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

4.1 Transmission and absorption in biological tissues

The interaction of neutrons with biological material cannot be discussed outside the context of ionizing radiation in general, and the reader is referred to the Overall introduction for a fuller discussion. Neutrons with the lowest energy distribution, in

Table 16. Incidences of liver tumours in F_1 offspring of male C3H mice exposed to ^{252}Cf neutrons and mated with unexposed C57BL mice two weeks or three months after irradiation

Paternal dose (Gy)	Sex of offspring	Two weeks (postmeiotic)		Three months (spermatogonial)	
		No. of mice	Liver tumours (%)	No. of mice	Liver tumours (%)
0	Male	31	3.2	33	9.1
0.5		44	43.2*	20	30.0
1		39	15.4	22	22.9
2		0		19	5.3
0	Female	30	3.3		
0.5		58	1.7	18	5.6
1		35	0	24	0
2		0		14	0

From Takahashi *et al*. (1992); Watanabe *et al.* (1996); *$p < 0.01$

thermal equilibrium with their surroundings, are called 'thermal neutrons' and typically have an energy < 0.5 eV. Neutrons with energies between 0.5 and 100 eV are known as 'epithermal', or 'resonance' neutrons. Neutrons with energies up to about 500 keV are usually considered 'intermediate' in energy, and neutrons above 500 keV are called 'fast'. Most neutrons emerging from a fission reaction are fast, but in a reactor their energies are slowed down (moderated) to thermal energies to allow a chain reaction to proceed. The neutron energy spectrum outside a reactor is typically dominated by intermediate energy neutrons. The distribution of energy from neutrons in tissue is different from that from X- or γ-radiation. At low doses, only a small fraction of cells in a tissue is traversed. For example, l cGy of 1-MeV neutrons will traverse about one in 20 cells, whereas low-LET radiation may give rise to five traversals per cell (see Overall introduction, section 3.1).

Techniques for measuring exposure to neutrons are described in the Overall introduction (section 2.1.1).

4.2 Adverse effects other than cancer

Less information is available about the deterministic effects in humans of neutron radiation than of low-LET radiation because fewer patients are treated with neutrons than with low-LET radiation. Although there was a neutron component present in the radiation released by the nuclear explosion at Hiroshima, the effects of neutrons alone

are difficult to separate out accurately. The information about the biological effects of neutrons is derived from studies of patients treated with neutrons of various energies and from experimental studies with animals exposed to neutrons of similar energies and to fission neutrons (for reviews see UNSCEAR, 1982; ICRP, 1990, 1991; Engels & Wambersie, 1998).

4.2.1 *Modifying factors*

A characteristic property of neutrons is that their effects are modified considerably less by dose rate, dose fractionation, oxygenation and cell cycle stage than are the effects of low-LET radiations.

(*a*) *Dose rate and fractionation*

In the case of dose rate and fractionation, the difference between neutrons and low-LET radiation can be attributed to the difference in the capability of the exposed cells to repair the damage induced by the different radiation qualities. With increasing LET, the size of the shoulder on the survival curve decreases and the slope increases. The characteristic reappearance of a shoulder, which is observed with fractionated exposure to low-LET radiation, is either much less pronounced or absent with neutrons. The reduction in repair appears to become maximal as the LET approaches 100 keV μm^{-1}.

(*b*) *Effect of oxygen*

In general, cells and tissues are more radiosensitive when exposed to low-LET radiation in the presence of oxygen than under hypoxic conditions. The 'oxygen enhancement ratio' is the ratio of the doses required to produce a given level of a specific effect in the presence and absence of oxygen. The ratio for photons is in the range of 2.5–3.0. With increasing LET values above 60 keV μm^{-1}, the oxygen enhancement ratio for survival of human kidney cells decreases until it becomes 1 at LETs of about 180 keV μm^{-1} and higher. It was the low oxygen enhancement ratio that encouraged use of neutrons in cancer therapy (Field & Hornsey, 1979).

(*c*) *Cell cycle*

Radiosensitivity varies with the age of a cell, with maximum resistance to cell killing late in S phase. The variation in radiosensitivity is less for neutrons than for low-LET radiation. In synchronized Chinese hamster cells exposed to neutrons, the D_0 for S-phase cells was about 25% higher than that for cells in G_1, whereas with X-radiation the difference was nearly 90% (Sinclair, 1968). In clonogenic cells of the jejunal crypt, the variation in cell survival throughout the cycle was about 30% greater with γ-radiation than with 50-MeV neutrons (Withers *et al.*, 1974).

4.2.2 *Effects in normal tissues*

There is considerable sparing of tissues after exposure to low-LET radiation because they can recover from sublethal damage; markedly less sparing is seen with exposure to neutrons. Since exposure frequently involves a number of relatively small dose fractions, the RBE for damage to tissues may be relatively high. Furthermore, slow repair may occur in slowly dividing or late-responding tissues after low-LET but not after high-LET radiation. Table 17 shows the RBE_m values (see section 1.2), calculated on the basis of the linear–quadratic model, for a number of representative end-points in tissues. The RBE_m values are higher and the α:β ratios are lower for the late-responding tissues than for the early-responding tissues, which have more rapid cell renewal. In reviewing their experience of the radiosensitivity of tissues in patients undergoing neutron radiotherapy, Laramore and Austin-Seymour (1992) stressed the steepness of the dose–response curves for the induction of damage to normal tissue, which renders the therapeutic window rather narrow.

(*a*) *Skin*

The responses of mouse skin to high- and low-LET radiations are qualitatively similar, as are the time courses of the effects. The influence of the neutron energy is reflected in the RBE, which is about 7–8 for 2–3-MeV neutrons and about 3–5 for 15–25-MeV neutrons (Denekamp *et al.*, 1984). The RBE for late effects (3.2–3.4) is greater than that for early effects in pig skin exposed to γ-rays or 50-MeV (Be) neutrons (Withers *et al*., 1977).

(*b*) *Gastrointestinal tract*

(i) *Oesophagus*

Death within 8–40 days due to either obstruction or perforation of the oesophagus can occur in mice exposed to high doses of neutrons to the thorax. Geraci *et al*. (1976) reported an RBE of 1.9 for 8-MeV neutrons generated by bombarding a beryllium source with 22-MeV deuterons. Phillips *et al*. (1974) obtained an RBE of 4 with 15-MeV monoenergetic neutrons.

(ii) *Small intestine*

The murine crypt microcolony assay (Withers & Elkind, 1970) has been used to determine the RBE of single and fractionated doses of neutrons. Gueulette *et al.* (1996) reported RBE values for single doses of fast neutrons used for therapy at seven facilities in five countries, determined from the doses of each neutron source that resulted in 20 crypt microcolonies per circumference of the small intestine relative to the dose of ^{60}Co γ-radiation that caused the same effect. The RBEs were 1.5–2.2. Withers *et al*. (1993) reported RBE values in mice of 3.2–4.6 for neutrons produced by cyclotrons with deuteron energies of 16, 22, 35 and 50 MeV. Composite survival curves for crypt clonogenic cells after exposure to single doses were constructed from

Table 17. Maximum values of relative biological effectiveness (RBE_m) for tissue damage induced by fast neutrons and α:β ratios of the dose–response relationships for reference radiation (X- or γ-rays)

Tissue	End-point	Species	Neutron energy (MeV)	α:β (Gy) photons	RBE_m	Reference
Skin	Moist desquamation	Human	7.5	10.0	4.5	Field & Hornsey (1979)
Haematopoeitic system	LD_{50} at 30 days	Mouse	14.0	5.0	2.0	Broerse & Barendsen (1973)
Respiratory system	LD_{50} at > 30 days	Mouse	7.5	3.0	6.8	Field & Hornsey (1979)
Central nervous system	Late effects	Rat	14.0	3.0	7.2	Van der Kogel (1985)
Kidney	Late effects	Mouse	7.5	2.2	8.6	Joiner & Johns (1988)

data obtained with multiple fractions, and RBEs were calculated from the ratio of the α values for each neutron energy and γ-radiation. These ratios were considered to be RBE_m values. The RBE increased with decreasing neutron energy, which is consistent with the results of other studies (Hall *et al.*, 1979; see ICRP, 1990).

(*c*) *Haematopoietic system*

The effects of neutrons and comparisons of their effectiveness with that of low-LET radiation have been determined from survival curves for progenitor cells, such as colony forming cells in the haematopoietic system, or from dose–response relationships for lethality expressed as LD_{50} at 30 days. Broerse *et al*. (1978) determined an RBE of about 2.0 for the occurrence of bone-marrow syndrome in rhesus monkeys exposed to fission neutrons. In studies of the effects of neutrons and mixed-field radiation in large animals in the 1950s and 1960s (see Alpen, 1991 for review), the RBE for fast neutrons, based on the LD_{50} at 30 days in dogs and goats, was about 1.0. In contrast, the RBEs for lethality in small rodents were about 2.0–2.5. Two factors are important: the characteristic effects of radiation are less affected by body mass in small animals than in large animals, and the RBE of neutrons for lethality is based on damage to the gut in rodents whereas the bone-marrow syndrome predominates in large animals such as dogs.

