ANNEX B

Adaptive responses to radiation in cells and organisms

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INTRODUCTION

1. The scientific community has been aware for many years of the possibility that low doses of radiation may result in changes in cells and organisms, which reflect an ability to adapt to the effects of radiation. In lower organisms, for example, enhanced proliferation in the presence of radiation at doses of a few microgray per day to a few milligray per day has been observed in experiments involving cultures of prokaryotes and eukaryotic cells [C1, C2, C3, C4, C5, C6, I1, I2, L4, P2, P3, T11].

2. The biological expression of adaptive and stimulatory responses in seeds and plants (e.g. [C14, H9, H10, R6, R7, S43]) has also been described. The extensive literature up to 1976 supporting radiation-associated adaptive effects was reviewed by Luckey [L1]. A more recent publication by the same author summarizes the literature between 1976 and 1991, involving about one thousand reports judged by him to demonstrate beneficial responses in animals and in human populations [L2].
3. It has been suggested in recent years that the conventional estimates of stochastic effects following exposure to low doses of ionizing radiation may have been overstated because no allowance was made for the possibility that small doses of radiation may condition cells so as to induce processes that reduce either the natural incidence of cancer in its various forms or the likelihood of excess cancers being caused by further radiation exposure.

4. An important observation from mammalian cell studies in support of these processes is that mitogen-stimulated human blood lymphocytes exposed in vitro appear to suffer less damage than would be expected following acute exposure to a few gray of low-LET radiation if they are first exposed to a dose of a few tens of milligray. This response to low-dose exposure, which remains effective for several hours, is referred to as an adaptive response.

5. Adaptive responses have been observed in other mammalian cell types, such as bone marrow cells and fibroblasts, but not consistently in spermatocytes or at all in embryo cells exposed in the pre-implantation stage. Changes in the composition of the culture medium can alter the adaptive response. In a wider context, it is known that changes similar to those observed in radiation-induced adaptive response can occur as a result of metabolic disturbances and after damage resulting from exposure to a variety of physical and chemical agents. The consequences of these cellular changes are referred to collectively in the literature as stress response or response to genotoxic stress.

6. Evidence of adaptive response has also been described in studies using laboratory animals, in which the animals were exposed either to single acute doses from a few tens of milligray to a few gray, or accumulated doses of up to a few gray over a lifetime. Reported manifestations of this form of adaptive response described in mammals after exposure to low doses of radiation include an accelerated growth rate in the young, an increase in reproductive ability, an extended life span, stimulatory effects on the immune system and a lower-than-expected incidence of spontaneous tumours. A satisfactory explanation of mechanisms that might be responsible for such effects, which have not been consistently observed in different investigations, is not obvious. It may involve a DNA repair mechanism similar to that proposed for the cellular adaptive response, implying its immediate availability if cellular damage randomly occurs during the animal's lifetime. Involvement of the immune system has also been proposed.

7. Four conferences (one on radiation hormesis, held at Oakland, California, in 1985 [S1]; one on low-dose radiation and the immune system, held at Frankfurt in 1987 [S2]; one on low-dose irradiation and biological defense mechanisms, held at Kyoto in 1992 [S3]; and one on low-level exposures to radiation and related agents, held at Changchun, China, in 1993 [S4]) have provided an opportunity for scientists working in the field of low-dose effects to present their experimental findings and debate the possible mechanisms involved in radiation-induced adaptive response.

8. This Annex, based mainly on recently published data, has been compiled by the Committee with a view to identifying the cellular mechanisms that may be involved in the adaptive response at low doses. It should be considered as a continuation of the discussions on radiation response contained in the UNSCEAR 1993 Report [U1]. One problem in identifying common mechanisms of response is that there are differences between the doses and dose rates used in cellular studies and those used in in vivo studies of laboratory animals. A further complication is that there are few studies available in which doses of a few milligray per year above the natural background radiation level have been used. Low doses were defined in the UNSCEAR 1993 Report [U1], Annex F, "Influence of dose and dose rate on stochastic effects of radiation", with the values depending on the level of investigation. At a microdosimetric level a low dose is defined as about 0.0002 Gy. In a similar context, the International Commission on Radiological Protection [I6] considered that low doses and low dose rates imply situations in which it is very unlikely that more than one event of energy deposition will occur in the critical parts of a cell within the time during which repair mechanisms in the cell can operate. For mammalian cells in culture, a low dose is defined as less than about 0.02 Gy. For the induction of human tumours, a low dose is defined as less than about 0.2 Gy. The same criteria can be applied in this Annex, although it should be recognized that much of the experimental data on effects in cells and animals is based on doses in excess of about 0.5 Gy.

