation syndromes. The more severe

Table 35. Effects and outcomes after exposure to ionizing radiation

Dose range (Gy)	Prodromal effects	Tissue effects	Survival
0.5–1.0	Mild	Small decrease in blood cell count	$LD_{0/60}$ (normal subject)
2.0–3.5	Moderate	Moderate-to-severe damage (bone marrow)	$LD_{5/60}$–$LD_{50/60}$
3.5–5.5	Severe	Severe damage (bone marrow)	$LD_{90/60}$–$LD_{99/60}$[a] (death, 3.5–6 weeks)
5.5–7.5	Severe	Ablation (bone marrow)	Death, 2–3 weeks[a]
10–20	Severe	Severe damage (gastrointestinal)	Death, 5–12 days
100	Severe	Cerebrovascular damage	Death, within 2 days

Adapted from Young (1987)

[a] Treatment may increase survival by raising the dose that is lethal by 50% but to a lesser extent in the case of the gastrointestinal syndrome.

effects are preceded by a prodromal phase, which is mediated by a poorly understood effect on the autonomic system. Apart from the signs and the symptoms of the prodromal phase and the central nervous syndrome, the early effects of radiation are due to cell killing in tissues with rapid cell turnover such as the bone marrow and the gut. Cell killing is also the major determinant in tissues such as lung and skin that incur early deterministic effects, but later. The relative radiosensitivity of the clonogenic cells in various solid tissues is shown in Table 36.

Table 36. Radiosensitivity of clonogenic cells in solid tissues, as indicated by the D_0

Tissue	D_0 (Gy)	Reference
Jejunum	1.30	Withers & Elkind (1970)
Testis	1.36	Withers *et al*. (1974)
Kidney	1.53	Withers *et al*. (1986)
Skin	1.35	Withers (1967)
Colony-forming units (haematopoietic)	0.95	McCulloch & Till (1962)
Breast	1.22	Gould & Clifton (1979)
Thyroid	2.0	Mulcahy *et al.* (1980)

D_0, reciprocal of the final slope of the curve of survival as a function of dose, representing cell killing due to multiple events

Death from bone-marrow damage occurs at lower doses than death from damage to the gut and longer after exposure. This reflects differences in the cell kinetics and design of the two cell renewal systems and to some extent the inherent radiosensitivity of the stem cells. In the haematopoietic system, the lifespan of the functional cells varies with cell type: the megakaryocyte–platelet and leukocyte populations are at highest risk because of their short lifespan.

Two main types of cell death are induced by radiation: (1) death associated with mitosis because of DNA damage, in many cases causing chromosomal alterations that make the first or subsequent post-irradiation cell division lethal, and (2) death through apoptosis in interphase, in some cases before the irradiated cells reach mitosis and in other cases after they have undergone mitosis. The probability that a cell will die through apoptosis depends largely on the type of cell. For example, in some types of lymphocytes, damage to the cell membrane can trigger a cascade of enzymatic events that ultimately result in scission of the DNA strands. In contrast, apoptosis is not frequently induced in fibroblasts. While apoptosis may occur in non-cycling cells, most such cells remain functional even though they carry DNA damage that is lethal when the cell attempts division.

Individual cell loss may be random, but it is the overall effect of killing a critical number of cells that causes the deterministic effect, which may be expressed either early or late. In lung and especially skin, some effects, such as erythema, occur relatively soon after exposure, but others, such as fibrosis, are observed many months later.

4.2 Late deterministic effects of ionizing radiation

The late effects of radiation are not fully explained, and the relative importance of depletion of parenchymal cells, which directly affects the functional and proliferative capacity of tissues, and of damage to the microvasculature, which indirectly affects the parenchymal cells, is a matter of discussion. The initial model of late effects was based on radiation-induced changes in the microvasculature of organs. Endothelial cells can be lost as a result of interphase death or death associated with mitosis as the slowly cycling cells come into division. The loss of vascular integrity in turn leads to fibrosis and loss of parenchymal cells (Rubin & Casarett, 1968; Casarett, 1980). An alternative model (Withers, 1989) stresses the importance of the loss of functional subunits, the architectural arrangement of organs and their stem cells. For example, the nephron in the kidney consists of epithelial cells; if a sufficient number are killed, the functional unit is lost because it cannot be repopulated from neighbouring nephrons. The function of the kidney is critically compromised as the loss of functional subunits increases. Similarly, in the spinal cord, the functional subunit essential for myelination and therefore for the function of the neurons is the minimum number of glial cells required for maintaining the integrity of the myelin. It is clear that not only radiosensitivity but also the volume of tissue irradiated is important. Withers (1989) contended that the severity of a radiation-induced late effect in an organ is

determined by the radiosensitivity of the stem cells and the arrangement into functional subunits. In those organs for which it has been determined, the radiosensitivity of the stem cells is fairly similar, with the exception of the more radiosensitive haematopoietic system. The most useful characteristic of the dose–response relationship is the α:β ratio (see section 5.1, Overall introduction), which is generally lower for late effects than for early effects and reflects the proportion of the damage repaired. The current approach to radiotherapy has gained from the idea that the quantal responses of tissues could be considered in terms of tissue-rescuing units (Hendry & Thames, 1986). It seems likely that damage to both the parenchymal cells and the microvasculature plays a role in the late deterministic effects, one being more important in some organs and less in others.

Most of the information about late effects comes from studies of patients undergoing radiotherapy. The success of radiotherapy comes at the risk of potential late effects, and dose fractionation is used to exploit the differential of repair and recovery between normal and cancerous tissues (see Thames & Hendry, 1987).

Atomic bomb survivors constitute the largest population that has been exposed to whole-body irradiation; they have been monitored for almost five decades (Shimuzu *et al*., 1999). During the period 1950–90, some 27 000 deaths occurred from causes other than cancer. The emphasis of the follow-up has been on diseases of the respiratory, cardiovascular and digestive systems, the rates of which increased 5–15% among people who received a dose of 1 Sv at these organs. This is a smaller increase than that for cancer. The most frequent causes of these deaths were stroke and heart disease, which accounted for about 54% of the total. It is not possible to distinguish statistically between a linear dose–response curve, a curvilinear response or the presence of a threshold. Late effects in the eye have also been studied, and the incidence of cataracts is discussed below.

The other relatively large population that has received whole-body irradiation is composed of patients who were exposed preparatory to bone-marrow transplantation. Late deterministic effects have been found in a number of tissues, including the lens of the eye, but no information is available on dose–response relationships, and the findings are confounded by prior chemotherapy in many patients. Future reviews of more homogeneous populations may provide more useful data.

The effects of radiation on organs for which some evidence of effects exists are described below.

4.2.1 *Skin*

The first reports of radiation-induced deterministic effects—erythema and radiodermatitis—in the X-ray technicians and physicians involved in the early days of what would become radiology appeared within months of Röntgen's discovery of X-rays. The ease with which the effects of radiation on the skin could be detected made it the obvious indicator of exposure for the purposes of radiation protection.

In 1925, the concept of the ‘tolerance dose’ was introduced for use in setting limits on exposure to radiation, and was expressed as 1% of the threshold dose for inducing erythema per month for whole-body exposure to X-rays (Taylor, 1981). The ease with which effects could be detected in the skin proved to be of no advantage when it was realized that cancer could be induced by doses of penetrating radiation below those that induce deterministic effects in the skin. The classification of early and late deterministic effects in the skin is shown in Table 37.

Table 37. Radiation-induced deterministic effects in skin and time of appearance after exposure

Effect	Time of appearance after exposure
Early transient erythema	Hours
Main erythematous reaction	About 2 weeks
Dry desquamation	3–6 weeks
Moist desquamation	4 weeks
Late erythema	8–20 weeks
Secondary ulceration	10 weeks
Dermal necrosis	10 weeks
Dermal atrophy	26 weeks
Telangiectasia	52 weeks

From ICRP (1991c)

The tolerance of the skin depends on the area of the exposed field, the total dose, the fraction size and the interval between fractions. Unless the fields are large, erythema occurs only after exposure to 5–6 Gy or to about 12 Gy if the dose is fractionated; transient loss of hair may also occur. Moist desquamation may occur after a single dose of 18 Gy or after 40–50 Gy in about 25 fractions over about five weeks. The skin has a remarkably large capacity to recover from the damage induced by large total doses (tens of grays) if the dose is spread over a number of fractions, which allows time for repair of sublethal damage and for repopulation.

The early or acute effects of radiation on the skin include erythema, which occurs in varous phases. Erythema may be seen within hours of exposure of large fields to doses in the range used in radiotherapy, about 2 Gy, reflecting increased permeability of the capillaries and the early onset of inflammation. This phase is transient, and the erythema disappears within 24–48 h. The more significant phase, known as the main erythematous reaction, usually appears during the third week of a fractionated regimen. This phase is due to the inflammatory reaction that follows the death of cells in the basal layer of the epithelium. A few days after irradiation, cell proliferation may

have stopped. Although the number of basal cells decreases, the integrity of the skin is maintained; however, dry desquamation may occur. With higher doses—about 30–40 Gy in multiple fractions—moist desquamation occurs. Desquamation is caused by inactivation of a critical number of clonogenic cells in the basal layer and follows within four to six weeks of exposure. Severe desquamation can lead to ulceration of the dermis. If the damage to the dermal vasculature is extensive, dermal necrosis may ensue within 10 or more weeks.

The responses to fractionated dose regimens are complex. In experimental studies of fractionated and prolonged irradiation of mouse skin, greater skin sensitivity was observed when 3-Gy fractions were given at an interval of 48 h than at either 6- or 24-h intervals. This effect was interpreted as the consequence of the increased radiosensitivity seen during the proliferative response induced by the radiation (Ruifrok *et al*., 1994).

A different form of acute ulceration is found after exposure of extremely small areas of skin (and other epithelial surfaces) to very high doses, as occurs when 'hot' particles, such as the very small fragments of steel activated by neutron irradiation in a reactor, stick to the skin or in the nose, where they can remain unnoticed long enough to deliver an appreciable dose of β-particles and γ-rays. Within about two weeks of exposure, a pale, circular area surrounded by a halo of erythema is seen, which is quite distinct from other skin lesions induced by radiation. Ulceration follows when the overlying epidermis separates to reveal a small area of necrotic dermis. The evidence suggests that endothelial cells and fibroblasts in the superficial dermis are killed in interphase. The dosimetry for this type of radiation damage was established in experiments on pig skin *in vivo*. The median effective doses for the induction of moist desquamation by exposure to circular sources of ^{90}Sr (a high-energy β emitter) of various diameters were 27.5 Gy for a 22.5-mm source up to 75 Gy for a 5-mm source; the 2-mm and 1-mm sources induced acute necrosis within three weeks, at median effective doses of 125 and 275 Gy, respectively (Hopewell *et al*., 1986).

Acute epithelial necrosis is induced by very-low-energy β-particles which cause interphase death in the suprabasal layer of the epidermis about 10 days after exposure. Radiation-induced lesions were studied in 56 workers, in particular firemen, at the Chernobyl facility who had incurred doses estimated to have been > 30 Gy at a depth of 150 mg cm^{-2} and over 200 Gy at about 70 mg cm^{-2}. The workers were exposed to high-activity fission products with a β-particle to γ-ray ratio of 10 to 30. Skin desquamation and subsequent infection in victims who received damage over 50% of their body surface area contributed to their deaths. All of these persons also had damage to their haematopoietic systems (UNSCEAR, 1988; Barabanova & Osanov, 1990).

Burns are induced by fall-out after detonation of nuclear weapons. For example, the doses to the skin received by Japanese fishermen exposed to the fall-out from one test were estimated to be 1.7–6.0 Gy. Erythema and necrosis were found in a few of the exposed men, and late effects were noted subsequently.

A late phase of erythema that gives the skin a dusky appearance is sometimes seen 8–20 weeks after exposure. It is seldom seen in patients receiving fractionated radiotherapy but was observed in victims of the Chernobyl accident who had received high doses 1.5 mm below the surface of the skin, where the deep dermal plexus of blood vessels is found. Loss of endothelial cells appears to be a major causal factor.

The other late effects of concern are dermal atrophy, telangiectasia and necrotic ulcer. The severity and the incidence of these lesions increase as the dose exceeds 30–40 Gy when given in fractions of 2 Gy. Dermal atrophy appears to develop in two phases, beginning 14–20 weeks after exposure and after about one year. The first phase is thought to be due to loss of endothelial cells, as in dermal necrosis (Hamlet & Hopewell, 1988), and to loss of fibroblasts (Withers *et al*., 1980), a significant loss of endothelial cells sometimes preceding that of fibroblasts. The second phase involves degeneration of the smooth muscle of arterioles.

Telangiectasia may occur in patients treated with fractionated doses about one year or more after therapy. The incidence and the severity increase with time in a dose-dependent manner.

4.2.2 *Lung*

Radiation pneumonitis and fibrosis are the main deterministic effects in the lung. Three types of pulmonary cell are involved in the responses to radiation: type-1 and type-2 alveolar cells and endothelial cells; the last two undergo renewal and are targets for radiation-induced damage (see review by Travis, 1987). Radiation pneumonitis occurs in experimental animals and in humans about 80–180 days after exposure and, depending on the dose, may be fatal. The human lung is slightly more sensitive than that of mice, with estimated LD_{50} values of 9–10 Gy of external irradiation for humans and 12–15 Gy for mice. Radiation pneumonitis is characterized by interstitial oedema, infiltration of inflammatory cells and desquamation of alveolar epithelial cells. At high doses, an exudate is found in alveolar air spaces. An alveolar infiltrate can be detected radiologically, and opacification is detected by computerized tomography in a high percentage of patients within about 16 weeks of receiving fractionated doses. Dyspnoea is a symptom of pneumonitis in both humans and mice.

The effects of total dose, the number of fractions and the total period of treatment on the incidence of radiation-induced pneumonitis in patients undergoing radiotherapy are shown in Table 38.

Fibrosis, the main long-term effect of radiation on the lung, may occur in patients in whom pneumonitis has not been detected. The loss of volume and of diffusing capacity depend, as in other tissues, on the size of the radiation field. The histological changes include an increased amount of collagen which replaces the alveolar septa, a decrease in the number of functioning capillaries, atypical alveolar epithelial cells and loss of alveoli due to fibrotic changes which may lead to atelectasis. Lung fibrosis

Table 38. Incidence of radiation pneumonitis in patients undergoing radiotherapy, according to dose regimen

Total dose (Gy)	No. of fractions	Length of treatment (weeks)	Incidence of pneumonitis (%)
6–7	1		0 (threshold)
10	1		84
26.5	20	4	5
20	10	2–4	5
30.5	20	4	5
30.0	10	2	100

Data from Mah *et al.* (1987); UNSCEAR (1988)

may appear about one year after irradiation, and the changes are usually irreversible (Travis, 1987).

4.2.3 *Gonads*

(*a*) *Ovary*

The ovary is a radiosensitive organ, but its radiosensitivity to the induction of sterility is age-dependent (Table 39). Radiation-induced ovarian failure gives rise not only to reduced fertility or sterility but also to reduction or cessation of hormone production, which may lead to premature menopause in younger women (Meistrich *et al*., 1997). Amenorrhoea has been reported in 10% of patients exposed during childhood to 0.5 Gy to the ovaries and in about 66% exposed to 3.0 Gy (UNSCEAR, 1993). A dose of 1.0–1.5 Gy appears to be the threshold for an effect on fertility. Ovarian failure occurs in 40% of 20-year-old women and in 90% of 35-year-old women receiving a dose of 4.5 Gy. The effect is reduced by dose fractionation and protraction of radiotherapy (Meistrich *et al*., 1997).