Accidental exposures and the atomic bombing of Hiroshima exposed humans to a mixture of fission neutrons and γ-radiation. The effect of mixed radiation on the haematopoietic syndrome has been studied in dogs (MacVittie *et al*., 1991) in which the RBE for the LD_{50} at 30 days was about 1.7 on the basis of midline doses of ^{60}Co γ-radiation relative to mixed neutron and γ-radiation, the neutrons having an average energy of 0.85 MeV. The RBE based on the D_0 for granulocyte–macrophage colony-forming cells harvested from rib and pelvic bone-marrow aspirates 24 h after exposure of the dogs was reported to be about 2.

In a study of the survival of canine bone-marrow progenitor cells after exposure *in vitro* to ^{60}Co γ-radiation and fission neutrons (mean energy, 0.85 MeV), the D_0 values were about 77 cGy and 28 cGy, respectively, giving an RBE of about 2.8. The higher RBE of fission neutrons is consistent with neutron energy-dependence and with the RBE values of 1–2 reported for higher neutron energies. The RBE values for effects on the haematopoietic system are generally lower than those for solid tissues, which is consistent with the relatively small amounts of sublethal damage and repair in bone-marrow cells exposed to γ-radiation. The D_0 of the survival curve after γ-irradiation of bone-marrow progenitor cells isolated from dogs exposed *in vivo* to 7.0 cGy of γ-rays per day for 500 or 1000 days was reported to be significantly higher (2–2.5-fold) than that of cells from unirradiated dogs, whereas the increase in radioresistance to neutrons was much smaller. The mechanism of the acquired resistance is not known (Seed & Kaspar, 1991).

The determination of RBEs in deep tissues of large animals, including humans, requires accurate estimation of the doses of neutrons and of the reference radiation at

the target tissue. In the experiment of MacVittie *et al.* (1991), the neutron:γ-radiation ratio was 5.4:1 in air but 1.7:1 at midline. The absorbed dose to the bone marrow and the resultant change in the neutron:γ ratio are not known. Inhomogeneity of the dose to the bone marrow is a confounding factor.

The effects of single and fractionated doses and low dose rates of fission neutrons on the survival of colony-forming units in the bone marrow were studied in $B6CF_1$ mice. The RBE was 2.6 for inactivation by a single dose but somewhat higher for fractionated doses. When mice were exposed to 0.96 Gy of neutrons or 2.47 Gy of γ-radiation in nine fractions, the populations of colony-forming units in femur cells had not returned to control levels by three months, but this sustained depression of progenitor cells contrasted with the number of circulating leukocytes, which was maintained at a normal level by some compensatory mechanism (Ainsworth *et al*., 1989).

(*d*) *Central nervous system*

The brain is considered to be relatively radioresistant, but damage to normal tissue has been a limiting factor in the treatment of brain tumours with neutron radiotherapy. In a small number of patients treated with 15.6 Gy of 16-MeV neutrons, severe injury and progressive dementia occurred. When the contaminating γ-radiation dose was included, the total dose was about 17.6 Gy. Damage to the vasculature was thought to account for lesions in normal brain tissue. A neutron dose of about 13 Gy can cause changes such as cerebral oedema (UNSCEAR, 1982).

Van der Kogel *et al*. (1982) described a so-called early type of damage that takes about five to six months to develop after exposure to low-LET radiation. The target is the glial cells responsible for myelinization. Late injury to the vasculature develops within two to five years after single doses of low-LET radiation. Similar lesions and particularly the earlier type of damage occur after neutron irradiation. RBE_m values of about 5–7 have been estimated for 7.5-MeV and 14-MeV neutrons (Van der Kogel, 1985), and values of 6–10 were determined for degeneration of the white matter (White & Hornsey, 1980; Hornsey *et al*., 1981).

(*e*) *Reproductive system*

The effects of neutrons on the testis and in particular on the survival of type B spermatogonia, a highly radiosensitive cell type, have been reported. D_0 values of about 28 cGy for γ-radiation and 4–9 cGy for neutrons were observed (Hornsey *et al*., 1977). Loss of testicular weight as a function of the dose of high-LET radiation and the survival of various types of spermatogonia have been used to assess the effects of neutrons (for references to individual studies see UNSCEAR, 1982; ICRP, 1990).

The effectiveness of 1-MeV, 2.3-MeV and 5.6-MeV fast neutrons in killing type B spermatogonia in mice was determined by scoring the number of preleptotene spermatocytes 48 h after the start of irradiation, because the surviving type B spermatogonia would have developed to this stage at that time. A decrease in the number of sperma-

tocytes was considered to indicate accurately the loss of spermatogonia to the spermatogenesis process. D_0 values were determined from the loss of spermatogonia as a function of neutron and X-radiation dose. The survival curves were exponential. The RBE_m values were 5.7 for fission neutrons of 1.0 MeV mean energy and 4.6 and 3.0 for the 2.5-MeV and 5.6-MeV neutrons, respectively (Gasinska *et al*., 1987).

(*f*) *Renal system*

Stewart *et al*. (1984) used local irradiation of the kidney in mice to determine RBE values for changes in urine output, isotope clearance and haematocrit induced by single and multiple fractions of 3-MeV neutrons. The repair capacity of the kidney was very limited: the RBE for a single dose of about 6 Gy was approximately 2.4 and increased to 4.5–5.1 with eight fractions of about 1 Gy of neutron radiation.

(*g*) *Respiratory system*

Damage to the lung induced by neutron radiation occurs both early, described as pneumonitis, and late after exposure, in the form of fibrosis. In contrast to most other tissues, the lung does not show significant differences in the RBE values for early and late effects. The RBE values based on the LD_{50} 60–180 days after exposure of mice to 7.5-MeV neutrons were reported to be 1.5 after single doses and about 3.4 after 15 fractions (Hornsey *et al*., 1975). Parkins *et al*. (1985) studied the effects of irradiation of the mouse thorax with up to 20 fractions of 3-MeV neutrons or 240-kVp X-radiation on relative breathing rate and found an RBE_m value of about 7.

(*h*) *Ocular lens*

The effects of neutrons on the lens in humans and experimental animals were reviewed by Medvedovsky and Worgul (1991), who reported that neutron-induced changes in the lens are indistinguishable from those produced by low-LET radiation, but neutrons are quantitatively more effective, the incidence being higher and the latent period shorter per unit dose. Reduction of the dose rate has little or no influence on the effectiveness of neutrons to induce cataracts.

The induction and development of lens opacities depend on how much of the total volume of the lens is irradiated and on the dose, the age at exposure and the radiation quality. The effectiveness of neutrons depends on their energy, the most effective energy being ≤ 1 MeV. The induction of various types of lenticular lesions has been used to assess the effect of radiation for the purposes of radiation protection, in order to prevent the induction of cataracts.

(i) *Cataracts in humans*

Some of the physicists involved in testing the cyclotron developed cataracts (Abelson & Kruger, 1949). Although the doses were not measured precisely, it was estimated that the lens opacities occurred as a result of exposure to < 1 Gy of mixed γ- and neutron radiation. If this estimate is correct, the threshold dose was one-half to

one-fifth that estimated for γ-radiation alone, which would indicate an appreciable RBE for the neutron component.

Data on the induction of cataracts in humans by high-LET radiation come from two sources. Roth *et al*. (1976) reported on the incidence of cataracts in patients treated with 7.5-MeV neutrons and found slight, permanent loss of vision in patients exposed to a total dose of 2.2 Gy in 12 fractions, with an RBE estimated to be about 2.5. The second source of information on the effects of neutrons on the eye is studies of the survivors of the atomic bombings, who have been examined for over three decades. The estimated threshold doses to the eye were reported to be 0.06 Gy (95% CI, 0–0.16) of neutrons and 0.73 Gy (95% CI, 0–1.39) of γ-rays, and the RBE was calculated to be approximately 32 (95% CI, 12–89) (Otake & Schull, 1990). Concern has been raised about errors in the dosimetry for this population in general but about the neutron component of the radiation released during the nuclear explosion over Hiroshima in particular (see section 1.3.3). The risk for cataract per unit dose was studied in persons who reported epilation after the atomic bombing and in those with no epilation, in two studies. The authors of one study attributed the difference between the two groups of survivors to a 48% random error in the dose estimates (Neriishi *et al.*, 1995), while the others concluded that it was not possible to decide whether the differences in the frequency of cataracts was due to differences in individual radiosensitivity or to random errors in the dose estimate (Otake *et al*., 1996). Because of these uncertainties, the data for experimental animals are important.

(ii) *Cataracts in experimental animals*

Bateman *et al*. (1972) reported high RBE values for radiation-induced lens opacities in mice on the basis of the presence of flecks and other minor changes, which also occurred in unirradiated mice but at later ages. Neutrons thus shortened the latency to the appearance of these lesions. Data for 430-keV neutrons suggested that the relationship of the RBE to the neutron dose (D_n) in grays could be described as:

$$RBE = 4\sqrt{1+1.5/D_n}\ .$$

At the lowest dose, the RBE was about 100.

Di Paola *et al*. (1978), using similar techniques, obtained RBE values of 9–21 with decreasing doses of 14-MeV neutrons from 0.38 to 0.01 Gy.

Despite differences in the methods of scoring lenticular opacities, Worgul *et al*. (1996) noted a reasonable degree of agreement in the results for neutron-induced cataracts in most species. They suggested that the RBE for cataractogenesis increases from < 10 at doses ≥ 1 Gy to > 100 at doses ≤ 10 mGy. The commonly used RBE of 20 is not consistent with their results for very low doses, because at a neutron dose of 2 mGy the RBE could be estimated to exceed 250. There is no evidence that the RBE for clinically significant cataracts in humans reaches such high values.