(*b*) *Testis*

The germinative cells of the seminiferous tubules are highly radiosensitive, whereas the Sertoli cells, which provide support and nutrition for the spermatogonia, and the Leydig cells, the source of testicular hormones, are considerably more resistant. Irradiation may reduce fertility or induce temporary or permanent sterility but has little effect on libido. The response of the testis has been studied in patients undergoing radiotherapy, radiation workers, volunteers in state penitentiaries, victims of nuclear accidents and atomic bomb survivors (see Meistrich & Van Beek, 1990). The sperm count remains within the normal range for about eight weeks after irra-

Table 39. Minimum fractionated doses to the ovary that induce sterility

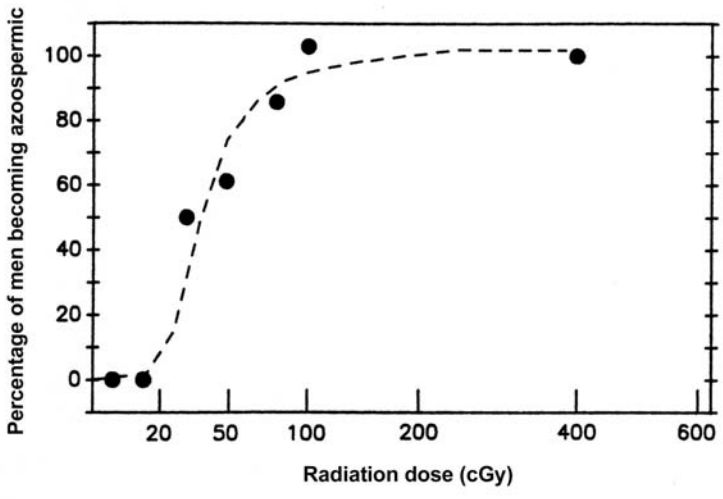
Dose (Gy)	Ovarian failure (%)	
	15–40 years of age	> 40 years of age
0.6	None	None
1.5	No risk	Small risk
2.5–5.0	60	100
5.0–8.0	60–70	NR
> 8.0	100	100

From Ash (1980) and Damewood & Grochow (1986);
NR, not reported

diation, but falls to its lowest level over the next three to eight months; aspermia may occur temporarily after a dose of about 0.2 Gy. The reduction in spermatogenesis is dose-dependent (Figure 13). A dose of 1.0 Gy causes aspermia in 90% of men. Fractionation does not reduce the effect and may increase it. The onset of recovery is also dose-dependent, occurring within about six months after a dose of 0.2 Gy but not until two years after a dose of 5 Gy. An analysis by Meistrich and Van Beek (1990) of the data obtained by Rowley *et al*. (1974) in a study of volunteers showed that type Ap and type B spermatogonia and early spermatocytes were the most radiosensitive cells and that late spermatocytes, spermatids and type Ad spermatogonia, considered to be the reserve stem cells, were somewhat less sensitive. It is difficult to estimate a threshold dose for temporary sterility, which depends on the time after exposure that fertility is assessed. A dose as low as 0.1 Gy has detectable effects in the young, and 0.15 Gy may cause oligospermia and temporary infertility in adults (UNSCEAR, 1993).

Two parameters have been used to assess the effect of radiation on the testis: loss of testicular weight and regeneration of the spermatogenic epithelium. The curve for loss of testicular weight as a function of dose has two components, and the logarithm of the loss of the radiosensitive component is linearly related to dose, with a D_0 of 0.9–1.0 Gy in mice, where D_0 represents cell killing due to multiple events (Kohn & Kallman, 1954; Alpen & Powers-Risius, 1981). The percentage of tubules that showed foci of repopulation by spermatogonial cells at 35–42 days after irradiation was used as a measure of stem-cell survival. At doses > 8 Gy, an exponential survival curve with a D_0 of 1.8 Gy was obtained (Withers *et al*., 1974). It is not clear why the values for D_0 vary by a factor of two. A detailed assessment of the sensitivity of cells in the development stages of spermatogenesis in mice showed that the range of sensitivities is broad, but in general the sensitivity decreases from the intermediate spermatogonial stage to the mature sperm (see Table 40; Oakberg & Clark, 1964).

Figure 13. Percentages of men developing azoospermia after various single doses of radiation



From Meistrich & Van Beek (1990). Doses are plotted after square-root transformation.

Table 40. Sensitivity of mouse spermatogenic cells to radiation

Cell type	LD_{50} (Gy)
Spermatogonia (types A_s, A_1–A_4)	2.0
Intermediate spermatogonia	0.2
Type B spermatogonia	1.0
Meiotic stages	2.0–9.0
Secondary spermatocytes	10.0
Spermatids	15.0
Spermatozoa	500.0

From Oakberg & Clark (1964); LD_{50}, median lethal dose

Although Leydig cells are generally considered to be relatively radioresistant, transient increases in serum follicular hormone concentrations were reported after exposure to doses as low as 0.2 Gy, while at 2.0 Gy the serum concentration of luteinizing hormone was increased. Both parameters are indicators of Leydig-cell dysfunction (Kinsella, 1989).

The outstanding features of the effects of radiation on the testis are the exquisite sensitivity of some testicular cells, the lack of sparing with fractionation and the long recovery time.

4.2.4 *Kidney*

Opinions about the relative radiosensitivity of the kidney vary (UNSCEAR, 1982). The importance of radiation-induced nephropathy and questions about whether the kidney should be shielded during whole-body irradiation before bone-marrow transplantation have renewed interest in the subject. The cells at risk in the three major components of the kidney, the renal tubules, the glomeruli and the complex, abundant vasculature, are mainly post-mitotic cells. This influences the response of the kidney to radiation and the sequelae. The late effects—nephritis, nephrosclerosis, tissue necrosis and fibrosis with subsequent hypertension and loss of renal function—are the main concerns (UNSCEAR, 1993).

Tests of renal function provide no evidence of renal damage during the first six months after radiotherapy with fractionated regimens of total doses < 23 Gy, but nephritis with signs and symptoms of renal damage may occur 6–12 months after treatment. Albuminuria and increased urea nitrogen in blood are common features. Renal failure and hypertension are later, more serious sequelae. The tolerance dose is about 23 Gy given in fractions over about five weeks to both kidneys. Doses of 20–24 Gy given over about four weeks may result in a 10–60% reduction in renal plasma flow and glomerular filtration rate.

The tolerance dose is lower in children than in adults, and radiation-induced nephropathy has been observed after bone-marrow transplantation in children. Anaemia, increased urinary creatinine concentrations and other signs of renal insufficiency have been observed after exposure to 12–14 Gy given in six to eight fractions. The precise contribution of radiation is difficult to assess because many such patients have had prior chemotherapy. It is also difficult to determine how much of the reduced tolerance, i.e. the delay before renal failure, is due to age or to chemotherapy. Experimental evidence in rats indicates that age is important and that tolerance increases with age at irradiation, within limits (Moulder & Fish, 1997).

The early histological changes seen in the kidneys after irradiation include hyperaemia, increased capillary permeability and interstitial oedema. The fine vasculature shows evidence of damaged endothelial cells and repopulation, which tends to occlude the lumen of the vessels. The glomerular arterioles are affected and blocked. The vascular occlusion and narrowing cause ischaemia in the cortex, and secondary degeneration of the tubular epithelium may follow. Damage to the tubules is the primary lesion, and dose–survival relationships have been determined in mice for the cells responsible for regeneration of the tubular epithelium. When regenerating tubules were scored in mice 60 weeks after irradiation, the D_0 was 1.53 Gy, which is comparable with that recorded for clonogenic cells in other tissues. The doses used in

the assay (11–16 Gy) may, however, have damaged the vasculature (Withers *et al*., 1986).

4.2.5 *Gastrointestinal tract*

The effects of radiation on the gastrointestinal tract have been the subject of extensive reviews (see e.g. Bond *et al*., 1965; Becciolini, 1987; Potten & Hendry, 1995), from which the following descriptions are derived. Because the structure and the kinetics of cell turnover differ in the various regions of the gastrointestinal tract, the response to radiation also varies from one site to another.

(a) *Oral cavity*

Effects on the oral mucosa provide a somewhat more sensitive indicator of radiation-induced damage than effects on the skin, and mucositis is widely used to assess the radiosensitivity of the oral cavity. The early changes are similar to those in the skin but occur sooner after exposure. In the second week of fractionated radiotherapy, dryness of the mouth and even dysphagia may occur. An interesting early effect is an alteration in sensitivity to taste, which appears to affect the taste of salt and bitter differentially from that of sour and sweet.

The late changes in the oral cavity are fibrosis in the submucosa, telangiectasia and fibrosis involving the mucous glands. Chronic ulcers of the mucosa can follow fibrosis in the vasculature. The environment of the oral cavity can be changed by exposure to radiation because the saliva from irradiated salivary glands is more acidic than normal, and dental caries may develop.

(*b*) *Oesophagus*

Fractionated doses of 20–30 Gy can cause transient oesophagitis. Stricture may occur four to eight months after radiotherapy with doses of 30–65 Gy, depending on the fractionation regimen.

(*c*) *Stomach*

Fractionated doses up to approximately 20 Gy have been used in the treatment of peptic ulcer. Irradiation suppressed gastric acidity for six months to many years and was well tolerated, but the risk for cancer increased subsequently. With conventional fractionated radiotherapy, the stomach can tolerate a dose of about 40 Gy, but the likelihood of ulceration and perforation increases rapidly above this dose. The delayed effects include dyspepsia, impaired gastric motility and chronic atrophic gastritis, due to fibrosis.

(*d*) *Small intestine*

The small intestine is radiosensitive because the functional cells undergo rapid renewal and have a short lifespan. Studies in experimental animals indicate that

damage to the intestinal epithelium occurs at doses > 1 Gy and that the degenerative changes are increasingly severe at doses of 5–10 Gy. Recovery depends on the survival of a sufficient number of clonogenic cells in the crypts before the villi and their vasculature lose their integrity. The acute radiation syndrome that occurs in humans after a single, high, whole-body irradiation is discussed in section 4.1. With fractionated radiotherapy, the probability of nausea, vomiting and diarrhoea is dependent on the dose per fraction and the frequency and number of fractions. Patients irradiated in the epigastric and abdominal regions experience nausea and vomiting, and when a dose of 25–30 Gy has been accumulated in radiation fields including the mid- and lower abdomen, loss of appetite, fatigue and diarrhoea are not uncommon. The malabsorption syndrome, involving reduced uptake of nutrients, may start during treatment and increase after therapy is completed. Patients vary in their sensitivity. Complications affecting the bowel after large-field abdominal radiotherapy have been reported to occur in 1% of patients receiving 35 Gy and in about 3% receiving higher doses. The late effects consist of excess collagen deposition in the submucosa and the typical radiation-induced changes in small vessels, such as intimal fibrosis (Becciolini, 1987).

(*e*) *Large intestine*

Because the cell turnover rate is lower in the large intestine than in the small intestine, the former is less radiosensitive. Acute transient changes in the mucosal epithelium of both the colon and the rectum may occur with doses > 30–40 Gy. The rectum is relatively radioresistant, but rectal bleeding may occur 6–12 months after irradiation with fractionated doses totalling 60 Gy. The late changes include fibrosis, shortening of the colon and strictures. As in other tissues, late changes in the vasculature, such as endarteritis and fibrosis, are characteristic (Becciolini, 1987).

The survival curves for clonogenic cells of the jejunum and colon after irradiation have been determined by the method introduced by Withers and Elkind (1970), which is based on the number of regenerating clones of crypt cells per cross-section of tissue three to four days after exposure to graded doses. The D_0 of the single-dose survival curve is about 1.3 Gy of 250-kVp X-rays. When the single-dose and the multiple-dose survival curves are separated, the 'shoulder' (see Figure 16, Overall introduction), assumed to indicate the amount of repair, is characterized by a D_q value between 4 and 4.5 Gy. Dose–survival curves for clonogenic cells in solid tissues can be determined experimentally only over a range of high doses, for example about 12–16 Gy in the jejunum. The survival curves for low doses must be obtained by reconstruction from data on fractionated doses.

4.2.6 *Haematopoietic system*

Death due to the acute radiation syndrome in the bone marrow is discussed in section 4.1. Depending on the dose, the prodromal stage is followed by the gastro-

intestinal syndrome; if the victim survives, the haematopoietic syndrome follows in the second week, and death may occur within two to three weeks after exposure to doses of 5.4–7.5 Gy and within four to six weeks after exposure to lower doses in the lethal range. The probability of death from bone-marrow damage depends on the treatment that is provided, more so than in any of the other syndromes. The prudent use of cytokines and growth factors has markedly improved the prospect of survival, although a fatal outcome becomes highly probable at doses ≥ 5.5 Gy. Survival of 10% of the haematopoietic progenitor cells is usually sufficient to prevent death.

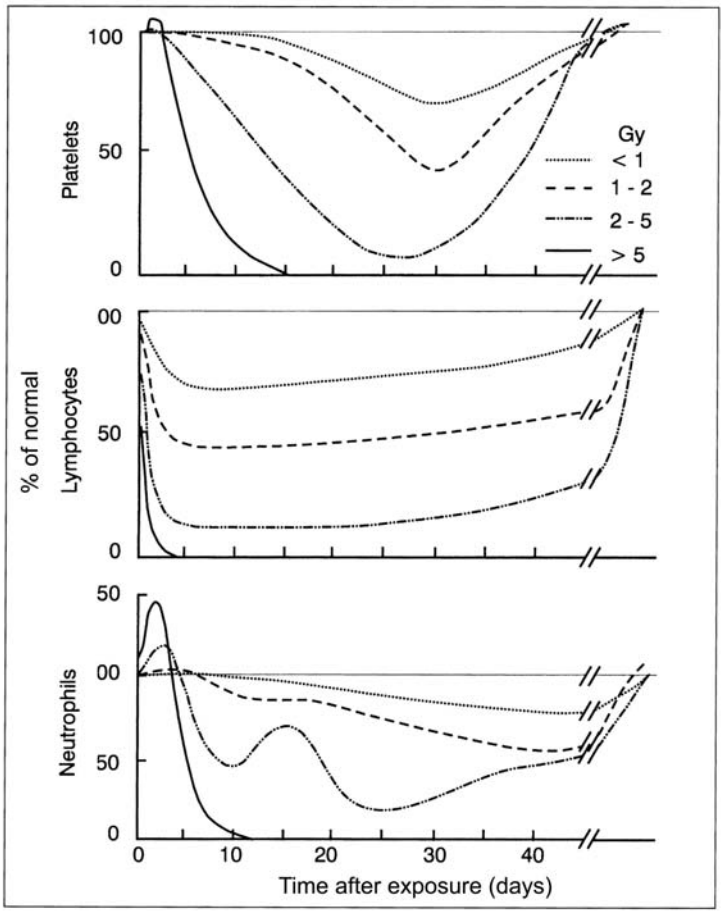
The radiation-induced loss of the functional elements of the blood and the subsequent response depend on the cell type and the cell kinetics. The short lifespan of the neutrophils and platelets is reflected in the decreases in their number before that of the long-lived red cells. The radiosensitive subpopulations of lymphocytes are also affected shortly after exposure. Within 8–10 days, the decreases in granulocytes and platelets become critical, and, at doses in excess of 5 Gy, pancytopenia may follow (Figure 14). Haemorrhage and infection may exacerbate the condition (Wald, 1971).

The bone marrow can withstand higher total doses of radiation when the dose rate is lower, the dose is fractionated or the size of the radiation field is reduced. For example, patients irradiated with single doses < 10 Gy on either the upper or the lower half of the body can recover within about eight weeks. The effect is more severe after irradiation of the upper half of the body, where about 60% of the active bone marrow is found.

McCulloch and Till (1960) developed a technique for determining survival curves of colony-forming units that contain progenitor cells capable of producing erythrocytes, myelocytic elements and platelets. Erythrocytes predominated in the colonies that grew in the spleens of irradiated mice transplanted with bone marrow. When the bone-marrow cells were irradiated *in vivo*, the D_0 was 0.95 Gy, with a small shoulder on the survival curve (extrapolation number (*n*), 1.5; see Figure 16, Overall introduction). Both the D_0 and *n* were higher when the cells were exposed *in vitro*. The survival curves of colony-forming units in humans and mice are similar.

Information about the late effects of radiation on the bone marrow comes mainly from studies of patients undergoing radiotherapy and, to a lesser extent, from reports of accidental exposure. The decrease in progenitor cells may persist, and the duration of depletion is dose-dependent; the counts of circulating blood cells, especially lymphocytes, may be depressed for months. In general, accidental exposure to high but sublethal doses is followed by recovery of the bone marrow, as was also observed in the survivors of the atomic bombings. In the case of localized exposures to high total doses, aplasia is followed by replacement of the bone marrow with fat cells and fibrosis.

Figure 14. Counts (percentage of normal) of platelets, lymphocytes and neutrophils as a function of time after exposure to and dose of radiation during accidents



From Wald (1971)

4.2.7 *Central nervous system*

The developing brain is most sensitive to radiation during gestation. As early as 1929, recognition of the fact that proliferating cells are more radiosensitive than differentiated cells led Goldstein and Murphy (1929) to study children born to women exposed to pelvic irradiation during pregnancy. They found some effects on the central nervous system. Miller and Mulvihill (1956) reported that children exposed *in utero* to atomic bomb radiation had small head sizes, an indication of damage to the central nervous system.

Observation of severe mental retardation and reduced intelligence quotients in children exposed to radiation *in utero* indicated that the most sensitive periods are 8–15 and 16–25 weeks after fertilization (Otake & Schull, 1984, 1998). The number of neurons increases rapidly during weeks 8–15 of gestation, and proliferation of the neurons of the cerebral cortex is virtually complete by 16 weeks; by 26 weeks, the neurons are differentiated. Accordingly, no cases of severe mental retardation have been found among individuals exposed to radiation before 8 weeks or after 26 weeks of gestation. Some cell proliferation continues in the brain, particularly in the cerebellum, during the first two years of life, and the proliferating neurons are radiosensitive. Glial cells, which proliferate actively during the early years of life, retain the ability to divide. Loss of glial cells can lead to demyelinization. In the developing brain, neurons not only proliferate but also migrate to specific sites. This migration occurs mainly between weeks 7 and 10 and 13–15 of gestation and is virtually complete at 16 weeks. Exposure to radiation during weeks 8–16 of gestation is thus likely to interfere with this process. In a study with explants of the cerebral cortex from rat embryos at day 16 of gestation, a dose as low as 0.1 Gy affected neuronal migration (Fushiki *et al*., 1993).

Most of the information on the effects of radiation on the brain postnatally comes from studies of patients—in particular, children treated for acute leukaemia. The degree of radiosensitivity depends on the effect and the age at exposure. In the adult brain, radiation-induced damage to the microvasculature is the major concern (for a review, see Gutin *et al*., 1991). The acute central nervous syndrome (see section 4.1) occurs with doses of 20–100 Gy, and the survival time is about two days or less, but damage to the membranes and the vasculature rather than neuronal cell killing is involved. In contrast, neurons can be induced to fire (as detected by electroencephalography) by doses as low as 0.01 Gy.

Four types of late effect of radiation in the central nervous system have been described: leukoencephalopathy, mineralizing microangiopathy, cortical atrophy and cerebral necrosis. Leukoencephalopathy is not strictly an effect of radiation as it is the result of an interaction between radiation and methotrexate. It gives rise to demyelinization, multifocal necrosis and gliosis, but the grey matter and the basal ganglia are spared. The histological changes are reflected by reduced mental ability, ataxia, dementia and even death. Radiation doses of ≥ 20 Gy plus methotrexate will cause these lesions, but fractionated radiotherapy with 18–24 Gy alone does not. Mineralizing microangiopathy affects the cerebral grey matter and less frequently the cerebellum. It is assumed to be due to damage to the microvasculature, which leads to calcification, obstruction of the vessels and necrosis. Headaches, seizures, ataxia and defective muscle control have been noted. The condition is seen in children treated with a total dose of at least 20 Gy. Cortical atrophy, caused by focal necrosis with a loss of neurons from all layers, occurs in about 50% of patients receiving more than 30 Gy of fractionated radiotherapy to the entire brain. Cerebral necrosis, which involves an amorphous fibrin exudate, often in the junctional tissues between the

white and grey matter, may appear 1–10 years after treatment. The incidence of cerebral necrosis increases rapidly at fractionated doses > 45 Gy (UNSCEAR, 1993).

The tolerance doses for brain damage are thus not known, but it is clear that the higher the dose per fraction the greater the probability of severe damage. In adults, a dose of 50 Gy to the brain in 2-Gy fractions over six weeks is considered to be critical, whereas the critical dose in children of three to five years of age is about 20% lower and that for younger children is even lower.

4.2.8 *Thyroid*

Hypothyroidism is the commonest late deterministic effect of radiation on the thyroid gland. It may be due to direct damage or, secondarily, to damage to the hypothalamic–pituitary axis (see UNSCEAR, 1993). Doses that are sufficient to affect function are more likely to be received during internal exposure from radionuclides such as ^{123}I, ^{125}I and ^{131}I for therapeutic treatment or as a result of a radiation accident.

Although there is conflicting evidence about the effect of age at the time of exposure, it is likely that the very young are more radiosensitive, as is the case for the induction of thyroid cancer (Ron, 1996). The activity of thyroid-stimulating hormone is frequently increased in children who have been irradiated for Hodgkin disease or brain tumours if the dose to the thyroid reaches about 24 Gy (Oberfield *et al*., 1986), but no increase was found after exposure to 15 Gy (Glatstein *et al*., 1971). Hypothyroidism was found in 20% of long-term survivors among children with acute leukaemia who received cranial or craniospinal irradiation with fractionated doses of a total of 18–25 Gy, the dose to the thyroid being about 3–8% of the total dose. No evidence of hypothyroidism has been found in children exposed to < 1 Gy (UNSCEAR, 1993). A study by DeGroot *et al*. (1983) indicated that chronic lymphocytic thyroiditis is relatively common in patients who received external irradiation in childhood. Hypothyroidism with increased serum levels of thyroid-stimulating hormone was found in 15% of patients who received < 30 Gy and in 68% of those who received higher doses (Kaplan *et al*., 1983).

4.2.9 *Eye*

The ocular lens and the skin are the two tissues for which specific dose limits have been set for the prevention of deterministic effects of radiation. The occupational dose limits are 150 mSv year^{-1} and 500 mSv year^{-1}, respectively. The effects of radiation on these tissues were recognized soon after the discovery of X-rays. Much of the early literature on radiogenic cataracts was reviewed by Bendel *et al*. (1978), and the responses of the human eye were detailed by Merriam *et al*. (1972).

The lens is the most important radiosensitive structure in the eye, but it is not the only tissue affected. Keratitis and oedema of the cornea can occur after exposure to

single doses of about 10 Gy, and damage to the lachrymal gland, the retina and the conjunctiva can be induced by higher doses (Merriam *et al*., 1972).

The development and progression of the effects of radiation on the ocular lens can be studied by non-invasive techniques. While the mechanism of cataract induction by radiation is not known, the evidence indicates that cataracts are caused by damage of cells in the germinative zone, resulting in abnormal differentiation of the developing lens fibres. The latent period from the time of exposure to the appearance of opacities is consistent with the time required for the differentiation and migration of abnormal fibres. The long-held hypothesis that cell killing is central to the formation of lens opacities is being questioned, and damage to the genome of the epithelial cells has been proposed as an underlying principle (Worgul *et al*., 1991). If this is so, cataract induction is probably a stochastic process with a threshold for a clinically significant lesion, and therefore differs from other deterministic effects. Like other deterministic effects, its incidence and severity increase with the dose of radiation.

The early stage of radiation-induced cataract is marked by changes in the posterior capsular area; subsequently, the anterior part of the lens is involved, and the posterior lesion expands. Opacities of the lens may develop and then cease to progress, and anecdotal accounts suggest that regression can occur. The latent period between exposure and detection of a cataract is dose-dependent but ranges from six months to several decades, with an average of two to three years (UNSCEAR, 1993).

Patients receiving radiotherapy are the main source of data for estimating the threshold dose for cataract induction and the increase in incidence with dose (Merriam *et al*., 1972). The threshold single dose was estimated to be about 2 Gy, and the threshold for a dose fractionated over 3–13 weeks was estimated to be about 5.5 Gy. Further evidence of an effect of fractionation comes from studies of patients irradiated before bone-marrow transplantation: the incidence of cataract after a single dose of 10 Gy was 80%, while only 19% of patients who had received fractions of 2–4 Gy over six or seven days (total dose, 12–15 Gy) developed cataracts (Deeg *et al*., 1984). At low doses above the threshold, the opacities are minimal and become static. The threshold dose for a progressive cataract is probably between 2.0 and 5.0 Gy.

In the survivors of the atomic bombings, the threshold dose for minimal opacities was reported to be 0.6–1.5 Gy, although the results are confounded by exposure of the survivors in Hiroshima not only to γ-rays but also to neutrons (see separate monograph; Otake & Schull, 1990). The data on radiation-induced cataracts in children treated for cancer are also confounded because in many cases the treatment consisted of a combination of radiotherapy and chemotherapy. Nevertheless, children appear to be more susceptible than adults. The data for the atomic bomb survivors also indicate age-dependency, the risk for cataract being two to three times higher in children under the age of 15 at the time of exposure than in older persons (UNSCEAR, 1993).

4.3 Radiation-sensitive disorders

Individuals who might be at enhanced risk for cancer caused by ionizing radiation include patients suffering from disorders that are associated with increased sensitivity to radiation at the cellular level. A paradigm of such disorders is xeroderma pigmentosum, in which enhanced sensitivity to the toxic effects of ultraviolet radiation (UV) parallels an enhanced risk for skin cancer after exposure to UV. The molecular mechanism underlying this phenomenon is reduced or absent repair of UV-induced DNA lesions, resulting in an increased frequency of mutations in the genome of cells from exposed parts of the body. These mutations can ultimately lead to cancer (see e.g. IARC, 1992). On the basis of this example, an enhanced risk for cancer induced by ionizing radiation might be expected in patients with a reduced capacity for repair of DNA damage and a smaller risk in those with conditions that result in disturbances in progression of the cell cycle. A further group of individuals who might be expected to show enhanced susceptibility to radiogenic cancer are those who have mutations of dominant tumour suppressor genes which are responsible for preventing the expansion of potentially malignant (initiated) cells. If radiation were to increase the number of these initiated cells, there would be an increased probability of their progression to frank tumours (for discussion, see National Radiological Protection Board, 1996).

4.3.1 *Ataxia telangiectasia*

The human genetic disorder ataxia telangiectasia is characterized by immunodeficiency, neurodegeneration, radiosensitivity and increased risks for developing a number of leukaemias and lymphomas and solid tumours (Boder, 1985; Sedgwick & Boder, 1991).

(*a*) *ATM gene and gene product*

The *ATM* gene (‘mutated in ataxia telangiectasia’) was identified by Savitsky *et al.* (1995). Full-length *ATM* cDNA was eventually cloned in two laboratories and shown to be capable of correcting aspects of the radiosensitivity of cells from patients with the disease as well as the defective cell-cycle checkpoints (Zhang *et al.*, 1997; Ziv *et al.*, 1997). Analysis of the *ATM* gene in patients with ataxia telangiectasia throughout the world showed over 300 mutations (see ataxia telangiectasia mutation database—http://www.vmmc.org/vmrc/atm.htm; P. Concannon and R. Gatti).

The ATM gene product is a highly phosphorylated nucleoprotein of about 370 kDa, which has a phosphatidylinositol 3-kinase (PI3) domain close to the C-terminus, through which it is related to a family of proteins involved in DNA damage recognition and/or cell cycle control (Hartley *et al.*, 1995; Anderson & Carter, 1996; Bentley *et al.*, 1996; Cimprich *et al.*, 1996). These proteins phosphorylate one or more substrates in response to DNA damage to activate signal transduction pathways and/or recruit

proteins to sites of DNA repair. In the case of ATM, substrates such as TP53, c-Abl, RPA, mdm2 and PHAS-1 have been identified (Banin *et al.*, 1998; Canman *et al.*, 1998; Khanna *et al*., 1998; Tibbetts *et al*., 1999).

Immunoblotting studies showed that the ATM protein is located predominantly in the nucleus in proliferating cells (Chen & Lee, 1996; Keegan *et al.*, 1996; Lakin *et al.*, 1996; Brown *et al.*, 1997; Jung *et al.*, 1997; Watters *et al.*, 1997), although cell fractionation followed by immunoblotting revealed that 5–20% of ATM is in a microsomal fraction (Lakin *et al.*, 1996; Brown *et al.*, 1997; Watters *et al.*, 1997). Immunofluorescence studies confirmed that ATM is predominantly nuclear in fibroblasts, with relatively uniform distribution throughout the nucleus, except for nucleoli (Watters *et al.*, 1997). A distinct pattern of punctate labelling was seen in the cytoplasm, and immunoelectron microscopy showed that the protein is localized in 60–250-nm vesicles (Watters *et al.*, 1997) and co-localizes with β-adaptin to endosomes (Lim *et al.*, 1998).

(*b*) *ATM and cell-cycle checkpoint control*

Cells from patients with ataxia telangiectasia are defective in activating both G_1/S and G_2/M phase checkpoints after irradiation, and DNA synthesis is inhibited to a lesser extent than in controls (Houldsworth & Lavin, 1980; Painter & Young, 1980; Scott & Zampetti-Bosseler, 1982; Nagasawa & Little, 1983; Beamish & Lavin, 1994).

Kastan *et al.* (1992) demonstrated that the response of the TP53 tumour suppressor protein in activating the G_1/S checkpoint after irradiation was defective in cells from patients with ataxia telangiectasia, and the induction of a number of *p53* effector genes was subsequently found to be reduced and/or delayed after irradiation (Canman *et al.*, 1994; Dulic *et al.*, 1994; Artuso *et al.*, 1995; Khanna *et al.*, 1995). Thus, *ATM* is initially activated in response to DNA damage by an unknown mechanism, which in turn activates *p53* (Shieh *et al.*, 1997; Siliciano *et al.*, 1997).

Cells from patients with ataxia telangiectasia are also characterized by radioresistant DNA synthesis (Houldsworth & Lavin, 1980; Painter & Young, 1980) and a defective G_2/M checkpoint after irradiation (Nagasawa & Little, 1983; Ford *et al.*, 1984; Rudolph *et al.*, 1989). The reduced inhibition of DNA synthesis appears to be due to the failure of these cells to recognize and respond to the damage. Hyperphosphorylation of replication protein A is induced after irradiation in normal cells but is significantly delayed in cell lines from patients with ataxia telangiectasia (Liu & Weaver, 1993). When cells from patients with ataxia telangiectasia are irradiated in G_2 phase, they progress into mitosis with less delay than normal cells (Zampetti-Bosseler & Scott, 1981), but when they are irradiated in G_1 or S phase they progress through these phases unhindered and block irreversibly in the subsequent G_2/M phase (Beamish & Lavin, 1994).

(*c*) *Sensitivity to ionizing radiation*

Clinical radiosensitivity in patients with ataxia telangiectasia was revealed when adverse reactions were observed during treatment with X-rays and other agents (Gotoff *et al.*, 1967; Morgan *et al.*, 1968; Feigin *et al.*, 1970). Increased sensitivity to radiation and radiomimetic agents was also demonstrated *in vitro* as reduced cell survival (Taylor *et al*., 1975; Shiloh *et al.*, 1982a; Morris *et al.*, 1983; Shiloh *et al.*, 1983) and an increased frequency of chromosomal aberrations in cells from such patients after exposure to ionizing radiation (Higurashi & Cohen, 1973; Cohen *et al*., 1975; Rary *et al.*, 1975). Defects in DNA repair in response to radiation damage were not found in early studies (Vincent *et al.*, 1975; Taylor *et al.*, 1976; Fornace & Little, 1980; Lavin & Davidson, 1981; Shiloh *et al*., 1983), but a defect in potentially lethal damage repair was observed (Weichselbaum *et al.*, 1978; Cox *et al.*, 1981; Arlett & Priestley, 1983). Evidence was subsequently provided for a defect in DNA strand-break repair in cells from patients with ataxia telangiectasia. Cornforth and Bedford (1985) reported the existence of residual breaks in these cells, as demonstrated by premature chromatin condensation 24 h after irradiation. Foray *et al.* (1997) demonstrated that approximately 10% of double-strand breaks in such cells remained unrepaired for up to 72 h after irradiation. The exact nature of the lesion recognized by the ATM protein has not been identified, but it is likely to be some form of strand interruption (Taylor *et al.*, 1975; Chen *et al.*, 1978; Shiloh *et al.*, 1982b).

Since cells from patients with ataxia telangiectasia are defective in all cell-cycle checkpoints after irradiation and since they eventually accumulate and die in G_2/M, it was suggested that these cell-cycle anomalies could account for the radiosensitivity of these cells (Beamish & Lavin, 1994). The sensitivity is more likely to be due to a defect in the recognition and repair of specific lesions in DNA, with consequent effects on the cell cycle. Since radiosensitivity is observed in non-dividing cells from patients with ataxia telangiectasia, a repair defect is probably involved, rather than defective cell-cycle control (Jeggo *et al.*, 1998). Lack of correlation between *P53* status, G_1/S phase arrest and radiosensitivity in a variety of human cells and the fact that cells from *p53*$^{-/-}$ mice are more resistant to radiation (Lotem & Sachs, 1993; Lowe *et al.*, 1993; Clarke *et al*., 1994) would appear to eliminate defective cell-cycle checkpoints as an explanation for sensitivy to radiation.

(*d*) *Cancers in patients with ataxia telangiectasia*

A major hallmark of patients with ataxia telangiectasia is a predisposition to develop a range of lymphoid malignancies (Boder & Sedgwick, 1963). Around 10% of all such patients develop cancer, most of which are of the lymphoid type (Morrell *et al.*, 1986, 1990). The association between a defective thymus, immunodeficiency and the high frequency of lymphoid malignancies initially suggested that these tumours arose as a consequence of the immunodeficiency (Peterson *et al.*, 1964; Lévêque *et al.*, 1966; Miller & Chatten, 1967), but the observations that the spectrum

of malignancies was not confined to those resulting from immunodeficiency and that chromosomal instability accompanied leukaemia in this syndrome provided an alternative explanation. Chromosomal rearrangements with specific breakpoints involving primarily chromosomes 7 and 14 are observed in up to 10% of T-lymphocytes from all patients with ataxia telangiectasia (Taylor *et al*., 1996). The breakpoints are largely located in the vicinity of immunoglobulin heavy chain and *TCR* (T-cell receptor) genes, preferentially involving four regions, 7p13, 7q33-35, 14q11-12 and 14q32 (Hecht & Hecht, 1985). Clones capable of proliferation can be generated from translocations involving *TCR* genes and non-immune genes or inversions of chromosome 14, and these clones have been shown to develop into leukaemias (Taylor & Butterworth, 1986; Baer *et al*., 1987; Davey *et al.*, 1988; Taylor *et al*., 1992).