It has become possible to detect very small radiation-induced lesions in the lens, and the estimates of threshold dose have become thresholds of detection. For the purposes of radiation protection, it is the threshold dose for clinically significant opacities (some loss

of vision) that is important. Fortunately, the treatment of cataracts has become so effective that the impact of radiation-induced cataracts has been reduced greatly. The experimental data for the induction of lenticular lesions by radiation are some of the best available for examining the relationship between RBE and dose and for testing the validity and consistency of models of the action of radiation. Lesions in the ocular lens can be assessed quantitatively at much lower doses of radiation than is the case for most, if not all, other tissues.

4.3 Radiation-sensitivity disorders

High-LET ionizing radiation kills mammalian cells more efficiently per unit dose than does X-radiation or γ-radiation (Cox *et al*., 1977a,b; Barendsen, 1985; Goodhead, 1988). Studies of the relationship between the RBE of various forms of radiation and energy deposition in cells can provide additional insight into the mechanisms of the early events in carcinogenesis, such as DNA damage and mutations. It is of interest, therefore, to consider the response to neutron radiation of cells in persons with syndromes such as ataxia telangiectasia, who are known to be sensitive to X-radiation and γ-radiation.

Hypersensitivity to low-LET ionizing radiation is a common characteristic of cells from patients with the chromosomal breakage syndrome ataxia telangiectasia (Taylor *et al*., 1975; Chen *et al*., 1978; Cox *et al*., 1978; see the monograph on 'X-radiation and γ-radiation', section 4.3.1). Cells from such patients have also been reported to be more sensitive than control cells to high-LET radiation, but the difference in sensitivity decreased as the LET of the radiation increased (Cox, 1982). Other characteristics of cells from patients with this syndrome include reduced inhibition of DNA synthesis after exposure to γ-radiation (Edwards & Taylor, 1980; Houldsworth & Lavin, 1980; Ford & Lavin, 1981) or to X-radiation (Painter & Young, 1980; De Wit *et al*., 1981) and greater and more prolonged accumulation of cells in the G_2 phase of the cell cycle after irradiation (Imray & Kidson, 1983; Ford *et al*., 1984; Bates & Lavin, 1989).

Exposure of control lymphoblastoid cell lines and cell lines from patients with ataxia telangiectasia to neutrons of a mean energy of 1.7 MeV affects cell survival and the incorporation of [^{3}H]thymidine into DNA. In addition, neutrons influence the progression of cells through the cell cycle. While high-LET radiation was considerably more effective in killing cells from the patients than from controls, the relative sensitivity of the two cell types was variable in the case of low-LET radiation. While fibroblasts from patients with ataxia telangiectasia were hypersensitive to X-radiation and γ-radiation, their radiosensitivity to α-particles was comparable to that of control cells (Lücke-Huhle *et al*., 1982). In a later study, Lücke-Huhle (1994) failed to observe increased killing by densely ionizing α-particles of cells from these patients when compared with control cells, indicating that the RBE for inactivation of cells from patients with ataxia telangiectasia is much less dependent on ionization density than that of control cells, for which it reaches a maximum of approximately 4 at a LET

value of 100 keV μm^{-1} (Cox *et al*., 1977a,b). In fibroblasts from these patients, the maximum RBE was ≤ 2 at 100 keV μm^{-1} (Cox, 1982). These data suggest that the lesions induced in DNA by high-LET radiation are inefficiently repaired in both cell types and the two can be distinguished only on the basis of DNA damage induced by low-LET radiation, which is readily repairable in controls. In a study with two lymphoblastoid cell lines from patients with ataxia telangiectasia, fast neutrons (mean energy, 1.7 MeV) were considerably more effective than γ-rays in inducing cell death. Fast neutrons inhibited DNA synthesis to the same extent in cells from patients with this syndrome as in those from controls (radioresistant DNA synthesis), but the long-term delay in G_2/M phase was greater in the cells from the patients, as was observed after γ-irradiation (Bates & Lavin, 1989). Thus, a correlation between G_2/M delay and cell killing was seen in these lymphoblastoid cells, regardless of the LET value of the radiation (Houldsworth *et al*., 1991); this was not the case with fibroblasts from these patients (Lücke-Huhle *et al*., 1982).

In keeping with the data on the survival of fibroblasts, marked differences in the rejoining kinetics of γ-radiation-induced double-strand breaks in DNA were found between control cells and those from patients with ataxia telangiectasia, but similar kinetics of rejoining of these breaks was observed after exposure to ^{241}Am α-particles (Coquerelle *et al*., 1987). When the production of micronuclei was determined in lymphocytes from such patients after irradiation, the increase over that in control cells was less pronounced after exposure to neutrons than after exposure to γ-rays (Vral *et al*., 1996).

4.4 Genetic and related effects

4.4.1 *Humans*

Chromosomal aberrations were examined in lymphocytes from eight men aged 24–56 who were exposed during a criticality accident to mixed γ-radiation and fission neutrons at doses estimated to range from 0.23 to 3.65 Gy. The neutrons contributed about 26% of the total dose. Five of the men received doses that were estimated to exceed 2.3 Gy, and the three others received lower doses. The blood samples were drawn about 2.5 years after the irradiation; blood from five unirradiated subjects was used as a control. Only chromatid-type aberrations were found in the controls. In the subjects exposed to the higher doses, the frequency of aneuploid cells was 7–23%, and gross aberrations, such as rings, dicentrics and minutes, were found in 2–20% of the cells. The men who received doses of 0.23–0.69 Gy also had abnormalities but at a much lower frequency (Bender & Gooch, 1962). Analysis of blood samples from the same persons 3.5 years after exposure showed that they still had chromosomal aberrations but in most cases at a somewhat lower frequency (Bender & Gooch, 1963).

Chromosomal aberrations in peripheral blood cells were scored in a study of 17 patients who received tumour therapy with 14-meV neutrons at a rate of about

0.2 Gy min^{-1} with a distance of 80 cm between the source and the skin. Treatment consisted either of daily doses of 0.65–0.80 Gy or of 12 exposures of 1.3 Gy in three fractions per week. The doses of contaminating γ-rays were 5–15% depending on the field size and the depth of the tumour. The intercellular distribution of dicentric chromosomes showed predominantly overdispersion. A positive correlation was found for dicentrics with a total skin dose of 0.8–15.6 Gy, and for total chromosome-type damage (dicentrics, centric rings and excess acentrics). The authors concluded that there was a significant correlation with therapeutic dose, despite the complex influences of biological and physical factors on the aberration yield (Schmid *et al.*, 1980).

[The reports summarized below became available after the meeting of the Working Group, although members of the Group were aware of the existence of some of these publications. In view of their importance for the evaluation, they are included in the monograph for completeness.

[The men studied by Bender and Gooch (1962, 1963) were further examined 7 (Goh, 1968), 8 and 10.5 (Goh, 1975) and 16 and 17 years (Littlefield & Joiner, 1978) after the accident. At 16–17 years, six of the men still had residual chromosomal aberrations; three men who had received the high doses had the highest frequency, and the two who had been exposed to the highest dose had around 10% aberrant cells.

[In a criticality accident in 1965 in Mol, Belgium, a man received doses to the bone marrow estimated to be 500 cGy of γ-radiation and 50 cGy of neutrons. Only 24 mitoses good enough for analysis were obtained. The aberrations included deletions, translocations, dicentrics and rings; some cells had two or even three dicentrics. On the basis of results available at the time on cells exposed *in vitro*, the total dose (mean homogeneous equivalent dose) corresponding in effect to low-LET radiation was estimated to be 470–500 cGy, in good agreement with the physical estimates (Jammet *et al*., 1980).

[An accident in Vinca, Yugoslavia, in 1958 resulted in the exposure of six persons to neutrons and γ-radiation. More than 50% of the dose was estimated to be neutrons, and the doses were estimated to be 165–227 cGy of neutrons and 158–209 cGy of γ-rays. Five years after the accident, the frequency of structural aberrations in the peripheral lymphocytes was 8–28% (Pendic & Djordjevic, 1968). Nineteen years after the accident, the frequency of aberrations in four men had declined somewhat to 10–22% (Pendic *et al*., 1980).

[The persistence of chromosomal aberrations in patients who received fractionated neutron therapy (average bone-marrow dose, < 100 to > 1000 cGy) to tumours located at various sites was evaluated recently (Littlefield *et al.*, 2000). Neutron-induced dicentrics and rings disappeared from the peripheral circulation within the first three years after exposure, while translocations persisted for more than 17 years.]

4.4.2 *Experimental systems*

(*a*) *Mutations* in vivo

(i) *Germ-cell mutations*

Visible dominant mutations: In mice, the spontaneous rate for visible dominant mutations is approximately 8×10^{-6} per gamete per generation. Exposure to fission neutrons (mean energy, 0.7 MeV) gave rise to a spermatogonial mutation rate of 25.5×10^{-5} per gamete per Gy (Batchelor *et al*., 1966).

Dominant lethal mutations: When male mice were exposed to fission neutrons four to five weeks before mating with untreated females (postgonial stage), the rate of dominant lethal mutations was approximately 25×10^{-2} per gamete per Gy (Grahn *et al*., 1979). When males were irradiated in the stem-cell stage, no effect of dose rate was observed after single or weekly exposures to neutrons, both of which gave a dominant lethal mutation rate of 40×10^{-3} per gamete per Gy (Grahn *et al*., 1979).