The lymphoid malignancies in patients with ataxia telangiectasia are of both B-cell and T-cell origin and include non-Hodgkin lymphoma, Hodgkin disease and several forms of leukaemia (Spector *et al.*, 1982; Hecht & Hecht, 1990). In a series of 119 patients with ataxia telangiectasia with neoplasms, 41% had non-Hodgkin lymphoma, 23% had leukaemia of any kind (usually acute lymphoblastic) and 10% had Hodgkin disease (Hecht & Hecht, 1990). In a smaller study in the United Kingdom of 17 children with ataxia telangiectasia, seven had leukaemias and 10 had lymphomas. The leukaemias were five T-cell acute lymphocytic leukaemias, a prolymphocytic leukaemia and a T-cell chronic lymphocytic leukaemia (Taylor *et al.*, 1996). In contrast, young adult patients with ataxia telangiectasia developed abnormal lymphocyte clones that converted with a high frequency into T-cell prolymphocytic leukaemia (Matutes *et al.*, 1991). Since the clonal expansions that give rise to lymphoid tumours in patients with ataxia telangiectasia are characterized by specific chromosomal breakpoints and rearrangements, it was considered likely that alterations in genes and/or their expression would contribute to the malignant phenotype. The breakpoints in chromosome 14 in patients with and without ataxia telangiectasia with T-prolymphocytic leukaemia occur in the vicinity of the *TCL-1* (T-cell leukaemia) locus (Baer *et al.*, 1987; Davey *et al.*, 1988; Mengle-Gaw *et al.*, 1988; Russo *et al.*, 1989; Virgilio *et al*., 1993). *TCL-1* is expressed at high levels in leukaemia cells characterized by rearrangements of chromosome 14, suggesting that it is deregulated as a consequence of these changes (Virgilio *et al.*, 1994). Transcriptional activation of the *Tcl-1* proto-oncogene in transgenic mice caused the appearance of proleukaemic T-cell expansion expressing *Tcl-1*, and leukaemia developed after a long latency (Virgilio *et al.*, 1998). These results suggest that *TCL-1* plays an important role in the initiation of T-cell prolymphocytic leukaemia.

Overall, therefore, patients who are homozygous for *ATM* are cancer-prone, and their cells are hypersensitive to the induction of chromosomal damage and death by radiation, but they are not hypersensitive to other end-points such as inhibition of DNA synthesis and induction of *HPRT* mutations. There is no evidence that they are prone to radiogenic cancer.

(*e*) *ATM mutations in cancers in patients without ataxia telangiectasia*

Clearly, the spectrum of leukaemias and lymphomas observed in patients with ataxia telangiectasia also occurs in the general population, albeit at low frequency. Since a higher incidence of these neoplasms is associated with loss of functional *ATM*, it was thought possible that sporadic cases of leukaemia, such as the rare T-cell prolymphocytic leukaemia, might show mutations in the *ATM* gene. Vorechovsky *et al.* (1997) used exon-scanning single-strand conformation polymorphism and described *ATM* mutations in 17/37 patients with T-cell prolymphocytic leukaemia. The pattern of mutations was complex, but most were missense mutations clustered in a region corresponding to the PI3-kinase domain of *ATM*. The mutations were predicted to interfere with either ATP binding or the catalytic activity of the ATM molecule. The pattern of mutations differed from those in patients with ataxia telangiectasia, the majority of which are predicted to give rise to truncated and unstable proteins (Gatti, 1998), and they did not tend to accumulate in specific regions of the molecule. Stilgenbauer *et al.* (1997) demonstrated loss of the q21–23 region of chromosome 11 (11q21–23) in 13/24 patients with T-cell prolymphocytic leukaemia. In six cases in which deletion of one *ATM* allele was shown, the second allele was also mutated and predicted to cause either absence, premature truncation or alteration of the ATM gene product. DNA fibre hybridization revealed structural lesions in both alleles of four T-cell prolymphocytic leukaemia samples (Yuille *et al.*, 1998). In a study of paired leukaemic and non-leukaemic cells, loss of heterozygosity at 11q22–23, including the *ATM* gene region, was detected in 10 of 15 cases. In cells from five T-cell prolymphocytic leukaemias with loss of heterozygosity, immunoblotting revealed that the ATM protein was either absent or decreased in amount. These changes in ATM protein were reflected in nonsense, aberrant splicing and missense mutations in the second allele (Stoppa-Lyonnet *et al.*, 1998). These studies suggest that *ATM* is a tumour suppressor gene which, when inactivated, leads to the development of T-cell prolymphocytic leukaemia.

A second leukaemia seen frequently in patients with ataxia telangiectasia is B-cell chronic lymphocytic leukaemia (Taylor *et al.*, 1996). Loss of heterozygosity in the *ATM* gene was found in five of 36 cases (Starostik *et al.*, 1998), and reduced ATM protein (> 50%) was seen in 34% (38/111) of cases of this cancer. Patients with this deficiency had shorter survival times and more aggressive disease. Stankovic *et al.* (1999) detected mutations in the *ATM* gene in six of 32 patients and reduced or absent protein expression in eight of 20 tumours. There was no evidence of loss of heterozygosity in the region of the *ATM* gene, suggesting that the effect on ATM protein was due to a mutation within the gene. Germ-line mutations were detected in two of the six patients, indicating their *ATM* carrier status, whereas the frequency of *ATM* heterozygosity in the general population is 0.5–1% (Swift *et al.*, 1991; Easton, 1994). DNA sequence analysis revealed a mutated *ATM* gene in four of six patients with B-cell

chronic lymphocytic leukaemia and an increased frequency of germ-line mutations (Bullrich *et al.*, 1999).

Loss of heterozygosity (loss of the wild-type allele leading to allelic imbalance) in the region of 11q23 has been reported in tumours of the cervix (Hampton *et al.*, 1994; Bethwaite *et al.*, 1995; Skomedal *et al.*, 1999), ovary (Gabra *et al.*, 1996), breast (Kerangueven *et al.*, 1997; Laake *et al.*, 1997; Rio *et al.*, 1998; Waha *et al.*, 1998), colon/rectum (Gustafson *et al.*, 1994; Uhrhammer *et al.*, 1998) and skin (melanoma) (Herbst *et al.*, 1995). While loss of heterozygosity in the 11q22–23 region is observed in T-cell acute lymphocytic leukaemia and ovarian cancer, no mutations in *ATM* have been reported in such cases (Takeuchi *et al.*, 1998; Koike *et al.*, 1999). These results suggest that epigenetic regulation of the *ATM* gene may play an important role in tumour development in some tissues. *ATM* is thus often mutated in some tumours that occur frequently in patients with ataxia telangiectasia but not in all.

(*f*) *Radiosensitivity,* ATM *mutations and cancer risk in people heterozygous for* ATM

Since ataxia telangiectasia is an autosomal recessive disorder, the *ATM* phenotype would not be expected to appear in gene carriers. Nevertheless, some penetrance does appear in carriers, namely intermediate sensitivity of their cells to ionizing radiation and increased risks for developing cancer and in particular breast cancer. Radiosensitivity of people heterozygous for *ATM* was first described by Chen *et al.* (1978), who used agar gel cloning and trypan blue exclusion to show that the radiosensitivity of six *ATM* heterozygous lymphoblastoid cell lines was intermediate between that of normal people and *ATM* homozygotes. Paterson and Smith (1979) subsequently described enhanced radiosensitivity, as determined by colony forming ability, and intermediate sensitivity to γ-radiation-induced DNA repair replication in fibroblasts from *ATM* heterozygotes. Such persons were subsequently reported to have greater radiosensitivity when taken as a group (Cole *et al.*, 1988). Dahlberg and Little (1995) demonstrated that the mean surviving fraction of irradiated control fibroblasts was significantly greater than that of *ATM* heterozygotes. Intermediate sensitivity in *ATM* heterozygotes has been shown in a number of other assays, including induction of chromosomal aberrations (Waghray *et al.*, 1990), production of micronuclei (Rosin & Ochs, 1986), flow cytometric analysis (Rudolph *et al.*, 1989; Lavin *et al.*, 1992) and by a cumulative labelling index (Nagasawa *et al.*, 1987). Heterozygotes as a group have been distinguished from controls by the radiosensitivity and accumulation of cells in the G_2 phase of the cell cycle (Shiloh *et al.*, 1986; Sanford & Parshad, 1990). A variety of measures of radiosensitivity distinguish *ATM* heterozygotes from controls, but there is considerable variation among heterozygotes and significant differences were found only when comparison was made between groups. None of the assays was specific for the detection of *ATM* heterozygotes.

Swift *et al*. (1991) concluded that diagnostic or occupational exposure to ionizing radiation probably increases the risk for breast cancer in women heterozygous for *ATM*. High doses of ionizing radiation, particularly before puberty, are known to increase the risk for breast cancer, but it is not yet known whether mammography leads to an increased risk for *ATM* carriers. A well-conducted mammographic examination involves an absorbed dose of about 0.3 cGy per breast, which, if applied annually over 35 years (between 40 and 75 years of age), would give rise to a lifetime radiation dose of 10.5 cGy—approximately the same as background radiation (Norman & Withers, 1992). An exposure of this order at the age of 40 would be estimated to increase the number of deaths from breast cancer by approximately 1/2000 women, which is insignificant when compared with the normal lifetime risk of 1/9 for breast cancer. If the increased sensitivity of *ATM* heterozygotes to radiogenic cancer were to parallel the hypersensitivity of their cells to radiation killing and the induction of chromosomal aberrations, i.e. an increase of 1.5–2-fold, a total dose of 10.5 cGy would not be expected to increase the lifetime risk for breast cancer in this group significantly. While the epidemiological studies point to a three- to fourfold increase in the risk for breast cancer, it is uncertain whether this is associated with mutation of the *ATM* gene.

(*g*) *Cancer risk in* Atm$^{-/-}$ *mice*

Several murine models for ataxia telangiectasia have been developed by disrupting the mouse homologue, *Atm*, by gene targeting (Barlow *et al.*, 1996; Elson *et al.*, 1996; Xu *et al.*, 1996; Herzog *et al.*, 1998). Targeting led to loss of Atm protein, since truncated forms are highly unstable. In another model, deletion of nine nucleotides gave rise to a relatively stable, near full-length protein. Mice with a disturbed *Atm* gene showed disease characteristics similar in many respects to those of its human counterpart: growth retardation, mild neurological dysfunction, male and female infertility, immunodeficiency, sensitivity to cell killing by radiation and a predisposition to develop thymic lymphomas (Barlow *et al.*, 1996; Elson *et al.*, 1996; Xu *et al.*, 1996). In none of these studies in *Atm*$^{-/-}$ mice were the neurodegenerative changes seen in patients with ataxia telangiectasia reproduced, nor the ataxia and other abnormalities resulting from cerebellar changes. Kuljis *et al.* (1997) used electron microscopy to demonstrate the degeneration of several types of neuron in the cerebellar cortex of two-month-old *Atm*$^{-/-}$ mice. This process was accompanied by glial activation, deterioration of neutrophil structure and both presynaptic and postsynaptic degeneration, similar to observations made in patients with ataxia telangiectasia. Most *Atm*$^{-/-}$ mice also develop thymic lymphomas by three months of age (Barlow *et al.*, 1996; Elson *et al.*, 1996). These lymphomas grow rapidly, metastasize and lead to organ failure and death.

4.3.2 *Nijmegen breakage syndrome*

A number of syndromes have been described that overlap with ataxia telangiectasia in some of their clinical, cellular or molecular features (Byrne *et al.*, 1984; Lange *et al.*, 1993). Nijmegen breakage syndrome is an autosomal recessive condition characterized by immunodeficiency, chromosomal instability, sensitivity to cell killing by radiation and predisposition to cancer (Weemaes *et al.*, 1981; Shiloh, 1997). Documented cases of malignancy have been reported in 42 patients, including 12 lymphomas, one glioma, one rhabdomyosarcoma and one medulloblastoma (Van der Burgt *et al.*, 1996), and a significantly increased incidence of malignant neoplasms has been observed among persons heterozygous for the *NBS* (Nijmegen breakage syndrome) gene (Seemanová, 1990). The clinical presentation of this syndrome includes microcephaly, distinctive facial appearance, growth retardation and normal serum α fetoprotein, with none of the neurocutaneous manifestations seen in patients with ataxia telangiectasia (Chrzanowska *et al.*, 1995; Shiloh, 1997). The overwhelming majority of the 42 patients in the registry in Nijmegen in 1996 were detected in eastern Europe, particularly in Poland and the Czech Republic (Van der Burgt *et al.*, 1996).

Mapping of the *NBS* gene to chromosome 8q21 confirmed that the disease is genetically distinct from ataxia telangiectasia (Stumm *et al.*, 1995; Komatsu *et al.*, 1996; Matsuura *et al.*, 1997; Saar *et al.*, 1997; Cerosaletti *et al.*, 1998). The *NBS1* gene was cloned, and positional cloning showed a truncating mutation in patients with the syndrome (Matsuura *et al.*, 1998; Varon *et al.*, 1998). The gene product was designated 'nibrin' or p95 (Carney *et al.*, 1998).

Prior to its identification, nibrin/p95 was identified as part of a complex with four other components: hMre11 (Petrini *et al.*, 1995), hRad50 (Dolganov *et al.*, 1996) and two unidentified proteins of higher relative molecular mass. hMre11 and hRad50 are highly conserved between yeast and humans; in yeast, the phenotype of mutants includes hyper-recombination, sensitivity to DNA-damaging agents and DNA repair deficiency (Ajimura *et al.*, 1993; Game, 1993). This phenotype closely resembles that seen in Nijmegen breakage syndrome, suggesting that these patients have a defect in double-strand break repair. The hypothesis that hMre11 and hRad50 are involved in double-strand break repair is supported by the co-localization of these proteins in nuclear foci in response to breaks in DNA (Petrini *et al.*, 1995; Dolganov *et al.*, 1996). While Mre11, Rad50 and p95 co-immunoprecipitate as part of the same complex, Mre11 and Rad50 maintain a complex in the absence of p95 in cell extracts from patients with Nijmegen breakage syndrome, although radiation-induced foci are not evident.

These findings suggest that p95 is required for localization of the complex to damaged DNA. The hMre11–hRad50–p95 complex has magnesium-dependent single-strand DNA endonuclease and 5′→3′ exonuclease activities, which could be important in recombination, repair and genetic instability (Lieber, 1997). Since the homologue

of hMre11 in *Saccharomyces cerevisiae* has nuclease activity, it is likely that the corresponding human protein is responsible for these cleavages (Cao *et al.*, 1990).

Radiosensitivity is a uniform feature of Nijmegen breakage syndrome. The results of cytogenetic analyses by Conley *et al.* (1986), Taalman *et al.* (1989), Barbi *et al.* (1991) and Stoppa-Lyonnet *et al.* (1992), reviewed by Weemaes *et al*. (1994), showed that the percentage of chromosome 7 and 14 rearrangements was significantly higher in patients with this syndrome than in patients with ataxia telangiectasia. The hypersensitivity of cells from patients with Nijmegen breakage syndrome to X-rays and bleomycin was demonstrated by Taalman *et al*. (1983) and Jaspers *et al*. (1988). The D_0 values of the survival curves were of the same order as those reported for cells from patients with ataxia telangiectasia, and reduced inhibition of DNA synthesis after irradiation was noted. The basis for the radiosensitivy appeared to be distinct from that in cells from patients with ataxia telangiectasia, as fusion of these cells with cells from patients with Nijmegen breakage syndrome fully abolished the X-ray hypersensitivy of the former to cell killing (Jaspers *et al.*, 1988).

A defect in the S phase checkpoint in cells from patients with Nijmegen breakage syndrome was first described by Taalman *et al.* (1983), who showed that suppression of DNA synthesis by ionizing radiation was less effective in these cells than in control cells.

Abnormalities in the activation of the *p53*-inducible response to ionizing radiation have been documented in Nijmegen breakage syndrome cells, with a reduced response in fibroblast and lymphoblastoid lines after exposure to 5 Gy (Jongmans *et al.*, 1997). Studies of G_1–S cell-cycle progression in Nijmegen breakage syndrome cells after exposure to ionizing radiation produced conflicting results (Antoccia *et al.*, 1997; Jongmans *et al.*, 1997; Sullivan *et al.*, 1997; Tupler *et al.*, 1997; Yamazaki *et al.*, 1998), which may be due in part to differences in the cell types being studied. Increased accumulation in G_2 phase after exposure to ionizing radiation has also been reported (Seyschab *et al.*, 1992; Antoccia *et al.*, 1997; Jongmans *et al.*, 1997).