Experimental evidence of the nature of radiosensitive targets in immature (resting) mouse oocytes led to new experimental designs that permitted measurement of radiation-induced genetic damage in these cells. Such damage has been detected after exposure to monoenergetic 0.43-MeV neutrons, and the genetic sensitivity of the immature oocytes has been compared with that of maturing oocytes. Recoil protons from 0.43-MeV neutrons produce short ionization tracks (mean, 2.6 μm) and can therefore deposit energy in the DNA without simultaneously traversing and damaging the hypersensitive plasma membrane. With these neutrons, dose–response relationships were obtained for both chromosomal aberrations and dominant lethal mutations in oocytes from females irradiated 8–12 weeks earlier, when the oocytes were immature. The intrinsic mutational sensitivity of immature mouse oocytes appeared to be similar to that of maturing oocytes (Straume *et al*., 1991).

Recessive visible mutations: In male mice, irradiation of post-spermatogonial stages with neutrons at doses of up to 1 Gy resulted in recessive visible mutation rates of $100–150 \times 10^{-6}$ per locus per Gy, with no effect of dose rate (Russell, 1965). In female mice, a rate of 145×10^{-6} per locus per Gy was reported for this type of mutation after single doses of fission neutrons (0.3, 0.6 and 1.2 Gy) (Russell, 1972).

Specific locus mutations: One system for studying mutation induction in mice comprises a series of 12 genes, most of which affect coat colour, six or seven of which are usually tested as a group (Cattanach, 1971). Neutrons show an inverse dose-rate effect, low dose rates of high doses being much more effective. In contrast to spermatogonia, oocytes are difficult to analyse for mutations (Batchelor *et al*., 1969). A complicating factor is the time of conception after irradiation: with neutrons at low dose rates, mutations could be recovered in litters conceived within seven weeks of irradiation, but later litters had no mutations (Russell 1967).

Comparison of the effects of high-LET and low-LET radiation: Male $B6CF_1$ mice were exposed to once-weekly doses of either fission neutrons or ^{60}Co γ-radiation for up to one year and mated periodically to screen for the induction of dominant lethal

mutations. The doses of neutrons were 0.0013–0.027 Gy week^{-1} and those of γ-radiation were 0.05–0.32 Gy week^{-1}. Data on both pre- and postimplantation fetal deaths were obtained. Age- and time-dependent factors made no consistent, significant contribution to the mutation rate; such factors could include changes in radiosensitivity and in spontaneous rates and any cumulative damage to the stem-cell population. Direct comparison of these data with data for males exposed to single doses confirmed that weekly neutron irradiation was significantly more effective than single doses in inducing postimplantation fetal losses, whereas single doses of γ-rays were more effective than the same dose divided into weekly fractions. The RBE of neutrons increased from 5 to 12 for single and weekly doses. The rates of preimplantation loss, although significant, were not considered to be a sensitive measure of genetic injury at the low doses used (Grahn *et al*., 1986).

Young adult male B6CF$_1$ mice were exposed to single whole-body doses of fission neutrons or ^{60}Co γ-radiation. Post-spermatogonial dominant lethal mutations, the incidence of reciprocal chromosomal translocations in spermatogonia, the incidence of abnormal epididymal sperm four to six weeks after exposure, and testicular weight loss three to six weeks after exposure were measured. The responses to neutron doses of 0.01–0.4 Gy and γ-radiation doses of 0.23–1.45 Gy were analysed in detail, although more limited data from a fourfold higher dose range were integrated into the analysis. Significant effects were seen at 0.01 and 0.025 Gy of neutrons, consistent with extrapolation from higher doses, with the exception of dominant lethal mutations, which occurred in significant excess of expectation. The dose–response relationships were linear or linear–quadratic, depending on the end-point, radiation quality and dose range. For translocation frequencies, the D^2 term in the linear–quadratic dose–response function (see section 5, Overall introduction) was negative for neutron and positive for γ-ray irradiations. The RBE values for testicular weight loss and abnormal sperm were between 5 and 6 over the full dose range and were between 7 and 9 at lower doses (< 0.1 Gy) for translocations. The RBE values for postimplantation loss and total dominant lethal rates were 5–6 at doses > 0.1 Gy and 10–14 at doses < 0.1 Gy. The values for preimplantation loss were between 15 and 25 at doses > 0.1 Gy and possibly higher < 0.1 Gy. The authors suggested that the unusual results at the lower doses may be explained by variation in cell sensitivity, cell selection, probability of neutron traversal per cell, variance of magnitude of the energy deposition events, dose rate and DNA repair (Grahn *et al*., 1984).

Male mice heterozygous for the Rb(11.13)4Bnr translocation were irradiated for 14.5 min with either 0.15 Gy of fission neutrons or 0.6 Gy of X-rays. These mice are known to show high levels of spontaneous autosomal non-disjunction (20–30%) after anaphase I. The effects of the irradiation on this process were determined in air-dried preparations of primary and secondary spermatocytes. The induced effects were studied at intervals of 2 and 3 h after the start of the irradiation and assessed by scoring: univalents in primary spermatocytes; deletions, aneuploid chromosome counts and precocious centromere separation in secondary spermatocytes; and chromatid gaps and

breaks in both cell types. The two types of radiation induced comparable levels of chromosomal damage. The RBE value for neutrons relative to X-rays was calculated to be 5.4 for the meiosis I stage and 3.3 for the meiosis II stage. According to the authors, the significantly higher incidence of cells showing damage at meiosis II than at diakinesis/meiosis I does not indicate a difference in radiation sensitivity, but is the consequence of the different chromosomal processes taking place during the time between irradiation and fixation (Nijhoff & de Boer, 1980).

(ii) *Somatic mutations*

Hprt: Mutation induction was measured at the *Hprt* locus in splenic lymphocytes of $B6CF_1$ mice 56 days after whole-body irradiation with fission-spectrum neutrons. Lymphocytes were cultured for 12–16 days in the presence of 5×10^4 feeder cells (syngeneic lymphocytes irradiated with 50 Gy γ-radiation). Animals were exposed to either single doses of neutrons (1.5 Gy) or fractionated doses delivered over two weeks (0.25 Gy × 6; total, 1.5 Gy). The frequency of *Hprt* mutant induction by the single 1.5-Gy dose was $5.98 \pm 1.51 \times 10^{-5}$ (SE). Multiple doses of neutrons (total, 1.5 Gy) gave rise to a mutation frequency of $8.71 \pm 5.39 \times 10^{-5}$ (SE) (Kataoka *et al*., 1993).

Oncogenes: Point mutations at codon 12 of the K-*Ras* oncogene were analysed by an 'enriched' polymerase chain reaction method in 25-year-old paraffin-embedded samples of normal lung tissue and lung adenocarcinoma tissue from mice that had been exposed to radiation. Significantly more K-*Ras* codon-12 mutations (100%) were observed in normal lung tissue from mice exposed 24 times to once-weekly neutron radiation than in normal lung tissue from sham-irradiated mice (50%; $p < 0.05$). Lung adenocarcinomas from these irradiated mice also had a significantly higher frequency of point mutations in codon 12 of K-*Ras* than lung adenocarcinomas from mice exposed to γ-radiation once a week for 24 or 60 weeks (50%), but the higher frequency was not significantly different from that in spontaneous lung adenocarcinomas from mice (75%; $p > 0.05$). Sequencing of two of the mutants revealed a K-*Ras* 13(Asp) point mutation (Zhang & Woloschak, 1998). [The Working Group noted that it cannot be concluded that the codon-12 mutations were induced by the radiation or arose in clones initially transformed by the radiation.]

N-*Ras* mutations were examined in DNA samples extracted from the spleens of CBA/Ca mice that had developed myeloid leukaemia after exposure to radiations of various qualities. Seventeen cases of myeloid leukaemia comprising five cases of neutron-induced and 12 cases of photon (three γ-radiation and nine X-radiation)-induced myeloid leukaemia were included, with 12 DNA samples from the bone-marrow cells of control mice. Mobility shifts revealed by polymerase chain reaction and single-strand conformational polymorphism indicated mutations only in exon II of the N-*Ras* gene. Such mutations were more prevalent in samples from mice exposed to fast neutrons. Silent point mutations, i.e. base transitions at the third base of codons 57, 62 or 70, were present only in mice that had developed myeloid leukaemia after

exposure to fast neutrons. The higher frequency of N-*Ras* mutations in neutron-induced myeloid leukaemia suggested that fast neutrons are more effective in inducing genomic instability at the N-*Ras* region of the genome. More importantly, N-*Ras* mutations appear not to be the initiating event in radiation leukaemogenesis. This conclusion was supported by the finding of N-*ras* mutations only in mice with an overt leukaemic phenotype and not in animals with minimal tissue infiltration of leukaemic cells, suggesting that the disease may be present before the N-*Ras* mutations (Rithidech *et al.*, 1996).

A protocol was developed to induce thymic lymphomas in RF/J mice efficiently by a single acute dose of neutron radiation. Activated *Ras* genes were detected in 4 of 24 of the tumours analysed. One of the tumours contained a K-*Ras* gene activated by a point mutation in codon 146. Activating *Ras* mutations at position 146 have not previously been detected in any known human or animal tumour. The spectrum of *Ras* mutations detected in neutron radiation-induced thymic lymphomas was different from that seen in thymic lymphomas induced by γ-radiation in the same strain of mice (Sloan *et al*., 1990). A novel K-*ras* mutation in codon 146 was also found in thymic lymphomas induced by neutrons (Corominas *et al*., 1991).

(iii) *Cytogenetic effects*

Sister chromatid exchanges were scored in bone-marrow cells from three-month-old rats as a function of time after exposure to 2 Gy of whole-body radiation with 1-MeV fission neutrons. This dose reduced the mean survival time to 445 days after irradiation and induced more than one tumour per animal; by 200 days after irradiation, all of the animals bore tumours at autopsy, but the bone-marrow was not a significant target for tumour induction. In controls, the mean number of sister chromatid exchanges per cell remained constant from 3 to 24 months of age (2.38 per cell; SD, 0.21), but irradiation induced two distinct increases in the frequency: the first occurred during the days following exposure and the second between days 150 and 240. Thereafter, the values levelled off at 3.37 per cell (SD, 0.39) until day 650. Between the two increases (i.e. days 15–150), the number of sister chromatid exchanges dropped to control values. Analysis of the distribution per cell showed that the changes were not confined to a particular cell population. These results suggest that, in irradiated rats, the second increase in sister chromatid exchange coincides with tumour growth, whereas the first increase may be due to DNA damage that is rapidly repaired (Poncy *et al*., 1988).

A modified mouse splenocyte culture system was standardized and used to evaluate the induction of micronuclei and chromosomal aberrations for the purposes of biological dosimetry after exposure to X-radiation and fission neutrons *in vivo* and/or *in vitro*. After irradiation with 1-MeV fission neutrons *in vivo* and culturing of mouse splenocytes, linear dose–response curves were obtained for the induction of micronuclei and chromosomal aberrations. The lethal effects of neutrons were shown to be significantly greater than those of a similar dose of X-radiation. The RBE was 6–8 in

a dose range of 0.25–3 Gy for radiation-induced asymmetrical exchanges (dicentrics and rings) and about 8 for micronuclei in a dose range of 0.25–2 Gy (Darroudi *et al*., 1992).

The induction of reciprocal translocations in rhesus monkey stem-cell spermatogonia was studied by analysing primary spermatocytes at metaphase. The animals were exposed to 1 Gy of γ-radiation at dose rates of 140 or 0.2 mGy min^{-1} or to 0.25 Gy of 2-MeV neutrons at 36 mGy min^{-1}. Reduction of the dose rate from 140 to 0.2 mGy min^{-1} did not lower the frequency of recovered translocations from 0.43% induced by the γ-radiation. The RBE for neutrons in relation to X-radiation was 2.1, which is clearly lower than the value of 4 obtained for mice (Van Buul, 1989).

(*b*) *Cellular systems*

(i) *DNA damage*

Radiolysis of water results in numerous products; the most reactive and the most damaging to DNA is the •OH radical. This radical either abstracts •H from deoxyribose and bases or reacts with the bases of all nucleotides. Consequential to these reactions, conformational changes occur in DNA, which lead to the generation of lesions. These lesions include single- and double-strand breaks and modifications of deoxyribose and bases (some of these are alkali-labile sites that are revealed as single-strand breaks after alkaline treatment), intrastrand and interstrand cross-links and DNA–protein cross-links (Burns & Sims, 1981). The RBE of neutrons (in relation to γ-radiation) for generation of these lesions is often higher than 2.5, but there is no qualitative difference in the results of exposure to these types of radiation.

Irradiation of pBR322 plasmid DNA in solution with neutrons or γ-radiation resulted in half the yield of single-strand breaks and a 1.5-times higher yield of double-strand breaks with neutrons as compared with γ-rays (Spotheim-Maurizot *et al.*, 1990, 1996). Scavenging of •OH radicals with ethanol inhibited all neutron-induced single-strand breaks but only 85% of the double-strand breaks, whereas with γ-irradiation the formation of both single- and double-strand breaks was completely inhibited. The results suggest at least three different origins for neutron-induced double-strand breaks. The occurrence of around 30% of these breaks can be explained by a radical transfer mechanism, as proposed by Siddiqi and Bothe (1987), for γ-radiation. In this model, a radical site is transferred from a sugar moiety of the cleaved strand to the complementary intact strand, which occurs with a probability of about 6%. Around 55% of neutron-induced double-strand breaks may be due to the non-random distribution of radicals in high-density tracks of the secondary particles of neutrons, which results in a simultaneous attack of the two strands by •OH radicals. The first two processes are both •OH-mediated and are therefore sensitive to ethanol. The direct effect of fast neutrons and their secondaries (recoil protons, α-particles and recoil nuclei) can account for the remaining 15% of double-strand breaks, which are not inhibited by scavengers (Spotheim-Maurizot *et al.*, 1990). Consistent with this

view, Pogozelski *et al*. (1999) found that the decrease in yields of strand breaks in plasmid pBR322 with increasing •OH scavenging capacities was not as pronounced for fission neutrons as for γ-rays. In contrast, damage to restriction fragments or oligodeoxyribonucleotides induced by fission neutrons can be almost completely suppressed by thiols (Savoye *et al.*, 1997; Swenberg *et al.*, 1997).

In an 80-base-pair DNA fragment exposed to fast neutrons, the probability of strand breakage at a given nucleotide site was not determined by the nature of the nucleotide but by its flanking sequence. The sequence-dependence is due to variations in the accessibility of the H4′ and H5′ atoms. Fitting the experimental results with the calculated reaction probabilities suggested that a C4′-centred radical develops into a strand break three times more efficiently than a C5′-centred radical, and that half of the breaks occur via the 4′ path and half via the 5′ path (Sy *et al.*, 1997).

DNA lesions induced by fast neutrons in L5178Y mouse lymphoma cells were classified into three types on the basis of their repair profiles: rapidly repaired breaks (half-time, 3–5 min), slowly repaired breaks (70 min) and unrepairable breaks. The rates of repair of the first two types of break were almost the same as those of corresponding damage induced by low-LET radiation. Neutrons induced less rapidly repaired damage, a nearly equal amount of slowly repaired damage and more unrepairable damage when compared with equal doses of γ-radiation or X-radiation (Sakai *et al*., 1987).

The induction and repair of breaks was studied by alkaline elution (Kohn & Grimek-Ewig, 1973) of DNA from Chinese hamster V79 and human P3 epithelial teratocarcinoma cells after exposure to fission-spectrum neutrons (mean energy, 0.85 MeV) and ^{60}Co γ-radiation in the biological dose range. The fission-spectrum neutrons induced fewer direct single-strand breaks per gray of absorbed dose than γ-radiation (Peak *et al.*, 1989). Measurements of cell survival had already indicated incomplete recovery of the cells after exposure to neutrons (Hill *et al.*, 1988). Whereas most single-strand breaks caused by exposure to fission-spectrum neutrons can be rapidly repaired by both hamster and human cell lines, a small but statistically significant fraction (about 10%) of the single-strand breaks induced by exposure to 6 Gy of neutrons was refractory to repair. In contrast, all measurable single-strand DNA breaks induced by 3 Gy of γ-radiation were rapidly repaired (Peak *et al.*, 1989).

Neutron irradiation has been reported to cause single-strand breaks, with RBEs varying from 0.3 to nearly 2 in assays with various cellular and extracellular systems and neutron energies (see, e.g. Van der Schans *et al.*, 1983; Prise *et al.*, 1987; Vaughan *et al.*, 1991). The RBEs for double-strand break induction by neutrons are usually about 1, although higher values have been reported. The breaks differ from those induced by γ-rays mainly in the fact that they are less readily repaired, as described below.

Monolayers of L-929 mouse fibroblasts were irradiated with fast neutrons or 250-kVp X-rays and treated simultaneously with dinitrophenol to prevent the DNA strands from rejoining; single-strand breaks induced in DNA were measured by the alkaline

sucrose sedimentation method. The RBE for single-strand breaks was about 1.6, which is essentially the same as that measured from cell survival (Moss *et al.*, 1976).

The effects on cellular viability and the kinetics of induction and repair of DNA strand breaks in HeLa cells were examined after exposure to a thermal neutron beam and compared with those after γ-irradiation. The survival curve had no initial shoulder. The RBEs of the neutron radiation were 2.2 for cell killing (ratio of D_0 values), 1.8 and 0.9 for single-strand breaks measured by alkaline sedimentation and alkaline elution, respectively, and 2.6 for double-strand breaks, determined by neutral elution (Bradley & Kohn, 1979). No difference was observed between thermal neutrons and γ-rays in respect of the repair kinetics of single- and double-strand breaks. It was suggested that the effect of the intracellular nuclear reaction, $^{14}N(n,p)^{14}C$, is mainly responsible for the high RBE values observed (Maki *et al*., 1986).

The effects of 2.3-MeV (mean energy) neutrons and 250-kVp X-rays on cell survival and DNA double-strand break induction and repair (measured by neutral elution) were investigated in Chinese hamster V79 cells. The lethal effects of neutrons were shown to be significantly greater than those of a similar dose of X-rays (RBE, 3.55 at 10% survival), but the RBE for double-strand break induction, in a dose range of 10–50 Gy, was 1. Radiation-dependent differences were found in the pattern of repair. A fast and a slow repair component were seen in both cases, but the former was reduced after neutron irradiation. Since the amount of slow repair was similar in the two cases, proportionally more unrejoined breaks were seen after exposure to neutrons. The results were similar when the elutions were conducted at pH 9.6 and pH 7.2 (Fox & McNally, 1988).