4.3.3 *Human severe combined immunodeficiency syndromes*

Bosma *et al.* (1983) first described a mouse mutant which had no detectable B or T lymphocytes. This severe combined immunodeficient (SCID) mouse was defective in recombination of the immunoglobulin heavy chain and *Tcr* genes and hypersensitive to ionizing radiation (Kim *et al.*, 1988; Biedermann *et al.*, 1991; Budach *et al.*, 1992), due to defective repair of double-strand breaks in DNA (Biedermann *et al.*, 1991), in which DNA protein kinase is involved (Blunt *et al.*, 1995; Araki *et al.*, 1997). To date, no human mutant in the catalytic subunit of DNA protein kinase has been described, but cell lines deficient in this protein and sensitive to radiation have been isolated from human tumours, including gliomas (Allalunis-Turner *et al.*, 1995). An extremely low level of ATM protein in these cells could also contribute to their radiosensitivity (Chan *et al.*, 1998), as dominant negative and anti-sense *ATM*

constructs led to sensitization of normal control cells as a consequence of decreasing endogenous levels of ATM (Morgan *et al.*, 1997; Zhang *et al.*, 1998).

Human SCID includes a spectrum of X-linked and autosomal recessive disorders characterized by abnormalities in cellular and humoral immunity (Rosen *et al.*, 1984; Puck, 1994). These syndromes include X-linked SCID, adenosine deaminase deficiency, Swiss-type agammaglobulinaemia and atypical syndromes, Omenn syndrome, purine nucleoside phosphorylase deficiency and immunodeficiency with short limb dwarfism. SCID is usually classified into two general groups according to the presence (B^+ SCID) or absence (B^- SCID) of B cells (Fischer, 1992). Some 70% of patients represent the former group. The incidence of classical SCID is between one in 5×10^4 and one in 7.5×10^4 births; the disease is detected by the occurrence of severe bacterial, viral and fungal infections and is fatal unless treated by bone-marrow transplantation. Some rare cases of SCID have been reported in which pre-B and mature B cells are absent (Ichihara *et al.*, 1988).

Little information has been reported on human SCID. Cavazzana-Calvo *et al.* (1993) described increased sensitivity to radiation of granulocyte macrophage colony-forming units in three patients without mature T or B cells and a twofold sensitization of the cells to X-rays. The D_0 value of the survival curve for fibroblasts from one of these patients was the same as that observed for granulocyte macrophages, indicating that the basis for the radiosensitivity overlapped with the immune defect. In the same study, increased sensitivity to radiation was also observed for granulocyte macrophages in a patient with Omenn syndrome, which includes a restricted T-cell repertoire and no B cells, but cell survival was normal in a patient with X-linked SCID who lacked only T cells. In a follow-up study, Nicolas *et al.* (1998) demonstrated increased sensitivity to ionizing radiation in fibroblasts and bone-marrow precursor cells in T^- B^- SCID patients. Sproston *et al.* (1997) described variable radiosensitivity of fibroblasts in a variety of SCID disorders. SCID strains were significantly more sensitive to radiation at both low- and high-dose rates. The cells most sensitive to radiation were from patients with T^- B^- SCID (D_0, 0.60 Gy), at a dose comparable to that reported by Cavazzano-Calvo *et al.* (1993). Lymphoblastoid cells from two patients with X-linked agammaglobulinaemia showed radiosensitivity equivalent to that of cells from patients with ataxia telangiectasia (Huo *et al.* 1994). Overall, SCID patients with no detectable B cells (30% of patients) are the most severely affected and have abnormalities in immunoglobulin gene rearrangements (Schwarz *et al.*, 1991; Abe *et al.*, 1994). These irregular rearrangements were subsequently shown to be due to mutations in the V(D)J recombinases RAG1, RAG2 or both in approximately 50% of B^- SCID patients (McBlane *et al.*, 1995; Akamatsu & Oettinger, 1998).

4.3.4 *Adverse responses to radiotherapy*

Severe chemosensitivity and acute radiation reactions were observed in a patient being treated for acute lymphoblastic leukaemia (Plowman *et al.*, 1990). Fibroblasts

from this individual were found to be indistinguishable from cells from patients with ataxia telangiectasia when exposed to ionizing radiation and were defective in repair of double-strand breaks in DNA (Plowman *et al.*, 1990; Badie *et al.*, 1995, 1997). The enhanced radiosensitivity was suggested to be due to a mutation in DNA ligase IV (Riballo *et al.*, 1999), as a patient was identified in whom DNA ligase was mutated in a conserved motif encompassing the active site. The defective protein was severely compromised in its ability to form a stable enzyme–adenylate complex. This individual, who appeared to be immunologically normal, had pronounced radiosensitivity, indicating that apparently normal individuals exist in the population who are radiosensitive due to a DNA-repair deficiency and may therefore be predisposed to leukaemia.

Individuals vary considerably in their ability to respond to radiation, as evidenced by the range of severity of the reactions of normal tissues of cancer patients exposed to radiotherapy; approximately 5% of patients show severe reactions (Norman *et al*., 1988; Ribeiro *et al*., 1993). Data on the survival of fibroblasts in culture have not predicted tissue sensitivity (West & Hendry, 1992; Budach *et al*., 1998); only the adverse effects of radiotherapy in patients with ataxia telangiectasia (Gotoff *et al*., 1967) were reflected in the hypersensitivity of the cells in culture to ionizing radiation (Taylor *et al*., 1975; Chen *et al*., 1978).

Chromosomal radiosensitivity has been observed in a number of syndromes characterized by a predisposition to cancer. Scott *et al*. (1998) drew attention to the importance of this characteristic as a biomarker for cancer, although sensitivity in these syndromes to various agents, including ionizing radiation, may not be the mechanism for cancer development. Using an assay to detect radiation-induced chromosomal damage in lymphocytes in G_2 phase, Scott *et al*. (1996) found that approximately 40% of an unselected series of breast cancer patients had elevated chromosomal radiosensitivity. Parshad *et al*. (1996) suggested that deficient DNA repair is a predisposing factor in breast cancer. When G_2/M cell-cycle arrest was determined 18–24 h after irradiation, lymphoblastoid cell lines from 22 of 108 breast cancer patients were shown to be radiation-sensitive (Lavin *et al*., 1994), and in a rapid assay for micronucleus formation in lymphocytes exposed to γ-rays with delayed mitogenic stimulation, 12 of 39 breast cancer patients and 2 of 42 controls were found to be hypersensitive to radiation (Scott *et al*., 1998). Thus, a substantial proportion of breast cancer patients showed cells that were sensitive to radiation *in vitro*. Severe clinical radiosensitivity, however, is observed in a considerably smaller proportion, approximately 5%, of breast cancer patients. Some of these patients may harbour a mutation in the *ATM* gene, particularly since there is substantial evidence that the sensitivity of at least some *ATM* heterozygotes to radiation is intermediate (Chen *et al*., 1978; Shiloh *et al*., 1986; Rudolph *et al*., 1989; Waghray *et al*., 1990; Lavin *et al*., 1992). No mutations were found in the *ATM* gene in 16 breast cancer patients with severe acute reactions to radiotherapy (Appleby *et al*., 1997) or in 15 patients who had developed severe late reactions to a standard radiotherapy schedule (Ramsay *et al*., 1998),

although the method used in the latter study would have missed up to 30% of non-truncating mutations, including missense mutations (Gatti, 1998). About 10% of *ATM* mutations are missense mutations. In this respect, it is of considerable interest that several rare allelic substitutions in *ATM* were observed in patients with various cancers but not ataxia telangiectasia (Vorechovsky *et al*., 1997). It is unclear whether these changes affect the function of the ATM protein in such a way as to influence either radiation sensitivity or cancer susceptibility.

4.3.5 *Tumour suppressor gene disorders*

(*a*) *Humans*

The term 'tumour suppressor gene' has been used to describe genes involved in growth control, differentiation and apoptosis, which undergo loss of function in the development of cancer (Stanbridge, 1990). Mutation in these genes would be expected to lead to a predisposition to cancer and a propensity to develop tumours in response to radiotherapy, but not necessarily to increased sensitivity of cells in culture.

(i) *Retinoblastoma*

Retinoblastoma is the most common intraocular malignancy in children and has served as the prototypic example of genetic predisposition to cancer (see Knudson, 1984; Newsham *et al*., 1998). Loss of one germ-line copy of *RB1* from all somatic cells predisposes to cancer in a dominant fashion because of the high probability of the loss of the remaining wild-type gene from a critical cell. It is estimated that 60% of cases are non-hereditary and unilateral, 15% are hereditary and unilateral, and 25% are hereditary and bilateral.

A significant proportion of children with the heritable bilateral form of retinoblastoma develop second cancers, most frequently bone and soft-tissue sarcoma. In an analysis of the treatment of 151 patients who developed a second neoplasm more than 12 months after the first, the second malignancy was considered to be associated with radiation in 61% of cases (Kingston *et al*., 1987). A dose–response relationship for the induction of bone and soft-tissue sarcomas in patients with the heritable form of the disease who were treated by radiotherapy has been documented. The relative risks for soft-tissue sarcomas showed a step-wise increase for all dose categories and were statistically significant at 10–29.9 Gy and 30–59.9 Gy. An increased risk for all sarcomas combined was evident at doses > 5 Gy, rising to 10.7-fold at doses ≥ 60 Gy ($p < 0.05$) (Wong *et al*., 1997). In a retrospective cohort study of mortality from second tumours among 1603 long-term survivors of retinoblastoma, follow-up was complete for 91% of the patients for a median of 17 years after diagnosis of the retinoblastoma. Of the 305 deaths, 167 were from retinoblastoma and 96 were from second primary tumours (relative risk, 30), with statistically significant excess mortality from second primary cancers of bone, connective tissue and malignant melanoma and benign and

malignant neoplasms of the brain and meninges. Radiotherapy for retinoblastoma further increased the risk of dying from a second neoplasm (Eng *et al*., 1993).

(ii) *Li–Fraumeni syndrome*

Li–Fraumeni syndrome is a rare disorder with a high penetrance in respect of a range of tumour types. It is often associated with a germ-line mutation in the *p53* tumour suppressor gene (Malkin *et al.*, 1990; Malkin, 1998). Patients with Li–Fraumeni syndrome or with a similar familial pattern of cancer are at increased risk for second cancers after irradiation, many of the neoplasms occurring in the irradiated field. Patients with familial patterns of cancer similar to those of the syndrome are found to form a significant fraction of those who develop bone sarcoma or acute leukaemia after radiotherapy for rhabdomyosarcoma (Heyn *et al*., 1993).

(iii) *Naevoid basal-cell carcinoma syndrome*

The carcinogenic effects of ionizing radiation in patients with naevoid basal-cell carcinoma syndrome were recognized more than 50 years ago when a five-year-old boy was reported to have developed more than 1000 pigmented basal-cell lesions in the irradiated field after radiotherapy for thyroid enlargement. DNA synthesis is abnormally rapid in X-irradiated cells from such patients, and it has been suggested that this might be related to the susceptibility to cancer after exposure to X-rays (Fujii *et al*., 1997). Taylor *et al*. (1975) and Stacey *et al*. (1989) reported no difference in survival between normal cells and those from patients with naevoid basal-cell carcinoma syndrome after exposure to γ-rays. Children with this syndrome who were treated for medulloblastoma developed multiple basal-cell carcinomas on irradiated skin (Atahan *et al.*, 1998; and see section 2.7).

(iv) *BRCA1 and BRCA2*

Mutations in a small number of highly penetrant autosomal dominant genes are responsible for approximately 5% of breast and ovarian cancers (Szabo & King, 1995; Stratton & Wooster, 1996; Easton, 1997). Mutations in two of these genes, *BRCA1* and *BRCA2*, lead to early-onset breast cancer (Futreal *et al*., 1994; Miki *et al*., 1994). In families with multiple cases of both breast and ovarian cancer, *BRCA1* mutations are primarily responsible for the disposition, while they make a smaller contribution in families with breast cancer only (Easton *et al*., 1993; Peto *et al*., 1996). The prevalence of *BRCA1* mutations has been estimated to be 1/800 in western populations and that of *BRCA2* to be less (Peto *et al*., 1996), although the prevalence can be as high as 1/100 in some inbred populations (Friend, 1996). The BRCA1 protein co-localizes in S-phase nuclei of human fibroblasts with Rad51 and interacts with this protein through a region encoded by exon 11 of *BCRA1* (Scully *et al*., 1997). It shares this property with BRCA2 (Sharan *et al*., 1997), which suggests that both proteins are involved in DNA repair and maintenance of genome integrity. In support of such a role, Gowen *et al*. (1998) demonstrated that *Brca1*$^{-/-}$ embryonic stem cells are defective in transcription-coupled repair of oxidative DNA damage and are hyper-

sensitive to ionizing radiation and hydrogen peroxide. Whether the sensitivity to ionizing radiation arises as a consequence of a defect in transcription-coupled repair or is due to defective strand-break repair through the Rad51 pathway or to a combination of the two remains unclear. Further evidence for a role of the BRCA1 protein in DNA damage repair was reported by Husain *et al*. (1998), who showed that *BRCA1* is overexpressed in a cisplatin-resistant breast cancer cell line (MCF-7) and that inhibition of *BRCA1* with antisense vectors increased the sensitivity, decreased the efficiency of DNA repair and enhanced the rate of apoptosis. Ramus *et al*. (1999) showed that *p53* mutations are significantly more frequent in ovarian tumours with mutations in either *BRCA1* or *BRCA2* than in controls. These results support a model of BRCA-induced tumorigenesis in which loss of cell-cycle checkpoint control coupled with inefficient DNA repair is necessary for tumour development.

(v) *Second tumours arising in response to radiotherapy*

Second malignant neoplasms occur at a higher frequency than expected after prior treatment with radiotherapy, particularly of childhood cancer (Tucker *et al.*, 1984; Hawkins *et al.*, 1987; de Vathaire *et al.*, 1989, 1999b). The studies of children with naevoid basal-cell carcinoma syndrome after being treated for medulloblastoma, discussed above, and other studies show that genetic background can influence the process of carcinogenesis in response to radiation. A case–control study has been reported in which 25 children from a cohort of 649 developed a second malignant neoplasm in response to radiotherapy during the period 1953–85. Children with one or more family relatives who had cancer had an odds ratio of 4.7 (95% CI, 1.3–17.1; $p = 0.02$) for a second malignant neoplasm when compared with children who had no family history of early-onset cancer. Thus, it is important to monitor children treated with radiotherapy, especially when there is a family history of early-onset cancer (Kony *et al.*, 1997).

(*b*) *Experimental models*

Several animal models have been used to mimic cancer-predisposing conditions in humans in which radiation is implicated as a tumorigenic agent. These include inbred strains susceptible to the development of tumours (Storer *et al.*, 1988) and animals with mutations in known tumour suppressor genes (Friedberg *et al*., 1998). In general, strains with a high spontaneous frequency of solid tumours also show an increased frequency of radiation-induced tumours (Storer *et al.*, 1988; Kemp *et al.*, 1994).

(i) pr53 *gene*

Mutation of the *p53* gene is among the most frequent genetic alterations in human tumours (Hainaut *et al.*, 1998). The TP53 protein is important in maintenance of a normal cellular phenotype owing to its involvement in cell-cycle control, as a promoter of DNA repair and programmed cell death (Ko & Prives, 1996). *pr53* knock-out mice provide a dramatic demonstration of the role of *pr53* in experimental

carcinogenesis: mice homozygous for a null *pr53* allele develop tumours at very high rates early in life, and the latent period for spontaneous tumours in *pr53* heterozygotes lies between that of the nulls and the wild types. The latent period for tumours in such mice can be significantly reduced by exposure to ionizing radiation (Kemp *et al.*, 1994), and the mice develop lymphoid tumours. The principal effect of *pr53* deficiency in the haematopoietic system of mice appears to be a constitutive abnormality that gives rise to an approximately 20-fold increase in the frequency of stable aberrations in *pr53* null mice and a 13-fold increase in *pr53* heterozygotes. The induction of stable aberrations was not increased by γ-rays, but *pr53* deficiency resulted in excess radiation-induced hyperploidy (> 10-fold the wild-type frequency) (Bouffler *et al.*, 1995).

(ii) *Murine adenomatous polyposis coli gene*

Min (multiple intestinal neoplasia) is a mutant allele of the murine *Apc* (adenomatous polyposis coli) locus that contains an *N*-ethyl-*N*-nitrosourea-induced nonsense mutation at codon 850 (Su *et al.*, 1992; Moser *et al.*, 1995). Heterozygosity for this mutation in the Min mouse is analogous to the genetic condition of familial adenomatous polyposis in humans (Joslyn *et al.*, 1991; Nishisho *et al.*, 1991), in that it predisposes to intestinal neoplasia. γ-Irradiation has been shown to increase the number of intestinal adenomas per mouse (Luongo & Dove, 1996; Ellender *et al.*, 1997). While these tumours were not observed in irradiated or untreated wild-type animals, the adenomas in the irradiated Min mice depended on the *Min* mutation, and the exposure caused chromosomal deletions involving loss of the *Apc* gene (Luongo & Dove, 1996).