DNA double-strand break induction and rejoining, measured by field-inversion gel electrophoresis, were compared by cell survival in mutant (XR-V15B) and wild-type parental (V79B) hamster cell lines after low-dose neutron and X-irradiation. Neutrons did not induce more double-strand breaks than X-rays. Even with low doses of neutrons, a visible increase was found in the formation of a smaller subset of DNA fragments, which arise only after very high doses of X-rays. In both cell lines, double-strand breaks induced by neutrons were rejoined more slowly than those induced by X-radiation. At long repair times (4 and 17 h), there were no significant differences between neutrons and X-rays in the fractions of unrejoined double-strand breaks. The authors proposed that neutron-induced double-strand breaks have a higher probability of becoming lethal because they are more likely to be misrepaired during the slow stage of rejoining (Kysela *et al*., 1993).

Irradiation of viable CHO AA8 cells on ice with 4–25 Gy of either ^{60}Co γ-radiation or d(20 MeV)Be neutrons (mean energy, 7.5 MeV) produced similar resistance to rewinding of nuclear DNA supercoils after treatment with ethidium bromide. The recovery from the effects of 12 Gy of either radiation was also similar, leaving no detectable residual damage. The discrepancy between these data and the reduced ability of neutrons to produce DNA breaks, as defined by the alkaline elution assay, is explained by the discontinuous deposition of energy associated with neutron irra-

diation. A microdosimetric analysis suggested that neutron radiation interacts with DNA at sites that are on average 5–10 times further apart than those that interact with γ-radiation. The long DNA sequences that result from neutron irradiation are consequently eluted inefficiently during alkaline elution, giving a reported RBE of approximately 0.3. Restrictions in the rewinding of individual supercoils are not dependent on the inter-ionization distance and thus give rise to an RBE of approximately 1. Furthermore, the complete removal of DNA damage, as measured by this technique, supports the hypothesis that the toxicity of neutrons is associated with incorrect, not incomplete, rejoining of the DNA molecule (Vaughan *et al.*, 1991).

The relative sensitivity of Chinese hamster ovary cells to fast neutrons and γ-rays was studied with a panel of mutants characterized by defects in the nucleotide excision repair pathway. These could be further subdivided into mutants that were defective in nucleotide excision repair alone, in base excision repair alone, in DNA-dependent protein kinase-mediated DNA double-strand break repair or in the distinct but overlapping pathway for the repair of DNA cross-links. None of the mutants defective in nucleotide excision repair showed different sensitivities to fast neutrons and γ-radiation. In contrast, deficiency in the base excision repair pathway resulted in significant primary sensitization to both types of radiation (2.0-fold to γ-radiation and 1.8-fold to neutrons). Deficiency in the double-strand break repair pathway mediated by DNA-protein kinase resulted in marked but again similar primary sensitization to γ-radiation (4.2-fold) and neutrons (5.1-fold). Thus, none of the repair pathways examined showed a preferential role in the repair of damage induced by low-LET and intermediate-LET radiations; this resulted in an essentially consistent RBE of approximately 2 in the cell lines studied (Britten & Murray, 1997).

(ii) *Chromosomal aberrations*

Many studies have been performed of radiation-induced chromosomal aberrations in mammalian cells—often human lymphocytes. Comparisons of the effects of radiation have often been based on the number of dicentric chromosomes induced, although premature chromosome condensation is also an end-point for comparison. The RBEs of neutron irradiation have been determined for dicentrics or for dicentrics plus centric rings in human lymphocytes isolated from peripheral blood exposed to neutrons with different energies (Table 18). Analysis of dicentrics revealed RBE values of 5, 6 and 14 for neutrons of mean energy 21, 14 and 6.5 MeV, respectively, produced on a beryllium target [^{9}Be(d,n)^{10}B] (Fabry *et al.*, 1985).

The yield of chromatid-type aberrations induced by either fission neutrons or X-radiation can be potentiated by post-irradiation treatment with hydroxyurea and caffeine when the cells are irradiated in G_2; however, the frequencies of neutron-induced chromatid-type aberrations are not potentiated by treatment with cytosine arabinoside, except at the highest dose used. In contrast, chromatid aberrations induced by X-radiation were strongly potentiated by cytosine arabinoside. These results indicate that

Table 18. Relative biological effectiveness (RBE) of neutrons for chromosome-type dicentrics (or dicentrics plus centric rings) induced in human peripheral lymphocytes irradiated *in vitro* (reference radiation, ^{60}Co γ-rays; constant dose rate, 0.5 Gy min^{-1}; Lloyd *et al*., 1975)

Source	Neutron energy (MeV)	Absorbed dose rate (Gy min^{-1})	Sampling time	RBE for 2.0–0.02 aberrations per cell	RBE_m	Reference
d, T						
Japan	~ 14.1	–	–	1.2–5.9[a]	14.5	Sasaki (1971)
Germany	~ 15.0 (γ < 4%)	0.12	48 h	1.1–3.6	9.0	Bauchinger *et al*. (1975)
Glasgow, Scotland	~ 14.7 (γ ~ 7.5%)	0.30	48 h	1.7–6.6	16.7	Lloyd *et al*. (1976)
Harwell, England	~ 14.9 (γ ~ 3%)	0.25	48 h (O_2)	2.2–6.6	16.2	Prosser & Stimpson (1981)
			(N_2)	1.2–2.1	4.3	
^{3}H(α,n)^{4}He						
Russian Federation (NG–150M)	14.7 (γ < 10%)	0.36–1.85	50–52 h	1.7–3.8	9.0	Sevan'kaev *et al*. (1979a,b)
d, Be						
Harwell, England (VEC)	~ 20	~ 0.50	52–72 h (with BrdU)	1.4–11.3	29.2	Barjaktarovic & Savage (1980)
Hammersmith, England (cyclotron)	~ 7.6 (γ < 10%)	0.30	48 h	2.1–11.9	30.4	Lloyd *et al*. (1976)
Louvain, Belgium (cyclotron)	~ 6.2 (γ low)	0.05	48–53 h	1.0–8.3	21.5	Biola *et al*. (1974)
Japan	~ 2.03	–	–	2.2–17.4[a]	43.3	Sasaki (1971)
Li/Be						
Russian Federation	~ 0.04 (γ < 7%)	0.01	50–52 h	2.4–6.8	16.5	Sevan'kaev *et al*. (1979a,b)
(KG-2.5 accelerator)	~ 0.09 (γ < 4%)	0.03		1.1–10.8	28.0	

Table 18 (contd)

Source	Neutron energy (MeV)	Absorbed dose rate (Gy min^{-1})	Sampling time	RBE for 2.0–0.02 aberrations per cell	RBE_m	Reference
Fission						
France (CEA/Crac)	Max, ~ 10 (γ very high + thermal)	–	46–53 h (data corrected for γ)	2.8–22.3	57.4	Biola *et al*. (1974)
France (CEN/Triton)	Max ~ 10 (γ ~ 30–50%)	0.03–0.07	46–53 (data corrected for γ)	2.7–21.6	55.7	Biola *et al*. (1974)
France (CEN/Harmonie)	Max ~ 1.5 (γ ~ 5%)	0.12	46–53 h	2.0–16.1	41.3	Biola *et al*. (1974)
Sofia, Bulgaria (IRT-2000)	Max ~ 3	–	52 h	0.8–6.5	16.9	Todorov *et al*. (1973)
Aldermaston, England	~ 0.9 (γ < 10%)	0.03	48 h	2.2–18.0	46.4	Lloyd *et al*. (1976)
Argonne, USA (JANUS)	~ 0.85 (γ ~ 3%)	0.06	48–50 h	2.3–18.3[a]	45.6	Carrano (1975)
Russian Federation (BR-10)	~ 0.85 (γ < 5%)	0.06–2.6	50–52 h	2.8–19.9	51.1	Sevan'kaev *et al*. (1979a,b)
Harwell, England (BEPO)	~ 0.7 (γ ~ 10%)	0.50	48 h	2.6–20.6	53.2	Lloyd *et al*. (1976)
Harwell, England (BEPO)	~ 0.7 (γ ~ 10%)	0.50	48–56 h	2.6–21	54.1	Scott *et al*. (1969)
Harwell, England (GIEEP)	~ 0.7 (γ ~ 15%)	0.0005 0.0011	48–46 h	2.5–20.4 3.1–25.2	52.2 65.0	Scott *et al*. (1969)
Italy (TAPIRO)	~ 0.4 (γ ~ 10%)	0.002–0.07	48 h	2.6–22.2	57.1	Vulpis *et al*. (1978)
Russian Federation (BR-10)	~ 0.35 (γ < 5%)	0.04–0.4	50–52 h	4.1–32.6	83.9	Sevan'kaev *et al*. (1979a,b)
Russian Federation (BR-10)	Thermal (γ < 5%)	0.005	50–52 h	1.3–20.6	53.3	Sevan'kaev *et al*. (1979a,b)

Table 18 (contd)

Source	Neutron energy (MeV)	Absorbed dose rate (Gy min^{-1})	Sampling time	RBE for 2.0–0.02 aberrations per cell	RBE_m	Reference
National Radiological Protection Board (^{252}Cf)	~ 2.13 MeV	0.12–0.17	48 h	1.8–14.8	38.2	Lloyd *et al.* (1978)

Adapted from Savage (1982)
[a] Dicentrics plus centric rings

neutrons produce a smaller proportion of lesions, the repair of which can be inhibited by this compound, than X-radiation (Antoccia *et al*., 1992).

Several radiosensitive Chinese hamster cell lines have been studied to explore the relationship between radiation-induced DNA lesions and chromosomal aberrations. The frequency of radiation-induced aberrations in *Xrs* mutants, which are deficient in double-strand break repair, was higher than in control cells. In a radiosensitive hamster cell line (V-C4), which has no detectable defect in double-strand break repair, the frequencies of X-radiation-induced aberrations are higher than those found in wild-type V79 cells. After treatment with fission neutrons, however, the frequency of aberrations is similar to that in V79 cells, indicating that V-C4 cells are defective in repair of X-radiation-induced lesions other than double-strand breaks. Apparently, these other lesions may also lead to aberrations (Natarajan *et al.*, 1993).

Chromosomal aberrations were scored in BHK21 C13 Syrian hamster fibroblasts exposed in stationary phase to ^{60}Co γ-rays, 250-kV X-rays, 15-MeV neutrons or neutrons of a mean energy of 2.1 MeV produced from the ^{9}Be(d,n)^{10}B reaction. No detectable difference was seen in the responses to ^{60}Co γ-rays and 250-kV X-rays. The RBE for the production of dicentrics, based on the 'one hit' component of the response, was 5 ± 2 for the 15-MeV neutrons and 12 ± 5 for the 2.1-MeV neutrons (Roberts & Holt, 1985).

Micronucleus formation induced by neutrons has been studied in a number of cell types, including human blood lymphocytes and two-cell mouse embryos exposed in late G_2 phase (Molls *et al*., 1981; Mill *et al.*, 1996; Vral *et al.*, 1996).

There is now substantial evidence that ionizing radiation can induce genomic instability in the form of chromosomal aberrations which appear several cell generations after irradiation. When the progeny of neutron-irradiated human epithelial MCF-10A cells were examined for chromosomal aberrations 5–40 population doublings after irradiation, an increase in the frequency of chromatid-type gaps and breaks was observed, but no such effect was observed for chromosome-type aberrations. Neutron-irradiated cells showed consistently increased frequencies of aberrations when compared with unirradiated control cells at all times examined, indicating that neutrons can cause chromosomal instability (Ponnaiya *et al*., 1997).

(iii) *Interchromosomal versus intrachromosomal aberrations*

Many attempts have been made to identify specific biomarkers of radiation as the causal agent of biological effects in cells and tissues. The search has included the examination of chromosomal aberrations for what has been termed a chromosomal 'fingerprint' that would indicate the type of radiation responsible for the aberration. Brenner and Sachs (1994) observed that high-LET radiation, in particular α-particles or fission neutrons, produces a remarkably low ratio of interchromosomal to intrachromosomal aberrations, which is two to three times lower than the ratio recorded after X- or γ-irradiation. The authors proposed use of this ratio as a fingerprint for exposure to high-LET radiation.

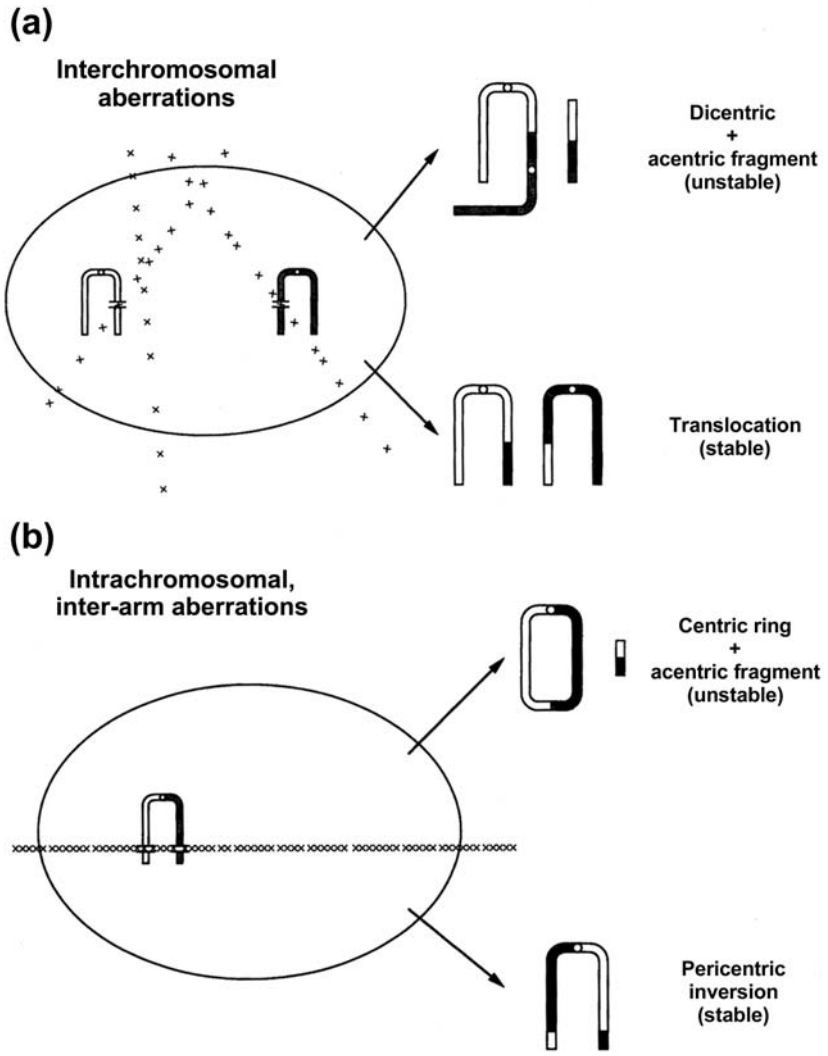
The two types of aberration are illustrated in Figure 5. Exchange-type chromosomal aberrations are interchromosomal if the DNA double-strand breaks that are the initial cause of the lesion occur on different chromosomes. If the double-strand breaks are on different arms of the same chromosome, the lesion is intrachromosomal. If the double-strand breaks were random and all the double-strand breaks were equally likely to interact with one another, the ratio *F* of the interchromosomal to intrachromosomal aberrations would be 90, assuming that all chromosome arms were of equal length. Since chromosome arms are not of equal length and there is an increased probability of interaction between double-strand breaks that are close together, the *F* value is lower and is indicative of lesions induced by low-LET radiations, such as X- and γ-radiation. High-LET radiations, which are densely ionizing because of the inhomogeneity of the energy deposition, induce double-strand breaks that are even closer than those produced by X- or γ-radiation, increasing the yield of intrachromosomal aberrations and resulting in a smaller *F* value. On the basis of many reports of the induction of chromosomal aberrations in humans and other experimental data, it was suggested that the *F* value for densely ionizing radiation was about 6 and that this was significantly lower than the values for X- and γ-radiation and for chemical clastogenic agents. If valid, this approach for determining *F* ratios would have potential use in epidemiological studies, such as those on atomic bomb survivors and persons exposed to radon, in establishing the type of radiation involved (Brenner & Sachs, 1994).

Other authors have both supported (Sasaki *et al*., 1998) and disputed (Bauchinger & Schmid, 1997, 1998) this hypothesis. The report of a workshop set up to examine the use of *F* values concluded that: (1) there was some evidence to suggest that ratios of different chromosomal aberrations might be used as a biomarker of exposure to high-LET radiations; (2) there are large interlaboratory differences in *F* values for the same type of radiation; (3) despite these variations, *F* values do not depend on dose or LET at doses above 1 Gy; (4) further studies are required to establish if *F* values can be used to identify a causal relationship between the observed chromosomal aberrations and specific exposure to radiation. It was suggested that the ratio of intrachromosomal intra-arm to interchromosomal aberrations (designated the *H* ratio) should be examined as a possible fingerprint of exposure to high-LET neutrons (Nakamura *et al*., 1998).

(iv) *Gene mutations*

Since mutation of a given gene is a relatively rare event, the majority of systems for studying radiation-induced mutations involve placing an irradiated cell population under selective pressure so that only the mutant cells are able to survive and can be enumerated. Mutation of genes in a hemizygous (single copy) or heterozygous (two copies but only one active) state is usually studied, to enable measurement. Commonly used mutation systems are based on the loss of enzyme activity, e.g. the enzyme HPRT, which renders cells resistant to the drug 6-thioguanine, the enzyme TK, which confers resistance to trifluorothymidine and the enzyme APRT which confers resistance to

Figure 5. Interchromosomal and intrachromosomal, inter-arm aberrations resulting from ionizing radiations of different quality



Adapted from Brenner & Sachs (1994)
Each cross represents an ionization cluster of sufficient localization and multiplicity to produce a double-strand DNA break. Panel **(a)** shows interchromosomal aberrations resulting, in the case shown here, from two independent, sparsely ionizing radiation tracks. This aberration could also result from two double-strand breaks caused by a single radiation track. Panel **(b)** shows intrachromosomal, inter-arm aberrations resulting, in the case shown here, from a single, densely ionizing radiation track.

8-azaadenine and 2-aminopurine (see section 4.4.2 in the monograph on X- and γ-radiation). The *Hprt* gene is located on the X chromosome, while the *Tk* and *Aprt* genes are on autosomes and must therefore be used in a hemi- or heterozygous state.