(iii) *Eker rat*

The Eker rat strain is characterized by heterozygosity for a germ-line mutation in the *Tsc* 2 tumour suppressor gene, which predisposes this animal to spontaneous renal-cell carcinoma (Eker & Mossige, 1961). The corresponding mutation in humans is associated with tuberous sclerosis syndrome and leads to an increased incidence of renal cancers and of blastomas of the skin, heart and nervous system (Al-Saleem *et al.*, 1998). Exposure of Eker rats to 9 Gy of radiation caused an 11–12-fold increase in the incidence of renal tumours. When comparison was made with wild-type rats, the relative risk for developing renal-cell carcinomas after irradiation was 100-fold greater in the mutant animals (Hino *et al.*, 1993). This study has some deficiencies, however, because the wild-type animals were monitored for only 11 months, a short period for estimating life-time risk.

(iv) *Brca2*

Although disruption of the *Brca2* gene in mice led to embryonic lethality, it was possible to establish that *Brca2* expression is transient and largely embryo-specific, with transcripts particularly prevalent in tissues with a high mitotic index. Evidence that the Brca2 protein might be involved in repair of damage to DNA stems from its

ability to bind to the MmRad51 protein, a key component in the repair of double-strand breaks in DNA. Furthermore, homozygous mutants in these genes show developmental arrest at a similar stage, and their expression patterns are similar (Sharan *et al.*, 1997). In keeping with the radiosensitivity of *MmRad51*$^{-/-}$ embryos (Lim & Hasty, 1996), exposure of blastocysts from *Brca2*$^{-/-}$ embryos to 4 Gy of γ-rays led to complete ablation of the inner cell mass. It was not possible to distinguish between *Brca2*$^{+/-}$ and wild-type embryos. Because of the involvement of *Brca2* in DNA repair and the sensitivity to irradiation of *Brca2*$^{-/-}$ embryos, it will be of interest to determine whether heterozygous animals are susceptible to tumours.

4.4 Genetic and related effects

4.4.1 *Humans*

Evaluation of the hereditary effects associated with exposure of human populations to ionizing radiation has been a major concern of UNSCEAR. Many approaches have been used to formulate optimal predictions of the extent to which a given dose of ionizing radiation will increase the naturally occurring rate of mutation of germ cells in humans and how such an increase would affect the health of future generations.

(*a*) *Background radiation*

The cytogenetic effects of chronic exposure to ionizing radiation have been studied among populations in areas with high background levels of natural radiation (see section 2.5.1). A group of 100 women aged 50–65 years living in Yangjiang County, China, with an annual whole-body dose of 0.18–0.28 cGy were compared with a control group of 100 women living in an area where the annual whole-body dose was 0.06–0.09 cGy. Peripheral blood lymphocytes were collected from all of the women and analysed for the presence of chromosomal aberrations. Overall and for each category of stable and unstable chromosomal aberrations, women in the area with high background radiation had more detectable abnormalities. The increase was statistically significant for unstable aberrations (dicentrics and rings; $p < 0.04$) and for the combination of stable and unstable aberrations ($p < 0.02$) (Wang *et al.*, 1990b).

Similar results were obtained in another study in the same region among people in a wider age group (15–65 years). The frequencies of dicentrics and rings were significantly higher in lymphocytes of inhabitants of the area with high background radiation than those from the area with low background exposure ($p < 0.05$). A higher frequency of stable aberrations was also reported among students aged 15–16 years ($p < 0.01$), and higher frequencies of stable and unstable aberrations were again found among women aged 50–65 years ($p < 0.05$ for both categories) (Chen & Wei, 1991).

(*b*) *Survivors of the atomic bombings*

The data on the survivors of the atomic bombings of Hiroshima and Nagasaki indicate that acute irradiation at moderate doses has a negligible adverse effect on the health of subsequent generations. Any minor effect that may be produced is so small that it is lost in the background noise of naturally occurring mutational effects: an increase above this background has not been demonstrated even by the refined epidemiological methods that have been used over the last five decades (Neel *et al*., 1988; Neel, 1991; UNSCEAR, 1993). Information on the following types of adverse effect has been accumulated: untoward pregnancy outcome (congenital malformations, stillbirths and neonatal deaths); deaths among children before reproductive age (exclusive of those resulting from a malignant tumour); cancer before the age of 20; increased frequencies of certain types of chromosomal abnormalities (balanced structural rearrangements, abnormalities in sex chromosomes); increased frequencies of mutations affecting certain characteristics of proteins; altered sex ratios and impaired physical development of children.

Although some changes in these effects were noted in comparison with a control group, no statistically significant effect of parental irradiation has been found. The average combined dose of acute ionizing radiation to the gonads received by the parents was approximately 0.4 Sv (Neel *et al*., 1988, 1990), which is similar to the dose that has been estimated to double the frequency of genetic effects in mice. This suggests that humans may be less sensitive to the genetic effects of radiation than mice. When it was assumed that some of the mutations did indeed result from the exposure to radiation from the atomic bombings, a doubling dose of 1.7–2.2 Sv was calculated (Neel *et al*., 1990; Sankaranarayanan, 1996), whereas the doubling dose for severe genetic effects after long-term exposure was estimated to be approximately 4 Sv. The notion that ionizing radiation must have some genetic effect was strengthened by the observation of an increase in the frequency of chromosomal damage in the lymphocytes of atomic bomb survivors (Awa, 1997).

(*c*) *Chernobyl accident*

(i) *Effects in somatic cells*

The accident in 1986 at the Chernobyl nuclear power station in the Ukraine resulted in acute irradiation from external and internal exposure to ^{131}I, with a half-life of eight days, and then to more stable isotopes, mainly ^{137}Cs. Between 1986 and 1992, peripheral blood samples were obtained from 102 workers who were on the site during the Chernobyl emergency or arrived there shortly thereafter to assist in the clean-up of radioactive contaminants and to isolate the damaged reactor. Blood was also taken from 13 unexposed individuals. The samples were analysed by flow cytometry with the allele-loss somatic mutation assay for glycophorin A (see Langlois *et al*., 1986). The frequency of N/O variant red cells increased in proportion to the estimated exposure to radiation of each individual. The dose–response function derived for this

population closely resembled that determined previously for atomic bomb survivors whose blood samples were obtained and analysed 40 years after exposure (Langlois *et al*., 1993), which suggests comparable mutation induction per unit dose in these two populations and long-term persistence of the mutational damage. Measurements on multiple blood samples from each of 10 donors taken over seven years showed no significant change in N/O variant cell frequency, confirming the persistence of radiation-induced somatic mutations in long-lived bone-marrow stem cells (Jensen *et al*., 1995).

A group of children exposed to the ionizing radiation released during the Chernobyl accident had an appreciable number of chromosomal breaks and rearrangements several years later, reflecting the persistence of the radiation-induced damage. The results suggested that the children were still being exposed to radioactive contamination from foods and other sources (Padovani *et al*., 1993). In a follow-up study, 31 exposed children were compared with a control group of 11 children. All underwent measurements with whole-body counters and conventional cytogenetic analysis. The frequency of chromosomal aberrations in the exposed children was significantly greater than that in the control group, confirming the earlier report that a persistently abnormal cytogenetic pattern was still present many years after the accident (Padovani *et al*., 1997).

A group of 125 workers involved in the initial clean-up operation (called 'liquidators', exposed mainly in 1986) and 42 people recovering from acute radiation sickness of second- and third-degree severity were examined in 1992–93 for cytogenetic effects. Increased frequencies of unstable and stable markers of exposure to radiation were found in all groups, showing a positive correlation with the initial exposure even as long as six to seven years after the accident. In a study of the mutagenic effects of long-term exposure to low levels of radiation, cytogenetic monitoring was also conducted among children, tractor drivers and foresters living in areas of the Ukraine contaminated by radionuclides released after the Chernobyl accident. All groups showed significantly increased frequencies of aberrant metaphases, chromosomal aberrations (both unstable and stable) and chromatid aberrations, and the number of aberrations in the children's cells correlated to the duration of exposure (Pilinskaya, 1996; see also section 2).

(ii) *Heritable effects*

After the Chernobyl accident, germ-line mutations at human minisatellite loci were studied among children born in heavily polluted areas of the Mogilev district of Belarus (Dubrova *et al*., 1996, 1997, 1998a,b). Many tandem-repeat minisatellite loci have a high spontaneous germ-line mutation rate, which allows detection of induced mutations in relatively small populations. Blood samples were collected from 79 families (father, mother, child) of children born between February and September 1994 whose parents had both lived in the Mogilev district since the time of the Chernobyl accident. The control sample consisted of 105 unirradiated white families

in the United Kingdom, the children being matched by sex to the exposed group of offspring. The mutation frequency was found to be twice as high in the exposed families as in the control group. When the exposed families were divided into those that lived in an area with less than the median level of ^{137}Cs surface contamination and those that lived in more contaminated areas, the mutation rate in people in more contaminated areas was 1.5 times higher than that in those in the less contaminated areas. Since the blood samples for the control group were collected in the United Kingdom, it is conceivable that the increased mutation rate in the group in Mogilev might reflect intrinsic differences in minisatellite instability between these two white populations. It is also possible that the group in Mogilev was exposed to relatively high levels of other environmental contaminants, such as heavy metals, in addition to radioactive contamination.

(*d*) *Accident at Goiânia (Brazil)*

A ^{137}Cs radiotherapy source (51×10^{12} Bq) was abandoned at a private hospital and picked up by a scrap dealer in Goiânia, Brazil, who destroyed the source capsule, thus releasing the radioactive material. The highest individual dose from internally deposited ^{137}Cs was accumulated at an initial rate of 0.25 Gy h^{-1}. The most highly exposed group received doses of 4–7 Sv, one receiving up to 10 Sv. The collective external dose amounted to 56 person–Sv and the internal dose to 4 person–Sv. Four people died within six weeks; of 112 000 people monitored, 249 showed detectable contamination, and 129 of them were found to have internal contamination and were referred for medical care. In order to estimate the absorbed radiation dose, the initial frequencies of chromosomal aberrations (dicentrics and rings) were determined in 110 exposed persons (Natarajan *et al.*, 1991a,b; Ramalho & Nascimento, 1991; Ramalho *et al.*, 1991; Straume *et al.*, 1991), and some were followed cytogenetically in a search for parameters that could be used for retrospective radiation dosimetry. The frequencies of translocations detected years after the accident by fluorescence *in situ* hybridization were two to three times lower than the initial frequencies of dicentrics, the differences being larger at higher doses (> 1 Gy) (Ramalho *et al.*, 1995; Natarajan *et al.*, 1998; Ramalho *et al.*, 1998). *HPRT* mutant frequencies were also monitored in T lymphocytes of this population, but no convincing increase in the mutation rate was detected (da-Cruz *et al.*, 1996; Saddi *et al.*, 1996; da-Cruz & Glickman, 1997; Skandalis *et al.*, 1997).

4.4.2 *Experimental systems*

(a) Mutations in vivo

Mice have been the main source of information on the genetic effects of ionizing radiation in mammals. Estimates of the spontaneous mutation rates for various genetic end-points are listed in Table 41, and those of induced mutation rates per centigray for the same end-points are given in Table 42, for both high and low dose rates of low-

LET radiation. The results for visible recessive mutations (specific locus test) indicate a conversion factor of 3 for acute to chronic irradiation (Russell & Kelly, 1982). This is the factor that has often been used to account for the difference between acute and protracted doses in humans, although a factor of 5–10 could equally well be proposed in view of the data shown in Table 42. The main results given in the tables are summarized in the text below.

Table 41. Estimated spontaneous mutation rates (mouse, unless otherwise indicated)

Genetic end-point and sex	Spontaneous rate
Dominant lethal mutations	
Both sexes	2×10^{-2}–10×10^{-2} per gamete
Recessive lethal mutations	
Both sexes	3×10^{-3} per gamete
Dominant visible mutations	
Male	
Skeletal	3×10^{-4} per gamete
Cataract	2×10^{-5} per gamete
Other	8×10^{-6} per gamete
Female	8×10^{-6} per gamete
Recessive visible mutations (seven-locus tester stock)	
Male	8×10^{-6} per locus
Female	2×10^{-6}–6×10^{-6} per locus
Reciprocal translocations (observed in meiotic cells)	
Male	
Mouse	2×10^{-4}–5×10^{-4} per cell
Rhesus monkey	8×10^{-4} per cell
Heritable translocations	
Male	1×10^{-4}–10×10^{-4} per gamete
Female	2×10^{-4} per gamete
Congenital malformations (observed *in utero* in late gestation)	
Both sexes	1×10^{-3}–5×10^{-3} per gamete
Aneuploidy (hyperhaploids)	
Female	
Preovulatory oocyte	2×10^{-3}–15×10^{-3} per cell
Less mature oocyte	3×10^{-3}–8×10^{-3} per cell

From Committee on the Biological Effects of Ionizing Radiations (BEIR V; 1990)

Table 42. Estimated induced mutation rates per cGy (mouse, unless otherwise indicated)

Genetic end-point, cell stage and sex	Low-LET radiation (dose rate)	
	High	Low
Dominant lethal mutations		
Postgonial, male	10×10^{-4} per gamete	5×10^{-4} per gamete
Gonial, male	10×10^{-5} per gamete	2×10^{-5} per gamete
Recessive lethal mutations		
Postgonial, male	1×10^{-4} per gamete	
Gonial, male	1×10^{-4} per gamete	
Dominant visible mutations		
Gonial, male	2×10^{-5} per gamete	
Skeletal	5×10^{-7} per gamete	
Cataract	$5–10 \times 10^{-7}$ per gamete	
Other	$5–10 \times 10^{-7}$ per gamete	1×10^{-7} per gamete
Postgonial, female	$5–10 \times 10^{-7}$ per gamete	
Recessive visible mutations (specific locus test)		
Postgonial, male	65×10^{-8} per locus	
Postgonial, female	40×10^{-8} per locus	$1–3 \times 10^{-8}$ per locus
Gonial, male	22×10^{-8} per locus	7×10^{-8} per locus
Reciprocal translocations		
Gonial, male		
Mouse	$1–2 \times 10^{-4}$ per cell	$1–2 \times 10^{-5}$ per cell
Rhesus monkey	2×10^{-4} per cell	
Marmoset	7×10^{-4} per cell	
Human	3×10^{-4} per cell	
Postgonial, female		
Mouse	$2–6 \times 10^{-4}$ per cell	
Heritable translocations		
Gonial, male	4×10^{-5} per gamete	
Postgonial, female	2×10^{-5} per gamete	
Congenital malformations		
Postgonial, female	2×10^{-4} per gamete	
Postgonial, male	4×10^{-5} per gamete	
Gonial, male	$2–6 \times 10^{-5}$ per gamete	
Aneuploidy (trisomy)		
Postgonial, female		
Preovulatory oocyte	6×10^{-4} per cell	
Less mature oocyte	6×10^{-5} per cell	

From Committee on the Biological Effects of Ionizing Radiations (BEIR V; 1990)

(i) *Visible dominant mutations*

The mutations detected in the F_1 progeny of the irradiated generation comprise skeletal abnormalities, abnormalities of the lens (cataracts) and other dominant mutations.

The mutation rates for skeletal abnormalities in mice after single doses of X-rays were estimated to be 1×10^{-5} per gamete per cGy for spermatogonia and 3×10^{-5} per gamete per cGy for the post-spermatogonial cell stages (corrected for unirradiated controls) (Ehling, 1965, 1966). Another study showed a mutation rate in mouse spermatogonial cells of 2.3×10^{-5} per gamete per cGy induced by ^{137}Cs γ-rays (Selby & Selby, 1977) when the radiation was given in doses of 1–5 Gy separated by an interval of 24 h. This procedure is often used to increase the mutation yield while avoiding excessive cell killing (Russell, 1962).

In X- and γ-irradiated spermatogonia, the mutation rate for abnormalities of the lens was $3–13 \times 10^{-7}$ per gamete per cGy (Ehling, 1985; Graw *et al*., 1986). No difference was observed between single and split-dose exposure. The mutation rate in post-spermatogonial stages appeared to be two- to fivefold higher than that in spermatogonia.

Other dominant mutations include those that result in changes in growth rate, coat colour, limb and tail structure, eye and ear size, hair texture and histocompatibility. No significant increase in mutation frequency at histocompatibility loci was detected in irradiated sperm or spermatogonia (Kohn & Melvold, 1976; Dunn & Kohn, 1981). This result could indicate reduced mutability of these loci or a greater susceptibility for lethal mutations than expected on the basis of known mutation rates for visible recessive mutations in mice.

The spontaneous rate of visible dominant mutations other than skeletal abnormalities and cataracts is approximately 8×10^{-6} per gamete per generation (see Table 41). Protracted treatment with ^{60}Co γ-rays yielded a spermatogonial mutation rate of 1.3×10^{-7} per gamete per cGy (Batchelor *et al*., 1966). In X-irradiated female mice, the induced rates were between 5×10^{-7} and 10×10^{-7} per gamete per cGy for single doses of 2, 4 and 6 Gy (Lyon *et al*., 1979). Studies with a different marker stock suggested a mutation rate as high as 3×10^{-6} per gamete per cGy, after two doses of 5 Gy of X-rays at a 24-h interval (Searle & Beechey, 1985, 1986).

(ii) *Dominant lethal mutations*

Dominant lethal mutations are scored, essentially by their absence, in the F_1 progeny of an irradiated generation. Thus, a deficiency in the number of offspring is measured from conception to the time of weaning, i.e. as pre-implant or post-implant losses and reductions in litter size. Dominant lethal mutations are attributed to the induction of chromosomal aberrations that interfere with cell and tissue differentiation during fetal growth. These aberrations are generally eliminated during mitotic cell division and do not persist in stem-cell populations.

Post-gonial stage: In many studies, male mice were exposed to low-LET radiation at a high dose rate and mated during the first four to five weeks after exposure in order

to obtain offspring derived from germ cells exposed at the postgonial stage. In general, mutation rates of about 10×10^{-4} per gamete per cGy were reported (Ehling, 1971; Schröder, 1971; Grahn *et al*., 1979, 1984; Kirk & Lyon, 1984), while the control value was $0.025–0.1 \times 10^{-4}$ per gamete per cGy. At low dose rates of radiation, mutation rates of 5×10^{-4} per gamete per cGy were observed (Grahn *et al.* 1979).

Few data are available on the induction of dominant lethal mutations in irradiated female mice. In one study, the average mutation rate 1–28 days after irradiation was similar to that seen in the male mice, 10×10^{-4} per gamete per cGy (Kirk & Lyon, 1982). In guinea-pigs, rabbits and hamsters, the rate of dominant lethal mutations in males appeared to be lower than that in male mice, but those in females were similar (Lyon, 1970; Cox & Lyon, 1975).

Stem-cell stage: Dominant lethal mutations generally do not persist in stem-cell populations because of chromosomal imbalance; however, balanced chromosomal translocations can be transmitted during the proliferative phase of gametogenesis, and such gametes behave like dominant lethal mutations. The average rate of mutations induced in spermatogonia by low-LET ionizing radiation at a high dose rate was reported to be 9×10^{-5} per gamete per cGy (Lüning & Searle, 1971). The dose-rate effect for γ-rays is significant, as the mutation rate fell to 3×10^{-5} per gamete per cGy with weekly exposures from 1.4×10^{-4} per gamete per cGy with continuous low-intensity exposure (Grahn *et al.*, 1979).

(iii) *Recessive autosomal and sex-linked lethal mutations*

Reviews of the rates of recessive autosomal lethal mutations in mice showed an average of 1×10^{-4} per gamete per cGy (Searle, 1974; Lüning & Eiche, 1976), but no information was available on the effects of dose rate.

The rate of sex-linked lethal mutations was first determined after the detection of a large inversion of the X chromosome. Two doses of 5 Gy of X-rays at a 24-h interval to the spermatogonia of mice gave a mutation rate of 3.7×10^{-6} per X chromosome per cGy (Lyon *et al*., 1982).

(iv) *Visible recessive mutations*

Visible recessive mutations have been studied in the specific locus test (Russell, 1951) with seven stocks of mice bearing six coat-colour mutants and one structural (ears) mutant. Irradiated wild-type male or female mice are crossed with stock bearing these mutations, and new mutations at any of the marker loci are observed in the F_1 progeny. The spontaneous mutation rate in the tester stock is $8–8.5 \times 10^{-6}$ per locus, based on pooled data from the three principal laboratories where this test is conducted, for > 800 000 control F_1 mice. Most of the radiation-induced mutations examined at the molecular level appeared to be deletions (Bultman *et al*., 1991; Russell & Rinchik, 1993; Rinchik *et al*., 1994; Johnson *et al.*, 1995; Shin *et al*., 1997).

The mutation rates induced in spermatogonia when male mice were exposed to low-LET radiation at a high dose rate was $21.9 \pm 1.9 \times 10^{-8}$ per locus per cGy with

single doses of 3–7 Gy and $7.3 \pm 0.8 \times 10^{-8}$ per locus per cGy with 0.35–9 Gy of low dose-rate radiation (Russell & Kelly, 1982). In post-spermatogonial stages, the mutation rate reached $65–70 \times 10^{-8}$ per locus per cGy in progeny conceived four weeks after exposure of the male parent to 3 Gy of low dose-rate X-rays (Sega *et al*., 1978). The mutation rate was increased by fractionation of 1 Gy into two equal doses at a 24-h interval, but not by a larger number of fractions or a shorter interval (Russell, 1962).

The spontaneous rate of recessive visible mutations in female mice was estimated to be 1.4 or 5.6×10^{-6} per locus, depending on whether two or eight spontaneous events had been observed, as six events that occurred in one cluster could have been treated as one event (Russell, 1977). Exposure of mature oocytes to single doses of 0.5–6 Gy of X-rays at 0.5 Gy min^{-1} gave a mutation rate of 39×10^{-8} per locus per cGy in progeny conceived during the first week of exposure, whereas at lower dose rates values of $1–3 \times 10^{-8}$ per locus per cGy were observed. From these results it is clear that the dose-rate factor—the ratio of the mutation rates at high and low dose rates—for females is at least 10, whereas it is 3 for males (Lyon *et al*., 1979; Russell 1977; see Table 42).

(v) *Somatic mutations*

Mouse spot assay: X-Radiation induced somatic coat colour mutations in C57BL × NB mice in a pioneering study by Russell and Major (1957). In a somewhat more recent system, somatic mutations were induced when embryos heterozygous for five recessive coat-colour genes from the cross C57BL/6 J Han × T-stock were X-irradiated with 1 Gy (Fahrig, 1975). The controls consisted of irradiated embryos resulting from wild-type C57BL × C57BL matings, which are homozygous for the genes under study, and untreated offspring of both matings. The colours of the spots on the adult fur were due either to expression of the recessive genes or were white because of cell killing. Irradiated offspring of the C57BL matings had only white spots, which were always midventral. No spots were seen in untreated offspring of either mating. After correction for the white midventral spots observed in C57BL matings, the frequency of expression of a recessive colour gene after C57BL/6 J Han × T-stock matings was about 11% for embryos irradiated 11 days after conception and about 1% for embryos irradiated 9 days after conception.

Loss of heterozygosity: Genetic alterations that result in loss of heterozygosity play an important role in the development of cancer. The underlying mechanisms are mitotic recombination, mitotic non-disjunction, gene conversion and deletion (Smith & Grosovsky, 1993). Such events occur not only in genetically unstable cancer cells but also in normal human and mouse somatic cells (Hakoda *et al*., 1991a,b). The mechanisms of loss of heterozygosity have been studied in mice rendered heterozygous for the autosomal *Aprt* gene by gene targeting (Van Sloun *et al*., 1998), which allows the study of mutations in both the *Aprt* and X-chromosomal *Hprt* loci *in vivo*. $Aprt^{+/-}$ mice received up to 3 Gy whole-body irradiation with X-rays, and seven weeks later the *Hprt* and *Aprt* mutant frequencies were determined in the same splenic T-lym-

phocyte cell population. A dose-dependent increase was observed in *Hprt* mutant frequency, but that for *Aprt* was no different from that of controls, even though clear induction of mutations at the *Aprt* locus was observed after treatment with chemical carcinogens. Molecular analysis indicated that 70% of these mutations were caused by loss of heterozygosity. The hemizygous *Hprt* locus appeared to be a better target for the recovery of X-ray-induced mutants than the heterozygous *Aprt* locus. This result is unexpected, as X-rays induce predominantly multilocus deletions (Hutchinson, 1995), and deletion of an essential flanking gene from a hemizygous locus would be more detrimental for the cell. The results also suggest that loss of heterozygosity might not occur after ionizing irradiation, at least at the *Aprt* locus in mice (Wijnhoven *et al*., 1998).

(vi) *Minisatellite mutations*

Tandem repeat minisatellite loci in mice frequently have a high rate of germ-line mutations, and exposure to radiation increases the germ-line rate at a doubling dose comparable to that for other genetic end-points. The rate of induction cannot be explained by the occurrence of initial radiation damage within the minisatellite sequence, and suggests an unexpected mechanism involving radiation-induced damage elsewhere in the genome (Dubrova *et al.*, 1998a,b; Sadamoto *et al.*, 1994). Such minisatellite mutations have no known phenotypic effect or any direct relation to carcinogenesis. Their importance is that they illustrate the amplification of radiation-induced damage which results in the occurrence of mutation in a remote DNA sequence (Morgan *et al*., 1996; Little *et al*., 1997; see also section (c), below).

(vii) *Transgenic animals*

The development of transgenic mutagenesis systems has made it possible to study the mutagenic effects of ionizing radiation at both the molecular and the chromosomal level in the same animal. The responses of Big Blue® *LacI* transgenic mice to ionizing radiation were measured as induction of *LacI* mutations in the spleen. C57BL/6 Big Blue® transgenic mice were exposed to ^{137}Cs γ-rays at doses of 0.1–14 Gy and then allowed expression times of 2–14 days. Mutant plaques were analysed by restriction enzyme digestion. Of 34 mutations analysed, four were large-scale rearrangements, three of which were deletions within the *LacI* gene, while the fourth was a deletion that extended from within the α *LacZ* gene into downstream sequences. The other mutants did not involve major deletions (Winegar *et al*., 1994).

The Big Blue® *LacI* transgenic mouse reporter system was also used to investigate mutation induction in the testis, spleen and liver after whole-body irradiation of the mice with ^{60}Co γ-rays. The spontaneous mutation frequencies were $6–17 \times 10^{-6}$. No statistically significant induction of mutation was observed in testis or spleen 35 days after exposure, although the mutation frequencies tended to be increased by approximately 1.5-fold. In the liver, however, the mutation frequencies were elevated approximately 4.5-fold after exposure to 1 Gy of ^{60}Co γ-rays. When the data for all

organs were pooled, the mutation frequency was doubled, but no other significant increase was observed (Hoyes *et al*., 1998).

[The Working Group noted that neither of these systems would detect the large, multilocus deletions that constitute the predominant radiation-induced mutations in mammalian cells.]

(*b*) *Studies* in vivo/in vitro

The dynamics of the process of carcinogenesis and of the contribution of the initial carcinogenic insult to initiation and progression are difficult to study in intact animals and virtually impossible to study in humans. The main obstacles to understanding the fundamental processes involved in radiation-induced cancer in animal models until recently included the long latency and the complexity of the neoplastic process. In an effort to overcome these problems, animal models have been developed for the identification, isolation and characterization of radiation-altered or radiation-initiated cells from irradiated tissues shortly after exposure (Ethier & Ullrich, 1982; Clifton *et al*., 1986; Adams *et al*., 1987; Gould *et al*., 1987). These 'in-vivo/in-vitro' systems have been used to show that initiation of cells by ionizing radiation is a frequent event, of the order of 10^{-2}, which is much greater than would be expected if initiation were the result of a simple mutation. Subsequent analysis of initiated cells and detailed study of their progression led to the hypothesis that a critical early event in radiation-induced carcinogenesis is the induction of widespread genomic instability, which is apparent from increased cytogenetic damage and increased mutation rates in the progeny of irradiated cells many cell doublings after exposure (Ullrich & Ponnaiya, 1998). Support for this hypothesis comes from a number of observations.

In one model involving transplantation of mammary tissue or mammary cells into syngeneic hosts (DeOme *et al*., 1978; Medina, 1979), a differential effect of ionizing radiation was demonstrated on the growth of transplanted normal and hyperplastic mammary tissue (Faulkin *et al*., 1983).

In an assay to determine the effects of exposure to γ-radiation at 0.5 or 1 Gy and of the time that the cells remained *in situ* after the treatment, mammary epithelial cells were isolated from BALB/cAnNBd mice at various times between 24 h and 52 weeks after irradiation *in vivo* and assayed for the growth of epithelial foci *in vitro*. The cell populations that emerged had increased growth potential *in vitro* and enhanced tumorigenic potential with increasing time *in situ* (Adams *et al*., 1987).

In order to determine the radiation-induced transformation frequencies in sensitive BALB/C mice, in resistant C57BL mice and in resistant hybrid B6CF1 mice, independently of the host environment, ductal dysplasia was determined 10 or 16 weeks after injection of mammary epithelial cells from γ-irradiated (1 Gy from a ^{137}Cs source) donor mice into gland-free fat pads of recipient mice. The variations in radiation sensitivity of these mouse strains were shown to result from inherent differences in the sensitivity of the mammary epithelium to radiation-induced cell transformation (Ullrich *et al*., 1996).

Cells of the EF42 cell line, derived from the mammary tissue of a female BALB/C mouse four weeks after γ-irradiation (1 Gy from a ^{137}Cs source), become neoplastic with time *in vitro* and *in vivo*. Before acquisition of the neoplastic phenotype, however, multiple mutations occur in *p53*. This finding suggests that the mutations are not caused directly by the radiation treatment but arise several cell generations later as a consequence of radiation-induced genomic instability (Selvanayagam *et al*., 1995).

(*c*) *Cellular systems*

(i) *Genomic instability*

A characteristic of cancer cells is the presence of multiple mutations and chromosomal alterations. Although a single dose of ionizing radiation may induce a tumour, there is virtually no possibility that the changes needed to result in a malignant cell can be caused directly by a single exposure to the radiation. Nowell (1976) suggested that the chromosomal aberrations in cancer cells are associated with genomic instability. Loeb (1998) proposed that the acquisition of a mutator phenotype is central to cancer induction, in particular the genomic changes in tumour progression.

Radiation has been shown to induce genomic instability, a characteristic of which is the delay between exposure and the appearance of the effect, despite a number of mitotic divisions. Early observations of delayed heritable effects included small colony size of irradiated cells *in vitro* and a persistent reduction in the size of the cells that continued to grow *in vitro* (Sinclair, 1964).

The first report of delayed development of chromosomal aberrations was that of Weissenborn and Streffer (1988, 1989), who found that new aberrations were expressed in the second and third mitoses after exposure of one-cell mouse embryos to X-rays or neutrons. Pampfer and Streffer (1989) showed that irradiation of an embryo at the zygote stage induced genomic instability that later became apparent as chromatid and chromosome fragments in fibroblasts of fetal skin. In addition, delayed reduction in plating efficiency (Seymour *et al.*, 1986; Chang & Little, 1992) and delayed chromosomal alterations (Kadhim *et al.*, 1992, 1994, 1995; Marder & Morgan, 1993; Sabatier *et al.*, 1994) have been reported. Kadhim *et al.* (1992) found that α-particles were markedly more effective than X-rays in inducing delayed chromosomal aberrations in murine and human haematopoietic cells. In these experiments, 40–60% of the cells had chromosomal aberrations although only 10% of the surviving cells had been traversed by α-particles, indicating some indirect or 'bystander' effect. Marder and Morgan (1993) concluded that radiation-induced genomic instability probably results from deletion of a gene or genes responsible for genomic integrity.

Ponnaiya *et al.* (1997) showed that chromatid-type gaps and breaks appear in human epithelial MCF-10A cells as a delayed effect of irradiation. The aberrations were not found until about 20–35 cell population doublings after exposure to γ-rays.

The large number of cell doublings required to reveal genomic instability after exposure to X- or γ-rays may explain the reports of an absence of delayed chromosomal changes after exposure to low-LET radiation. There appears to be a LET-dependent difference in the time course of expression of radiation-induced genomic instability.

In mice, an association has been found between the probability of radiation-induced chromatid-type aberrations and susceptibility for induction of mammary cancer. Ullrich and Ponnaiya (1998) showed that BALB/c mice were more sensitive than C57BL/6 mice to induction of mammary cancer by radiation and also to the induction of delayed chromatid-type aberrations.

A possible role of genomic imprinting in the development of genomic instability and radiation-induced mutations was discussed by Schofield (1998). Genomic imprinting usually depends on post-replication modification of DNA, such as methylation, which regulates which of the two alleles of a gene is expressed or suppressed, depending on the gamete from which it was inherited. Thus, a cell becomes hemizygous for the expression of certain key genes. For example, in radiosensitive mice that are predisposed to gastroschisis, its induction is closely linked to a region on chromosome 7 in which a number of genes for imprinting are located. Genomic instability is thus apparently associated with the development of the malformation: it occurs only in the predisposed mouse strain and is transmitted to the next generation. Genomic instability therefore contributes to radiation-induced carcinogenesis and to other effects such as malformations.

The evidence for the induction by radiation of chromosomal instability is compelling, but the susceptibility of cell lines is clearly influenced by their genetic background. Neither the target, which appears to be large, nor the mechanism(s) of induction has been identified unequivocally. The probability of induction depends on the LET of the radiation; dose-dependence has been reported (Limoli *et al.*, 1999). Delayed appearance of *hprt* mutations has also been demonstrated after exposure to low-LET radiation *in vitro* in several systems (Little *et al.*, 1990; Harper *et al.*, 1997; Loucas & Cornforth, 1998).

The possibility that radiation-induced genomic instability contributes to radiation-induced carcinogenesis has important mechanistic implications. The characteristic delay between the event that initiates genomic instability and its expression is consistent with the long latent period between exposure to radiation and the appearance of a tumour. Better understanding of this phenomenon will be required before the implications of genomic instability for extrapolation of epidemiological findings to low-level exposures are fully understood.