The effects of the dose rate of high-LET radiation on mouse L5178Y cells were reported (Nakamura & Sawada, 1988) after exposure to ^{252}Cf (2.13-MeV neutrons). At the high dose rate of 1.2 cGy min^{-1}, ^{252}Cf irradiation produced a linear induction of *Hprt* mutants at relatively low doses but showed reduced effectiveness at higher doses. At the lower dose rate of 0.16 cGy min^{-1}, the initial slope for mutant induction (9×10^{-7} per cGy) was approximately the same as that at the higher dose rate, but the induction curve did not appear to 'turn over' at higher doses. Dose-dependent values for the RBE of high-LET radiation in excess of 10 were found for the low-dose rate in a comparison of neutrons from ^{252}Cf with ^{60}Co γ-radiation.

Human B-lymphoblastoid TK6 cells were used to examine the effectiveness of 4.2-MeV (^{230}Pu, Be) neutrons at dose rates of 0.00014–0.04 cGy min^{-1} for up to 20 days. Neutrons at dose rates < 0.0014 cGy min^{-1} were more effective at inducing mutants than were higher dose rates. The RBE of these low dose rates, relative to 100-kV X-rays, can be calculated to be about 10. When TK6 cells were exposed to beams ranging in atomic number from ^{20}Ne to ^{40}Ar over an energy range of 330–670 MeV per atomic mass unit (amu), mutation induction was evaluated for both the *TK* and the *HPRT* loci for a subset of these beams. The results obtained with the ^{20}Ne ions of 425 MeV per amu (LET, 32 keV μm^{-1}) and ^{28}Si ions of 670 MeV per amu (LET, 50 keV μm^{-1}) closely resembled those obtained after brief exposure to (^{230}Pu, Be) neutrons. Alterations in DNA structure within the *TK* locus of mutants induced by neutrons and by ^{40}Ar ions were similar and were dominated by allele loss. Multi-locus deletions inclusive of the c-*erb*A1 locus were common among the *TK*-deficient mutants induced by these densely ionizing radiations (Kronenberg & Little, 1989; Kronenberg, 1991).

A system involving human–hamster hybrid cells was developed by Waldren *et al*. (1979) from a stable hybrid containing the Chinese hamster genome and one copy of the human chromosome 11. The loss of several markers on this chromosome can be determined, and even complete loss of the chromosome is not lethal. This system has been used to measure mutant frequencies after irradiation with neutrons of various energies (0.33–14 MeV), at doses up to 200 cGy. Significant increases in mutant frequency were found at doses as low as 10 cGy, and dose-dependent RBEs of up to 30—for the 0.33-MeV neutrons—were calculated in comparison with ^{137}Cs γ-radiation (Hei *et al*., 1988).

Fast neutrons produced by proton bombardment of a beryllium target in a cyclotron were used to examine the energy dependence of the induction of mutants at the *Hprt* and *Tk* loci in V79 Chinese hamster cells. The beams of neutrons were produced from protons with 46, 30, 20 and 14 MeV of energy. Gradually increasing cytotoxic and mutagenic effects of the neutrons were noted as the energy decreased. The frequency of induced mutants at the *Tk* gene was higher than at the *Hprt* gene. In a human epithelium teratocarcinoma cell line (P3), the mutation frequency at the *HPRT*

locus, as in V79 cells, increased 2.5–4 fold with decreasing neutron energy (Zhu & Hill, 1994; Sharma & Hill, 1996).

A 1-Gy fission neutron dose from a ^{252}Cf source induced a maximal *Hprt* mutation frequency in synchronized L5178Y mouse lymphoma cells when delivered immediately after release from G_2/M block, whereas the maximal response to ^{60}Co γ-radiation was found in G_1 (Tauchi *et al*., 1993).

The biological effectiveness for mutation induction at the *Hprt* locus in confluent cultures of mouse m5S cells exposed to fission neutrons from ^{252}Cf, relative to γ-radiation, was increased from 4.9 to 7.4 when the dose rate was reduced from 1.8 to 0.12 cGy min^{-1}. The changes in RBE were due mainly to a reduction in the effect of γ-radiation. The authors noted that their observations contrast with reports of proliferating cell cultures and suggested that they could be ascribed to the cell growth conditions used in their experiments (Komatsu *et al*., 1993).

The toxic and mutagenic effects of X-rays and neutrons were compared in the Chinese hamster ovary cell line K1-BH4 and its transformant, AS52, which lacks the normal *Hprt* gene but instead contains a single autosomally integrated copy of the bacterial equivalent, the *gpt* gene. X-radiation and neutrons appeared to be equitoxic in the two cell lines, but both were 10 times more mutagenic to the *gpt* gene in AS52 cells than to the *Hprt* gene of K1-BH4 cells. The apparent hypermutability of AS52 cells probably results from better recovery of multi-locus deletion mutants in AS52 cells than in K1-BH4 cells, rather than a higher yield of induced mutants (Hsie *et al*., 1990).

Chinese hamster ovary cells were exposed to thermal neutrons, and the mutation frequency at the *Hprt* locus was determined. The Kyoto University Research Reactor, which produces thermal neutrons with a very low level of contaminating γ-rays and fast neutrons, was used as the source of radiation. The cells were irradiated in the presence or absence of boric acid. Thermal neutron irradiation was 2.5 times as mutagenic as γ-radiation without boron. In the presence of boron, however, thermal neutron radiation was 4.2–4.5 times as mutagenic as γ-radiation. When the mutation frequency was plotted against the surviving fraction, greater mutagenicity was observed in the presence than in the absence of boron, suggesting that the enhancement of thermal neutron-induced mutation with boron is strongly associated with α-particles released by the ^{10}B(n,α)^{7}Li reaction (Kinashi *et al*., 1997).

(v) *Cell transformation*

Ionizing radiation of low LET is an effective inducer of cell transformation in various systems (see section 4.4.2 in the monograph on X- and γ-radiation). A large number of studies have also been conducted with neutrons, which are even more effective than X- or γ-rays. The RBE values relative to X- or γ-radiation depend on the energy of the neutrons. Miller *et al*. (1989) examined the effect of low absorbed doses of monoenergetic neutrons with energies of 0.23–13.7 MeV on transformation in asynchronous mouse C3H10T1/2 cells. The dose–response curves were linear or

nearly linear for the various neutron energies and curvilinear for the reference X-rays. The RBE values were found to decrease with increasing dose for both cell transformation and survival. The maximal values varied from 13 for 5.9-MeV neutrons to 35 for 0.35-MeV neutrons. Rather lower RBE values were reported in a study with less pure neutron sources (Balcer-Kubiczek & Harrison, 1983): the maximum observed RBE for reactor fission neutrons (with 8–20% γ-ray component) was 3.8, and that for cyclotron neutrons (8% γ-ray component) was 1.2. A subsequent study on fission neutrons at various dose rates gave an RBE for cell transformation of 3 at a high dose rate (0.1 Gy min^{-1}) and 10 at the lowest dose rate studied (0.005 Gy min^{-1}) (Balcer-Kubiczek *et al.*, 1988). In mouse mS5 cells, ^{252}Cf neutrons showed RBE values for cell transformation of 3.3–5.1, depending on the dose rate (1.8–0.12 cGy min^{-1}) (Komatsu *et al.*, 1993).

The claim of Hill *et al.* (1984a,b) that neutron-induced transformation in the C3H10T1/2 system was enhanced by a factor of about 9 at low dose rates triggered much work on dose rates and dose fractionation with respect to the so-called 'inverse dose rate' problem. The effect was confirmed in the same system by several authors (see e.g. Miller *et al.*, 1990) and flatly denied by others (Balcer-Kubiczek *et al.*, 1988, 1991; Saran *et al.*, 1991; Balcer-Kubiczek *et al.*, 1994; Saran *et al.*, 1994). Syrian hamster embryo cells were also reported to show the effect (Jones *et al.*, 1989), and an inverse dose-rate effect of 2.9 was reported for the human hybrid system (HeLa × skin fibroblasts), with fission neutrons of an average energy of 0.85 MeV (Redpath *et al.*, 1990); however, no effect was found in confluent cultures of mouse m5S cells (Komatsu *et al.*, 1993). Several authors reported that the effect is specific to particular sources or energies of neutrons (Elkind, 1991; Miller & Hall, 1991), and there is still some confusion in the area (Masuda, 1994; Brenner *et al.*, 1996). Explanations of the inverse dose-rate effect have involved cell proliferation during irradiation and the postulated existence of a hypersensitive 'window' in the cell cycle (Elkind, 1991).

In experiments with synchronized mouse C3H10T1/2 cells, Miller *et al.* (1995) found that the G_1 phase of the cell cycle (4–6 h after mitotic 'shake-off') was the most sensitive to neutron-induced oncogenic transformation, in contrast to what has been observed with X-radiation where the peak was 14–16 h after 'shake-off', reflecting mostly G_2 cells. Less variation in the response during the cell cycle was seen for neutrons than for X-rays (Redpath e*t al.*, 1995; Pazzaglia *et al.*, 1996).

It is not clear what molecular changes induced by neutrons are responsible for cell transformation. In 5.9-MeV neutron-transformed foci of C3H10T1/2 cells, chromosomal aberrations have been found, but there were no N-*ras* or K-*ras* mutations (Freyer *et al.*, 1996), and it was reported that human keratinocytes transformed by neutrons do not contain mutations in either *RAS* or *p53* (Thraves *et al.*, 1994).