(ii) *Cell transformation*

Ionizing radiation was one of the first agents to be used in cell transformation systems (Borek & Sachs, 1966), and there is now an extensive literature (reviewed by Hall & Hei, 1985; Kakunaga & Yamasaki, 1985; Hall & Hei, 1990; Suzuki, 1997). The initial studies were carried out with primary Syrian hamster embryo cells, a fibroblast

cell system that has the advantage that the effects of radiation on initial immortalization (or transformation) and the other changes required for neoplastic transformation can be studied. Because of technical problems with these primary cells, the C3H/10T½ cell line developed by Reznikoff *et al.* (1973a,b) has been used more extensively. Very high transformation frequencies were obtained in C3H/10T½ and C3H/3T3 cells when the frequencies were expressed per initial number of cells plated (Terzaghi & Little, 1976), indicating that even dishes with very few cells would eventually yield a neoplastically transformed clone. The actual neoplastic transformation process appears to be delayed and a change occurs in a large proportion of cells even after very low doses, so that there is a finite probability that one of their descendants will have a transformed phenotype (Kennedy *et al.*, 1980). This may be an expression of induced genetic instability, and its mechanism is still obscure.

Human primary cells have proven very difficult to transform neoplastically. Human keratinocytes (Rhim *et al.*, 1990, 1993) and bronchial cells (Hei *et al*., 1994) immortalized by SV40 and papilloma virus, respectively, have been used to study neoplastic transformation. These systems have the advantage that the cells are human and epithelial, but they are immortalized and therefore do not allow study of the initial change in the carcinogenic process.

In a human hybrid cell system, HeLa × skin fibroblasts, the appearance of transformed foci is associated with apoptosis which begins about eight days after irradiation. The authors suggested that the instability process has two relevant outcomes: induction of apoptotic death and neoplastic transformation of a small subset of survivors. These survivors were shown to have lost fibroblast chromosomes 11 and 14, and the authors suggested that tumour suppressor gene loci might be located on these chromosomes. The yield of transformants was found to be modulated by serum batch and this was correlated with the extent of delayed death, possibly reflecting altered expression of the induced genetic instability (Mendonca *et al.*, 1995, 1998a,b).

The results obtained with the human hybrid system can be as difficult to understand as those from earlier systems. For example, cells exposed to a dose of 1 cGy (which is too low to induce either cell killing or neoplastic transformation) and held for 24 h at 37 °C before plating, showed fewer transformants on subsequent incubation than unirradiated cells (Redpath & Antoniono, 1998). This confirmed an earlier result with a specific clone of C3H/10T½ cells (Azzam *et al.*, 1996).

Little work has been done to elucidate the type of initial radiation damage that leads ultimately to cell transformation. Obe *et al*. (1992) argued that double-strand DNA breaks are the critical lesion, citing the results of Zajac-Kaye and Ts'o (1984), who showed that application of DNase I in liposomes to Syrian hamster embryo cells led to foci of transformed cells that gave rise to tumours when injected into newborn hamsters, and the work of Bryant and Riches (1989) who treated C3H/10T½ cells with the restriction enzyme *Pvu*II in the presence of inactivated Sendai virus and observed that the cells became morphologically altered.

While cell transformation systems may be useful for revealing the potential of radiation to induce changes that may be associated with carcinogenesis, it is not clear how some of the observations obtained *in vitro* should be extrapolated to the situation *in vivo*.

(iii) *Chromosomal damage*

Three classes of chromosomal aberration are known to occur in somatic and germ cells: numerical aberrations, chromosomal breaks and structural rearrangements (Savage, 1976, 1979). Numerical and structural aberrations are associated with congenital abnormalities and neoplasia in humans. Numerical aberrations in germ cells occur as a result of nondisjunction during female gametogenesis. In normal somatic cells, the frequency of changes in chromosome number is low and difficult to estimate, but in cancer cells such changes are rather common (Holliday, 1989). A single-strand break induced before DNA replication gives rise to a chromosomal break at the following mitosis. When breakage occurs after the S phase or during G_2, it will be observed as a chromatid break, but many such breaks rejoin rapidly and go unnoticed. Single-strand breaks, both chromosomal and chromatid, are readily induced by ionizing radiation, and their number increases linearly with dose. Unrepaired breaks generally result in cell death in normal (as opposed to transformed) cells.

Structural chromosomal rearrangements are the result of inappropriate joining of radiation-induced breaks at one or more sites. They comprise simple unstable forms such as rings and dicentrics, simple stable forms including inversions, interstitial deletions and translocations, and also more complex combinations. The conventional assumption has been that two sites of radiation damage are necessary to produce simple exchange aberrations, either linearly with dose by a single track or proportional to the square of the dose by pairs of tracks (ICRP, 1991a; see also section 3.4, Overall introduction). Alternatively, some evidence suggests that simple chromosomal exchanges result from single tracks, due to recombination with undamaged DNA, and that multiple-track damage can lead to more complex chromosomal aberrations (Goodhead *et al.*, 1993; Chadwick & Leenhouts, 1998; Griffin *et al.*, 1998). The ability of X- and γ-radiation to induce all types of chromosomal damage has been documented extensively (see UNSCEAR, 1988; Committee on the Biological Effects of Ionizing Radiations (BEIR V), 1990; UNSCEAR, 1993). Dose–response curves for dicentrics, the most useful aberration for dosimetric purposes, were reported by Lloyd and Purrott (1981).

Although ionizing radiation induces more DNA single-strand breaks than double-strand breaks, several observations indicate that double-strand breaks of variable complexity are the major lesions responsible for the induction of chromosomal aberrations. Direct evidence that simple double-strand breaks can lead to chromosomal aberrations comes from experiments in which restriction endonucleases were introduced into cells. Although restriction enzymes produce only simple double-strand breaks (with blunt or cohesive ends), the induction of chromosomal aberrations was

efficient and the observed aberration patterns were similar to those induced by ionizing radiation (Bryant, 1984; Natarajan & Obe, 1984). The structural chromosomal aberrations seen at metaphase are of two types: chromosome-type aberrations and chromatid-type aberrations. Ionizing radiation induces chromosome-type aberrations in cells exposed in G_0 or G_1 phase of the cell cycle and chromatid-type aberrations in cells exposed in the S or G_2 phase (Savage, 1976).

Since human T lymphocytes have a long lifetime—a small proportion survive for decades—and the rate of replacement is rather slow, the frequency of structural chromosomal aberrations can serve as an indicator of the dose received by the exposed individual. In early work, the frequency of dicentric chromosomes at the first metaphase after stimulation of human T cells was determined. With the introduction of fluorescent *in situ* hybridization and chromosome-specific probes, it became possible to quantify the frequency of chromosomal translocations accurately (Natarajan *et al*., 1996), and these approaches were used to estimate past exposure during radiation accidents (see section 4.4.1; Natarajan *et al.*, 1991a,b; UNSCEAR, 1993; Natarajan *et al.*, 1998). The development of additional chromosome arm-specific probes as well as specific probes for telomeres allowed detailed analysis of the spectrum of ionizing radiation-induced chromosomal aberrations in humans cells *in vivo* as well as *in vitro* (Natarajan *et al.*, 1996; Boei *et al.*, 1997, 1998a,b).

(iv) *Mutagenicity*

Ionizing radiation has held a special place in mutation research ever since Muller (1927) demonstrated that X-rays induce hereditary effects in the fruit fly, *Drosophila melanogaster*. His was the first report on the induction of germ-cell mutations by a toxic, exogenous agent. Since that time, many studies have shown that ionizing radiation is mutagenic in essentially all experimental systems in which it has been examined (see extensive reviews of UNSCEAR, 1988; Committee on the Biological Effects of Ionizing Radiations (BEIR V), 1990; UNSCEAR, 1993).

In most mammalian systems, the predominant mutations induced by ionizing radiation are deletions, which range in size from extensive regions visible by microscopy to single nucleotides. Southern blotting, a technique used frequently for detecting deletions, is sensitive for deletions of more than about 100 nucleotides (Southern, 1975).

Base-pair substitution mutations have been shown to be induced by ionizing radiation in bacteria (Bridges *et al.*, 1967), mediated by the same SOS system that is involved in mutation induction by ultraviolet light and most other DNA damaging agents (Bridges *et al.*, 1968). The mutation rates at various loci ranged from 1.5×10^{-11} to 1.5×10^{-10} per cell per cGy. While such rates are readily measured in bacteria, mammalian cells are much more sensitive to the lethal effect of radiation; thus, a mutation rate of the order of 10^{-10} per cell per cGy would cause increases in the mutation frequency that are too small to be measured at doses at which cell survival is sufficiently good.

The first demonstration of the mutagenic action of ionizing radiation in mammalian cells was *Hprt* deficiency in Chinese hamster cells (Bridges *et al.*, 1970). The observation has since been confirmed and extended (for a review, see Thacker, 1992). Various mammalian somatic cell systems have been used to compare the spectrum of radiation-induced mutations with that of spontaneous mutations. The genetic loci most commonly used for mutation analysis in human cells are those encoding *HPRT* (Albertini *et al*., 1982), adenine-phosphoribosyltransferase (*APRT*; Grosovsky *et al*., 1986), a histocompatibility gene (*HLA-A*; Janatipour *et al*., 1988), thymidine kinase (*TK*; Yandell *et al*., 1990) and dihydrofolate reductase (*DHFR*; Urlaub & Chasin, 1980). Another method for detecting mutations in humans is an assay of loss of the allele for glycophorin A, a surface protein of erythrocytes (Langlois *et al*., 1986). Ionizing radiation does not significantly increase the frequency of ouabain-resistant mutants, which are believed to be due to base-pair substitutions (Thacker *et al*., 1978).

Depending on the test system used, 80–97% of the spontaneous *HPRT* and *APRT* mutations are base-pair changes. The percentages are only 50–60% at the *HLA-A* locus and 5–20% at the *TK* locus because mitotic recombination contributes substantially to the spontaneous mutation spectra at these loci. With a few exceptions, most radiation-induced mutations in cultured cells are deletions and other gross changes that are visible in Southern-blot patterns: at the *Hprt* or *HPRT* locus, mutations of this type constitute 70–90% of those in Chinese hamster ovary cells, 50–85% of those in TK6 human lymphoid cells and 50–75% of those in human T lymphocytes, and deletions constitute 60–80% of *TK* mutations in the TK6 cell line, 80% at the *HLA-A* locus in T lymphocytes and 100% at the *Dhfr* locus in Chinese hamster ovary cells. Of the radation-induced changes in *Aprt* in Chinese hamster ovary cells, only 16–20% consisted of deletions or other changes. Mutations that do not show aberrant Southern blot patterns may, of course, still be small deletions (Sankaranarayanan, 1991).

The results of a study in which *Hprt* and *Aprt* mutations were analysed after exposure of two Chinese hamster ovary cell lines ($Aprt^{+/-}$ and $Aprt^{+/0}$, respectively) to ionizing radiation strongly suggested that radiation-induced mutational events often consist of deletions of more than 40 kilobases (the length of the *Hprt* gene) and that the difference in the frequency at the two loci in the two types of cell lines was due to the presence of essential sequences close the respective target genes, a deletion of which would be lethal to the cell (Bradley *et al*., 1988).

Spontaneous and induced *Aprt* deficiencies were studied in the mouse P19H22 embryonal carcinoma cell line, which contains two distinct chromosome 8 homologues, one derived from *Mus domesticus* and the other from *M. musculus*. The cell line also contains a deletion for the *M. musculus Aprt* allele, which is located on chromosome 8. The large majority (> 95%) of the spontaneous and γ-radiation-induced mutants showed *Aprt* gene loss, indicating that relatively large deletions had occurred and that homozygosity for these regions is not a lethal event. Loss of heterozygosity for

adjacent markers was found to be a common event in cells with *Aprt* gene loss (Turker *et al.*, 1995).

In Chinese hamster ovary K1 cells and 10T5 cells, a K1 derivative containing the bacterial gene xanthine-guanine phosphoribosyl transferase *(Gpt)*, mutants were analysed at the *Gpt*, *Hprt* and *Tk* loci. After X-irradiation, the mutation rates at the *Tk* and *Gpt* loci were 8–10 times higher than that at the *Hprt* locus. The greater sensitivity of the *Tk* locus compared to that of the *Hprt* locus to mutation induction by ionizing radiation is likely to be due, at least in part, to the recovery of an additional class of mutants, possibly ones containing larger mutational events giving rise to small colonies. Approximately half of the X-ray-induced *Tk* mutants were small-colony mutants (Schwartz *et al.*, 1991).

Reduction of the radiation dose rate generally diminishes the severity of the biological effect per unit dose. The influence of dose rate on the mutagenicity of ionizing radiation has been investigated extensively in cultured cells. In his review, Thacker (1992) concluded that the results of studies in cells and animals indicated that a reduction in dose rate could reduce mutagenic effectiveness by a factor of 2–4, with some notable exceptions. Changing the dose rate of low-LET radiation had no effect in certain cell types, such as human TK6 cells and certain repair-deficient rodent cell lines or for certain types of mutation. Thacker (1992) concluded that, insofar as it was possible, there was no deviation from linearity in the dose–response relationship measured at low doses and low dose rates, but the errors were inevitably large in such measurements.

(v) *DNA damage*

Ionizing radiation may act directly on the cellular molecules or indirectly through water molecules. The high energy (typically megavolts) of an incident particle or electromagnetic wave ultimately results in a large number of small energy deposits (typically 60–100 eV), each of which provides energy for one or a small number of ionizations. As a result, electrons, charged and neutral radicals and non-radical species are generated. In aqueous media these are e_{aq}^-, $^\bullet H$, $^\bullet OH$, H_{aq}^+ and H_2O_2, and they react with nearby molecules in a very short time, leading to breakage of chemical bonds or oxidation of the affected molecules. The hydroxyl radical in particular is highly reactive and is also the most active mutagen generated by ionizing radiation.

The major effects of ionizing radiation on DNA are the induction of base damage, breaks of either single strands or both strands and more complex combinations. Double-strand breaks and damage of varying complexity are considered to be biologically more important because repair of this type of damage is much more difficult, and erroneous rejoining of broken ends may occur (see e.g. Sikpi *et al.*, 1992; Jenner *et al.*, 1993; Goodhead, 1994; Prise, 1994; Löbrich *et al.*, 1995, 1998). These so-called ‘misrepairs’ may result in mutations, chromosomal aberrations or cell death. The types, frequencies and extent of repair of these lesions depend on the dose, the dose rate and the LET of the radiation. Most cells can survive a dose of about 1.5 Gy low-

LET radiation, despite the fact that hundreds of DNA strand breaks are induced in each cell. This means that repair processes play an important role in the cellular response to radiation (see e.g. Cole *et al.*, 1988).

The active oxygen species produced when ionizing radiation interacts with water are comparable to those that are generated continuously by the metabolic processes of aerobic organisms. Double-stranded DNA breaks may be produced, but rarely, by the active oxygen species associated with metabolism. Instead, oxidative damage is induced in individual nucleotides. In some cases, this leads to the removal of a base, which results in an apurinic or apyrimidinic site. These sites, if not removed by the relevant endonuclease repair system, can be mutagenic because an incorrect nucleotide will be incorporated into the opposite strand (Schaaper *et al*., 1982).

Free radical-induced DNA damage associated with exposure to ionizing radiation may give rise to a number of oxidized purines, of which 7*H*-8-oxoguanine and 7*H*-8-oxoadenine predominate. In a detailed quantum-mechanical study to assess the tautomeric preferences of the bases in aqueous solution, the 6,8-diketo form of guanine and the 6-amino-8-keto form of adenine were the major species. The estimated free energies of hydration indicate that mutagenically significant amounts of minor tautomeric forms exist in the aqueous phase and may be responsible for induction of both transversion and transition mutations (Venkateswarlu & Leszczynski, 1998).

Minor oxidative lesions induced in DNA by exposure to ionizing radiation are 5-hydroperoxymethyl-2′-deoxyuridine and its decomposition products 5-hydroxymethyl-2′-deoxyuridine and 5-formyl-2′-deoxyuridine. The first compound was a more potent mutagen than the other two in *Salmonella typhimurium*, the TA100 strain being the most sensitive (Patel *et al*., 1992).

Because of the ubiquitous presence of nucleotide damage resulting from endogenously generated active oxygen species, living organisms have evolved a comprehensive array of DNA repair systems to deal with such damage (see Friedberg *et al.*, 1995). At low doses of radiation, the yield of active oxygen species would be small in comparison with those occurring spontaneously, and most of the resulting nucleotide damage (base damage and single-strand breaks) would be expected to be removed by repair processes. This is consistent with the accumulated evidence that multiple damaged sites, including double-strand breaks, are responsible for the effects of ionizing radiation on DNA (Goodhead, 1988; see also section 3.4, Overall introduction). Such damage presents a severe and often insurmountable challenge to the cellular repair systems. Because of the non-homogeneity of the energy deposition and the ensuing clustered damage, the effects of radiation are quite different from those induced by endogenously generated active oxygen species.