9. Manifestations of adaptive responses in animals and in human populations are briefly addressed in this Annex. Evidence for the expression of an adaptive response in human populations exposed to low doses of radiation above the natural background level has not until now been clearly demonstrated nor has it been refuted. The possibility that exposure to low doses of radiation may affect the level of competence of immunosurveillance mechanisms in carcinogenesis is discussed. Details of the mechanisms of action of radiation in inducing cancers and serious hereditary effects in humans are not discussed. These aspects of the deleterious effects of radiation can be found in the UNSCEAR 1993 Report [U1], Annex E, "Mechanisms of radiation oncogenesis", Annex G, "Hereditary effects of radiation", and in this Report in Annex A, "Epidemiological studies of radiation carcinogenesis". These Annexes should be read in conjunction with this Annex to achieve a balanced view of the overall effects of low doses of radiation.
I. ADAPTIVE PROCESSES IN MAMMALIAN CELLS

10. Efforts have been made over the past decade to characterize the adaptive response induced by mutagens in mammalian cells. A response has been demonstrated in mitogen-stimulated, human blood T lymphocytes. Other cell types investigated for evidence of a response include proliferating lymphoblasts, bone marrow cells, spermatocytes, pre-implantation embryos and fibroblasts.

11. Cells respond to radiation-induced injury by the up-regulation of proteins involved in cell signalling and by the increased expression of genes involved in cell proliferation [A1, S3] and in the synthesis of DNA repair enzymes [L1, L5, M3, W2]. Qualitatively similar responses have been described as a result of cellular disturbance caused by temporary oxygen deprivation [B5, L7, M4, S9, S10] and glucose starvation [H1, S11].

12. The mechanisms involved in the adaptive response to low doses of radiation have been linked to a more general phenomenon in which the cells are able to respond to damage from a variety of physical and chemical agents. These agents include overheating [S8], UV-radiation [B3], trace amounts of mutagenic chemicals [B4, V1], local anaesthetics that alter membrane structure [L6] and heavy metals known to act as cellular poisons [C7].

A. EFFECTS IN HUMAN LYMPHOCYTES

1. Chromosome aberrations

13. It was reported in 1984 [O2] that when phytohaemagglutinin-stimulated human lymphocytes were grown in a culture medium containing tritiated thymidine and were exposed in the G2 phase of the cell cycle to 1.5 Gy from x rays, the yield of chromatid aberrations was significantly less than the sum of yields of the aberrations induced by tritiated thymidine and x rays separately (Table 1). The response was observed to occur at a concentration of tritiated thymidine low enough to give an estimated one beta disintegration in each cell volume between exposure to the tritium and the x rays. The reduction in the expected number of aberrations was not considered to be attributable to a radiation-induced delay in cell cycle progression at this low concentration. Nor was it considered to be due to the selective killing of a radiosensitive population of lymphocytes that had incorporated tritiated thymidine [W3].

14. When the tritiated thymidine was present in the blood cultures throughout the entire culture period, the results were quite variable. This was shown, however, to be attributable to the fact that, in blood, the amount of tritiated thymidine incorporated into cells is highly dependent on the catabolism of thymidine to a degraded form which cannot be incorporated. Later experiments in which the tritiated thymidine was pulse-labelled while the cells were in the S phase markedly reduced this variability, and the uptake of tritiated thymidine was maximized.

15. A similar response was shown to be induced by exposing phytohaemagglutinin-stimulated lymphocytes in the S phase to a low dose from x rays (referred to variously in the literature as the conditioning, inducing, priming or adapting dose), followed by exposure of the cells in the G2 phase to a high dose from x rays (called the challenge dose). This response was subsequently confirmed by some investigators [B6, B11, K4, L25, M30, O3, S13, S14, W4, W5], although not consistently by others who were using similar culture protocols [B7, H8, M21, S15, S16]. The lymphocyte cells from different donors show variable sensitivity, as is shown in Table 2.

16. It was postulated that the conditioning dose of radiation activated genes and that this was quickly followed by the synthesis of enzymes responsible for DNA repair. If these enzymes were available in adequate concentrations at the time the cells were exposed to a challenge dose, the extent of the repair of DNA damage was improved, so that fewer chromatid aberrations were observed than in cells receiving the challenge dose only. It was presumed that the repair enzymes were not immediately available to cells receiving the challenge dose only and that, in these circumstances, much of the damage was irreparable by the time a sufficient quantity of enzymes became available. This hypothesis was supported by the observation that the adaptive response could be blocked by the protein synthesis inhibitor cycloheximide (Table 3) and by 3-aminobenzamide (Table 4), an inhibitor of poly(ADP-ribose) polymerase, which is known to be induced during the repair of DNA strand breaks [A16].

17. Other characteristics of the in vitro lymphocyte adaptive response have been reported:

(a) the adaptive response to x rays requires a dose of at least 0.005 Gy delivered at a rate of more than 0.2 Gy min⁻¹ [S14]. The implication of this finding is that a certain number of DNA lesions, perhaps of a specific type, need to occur within a fixed time in order to initiate the signal for expression of the adaptive response. In fact, there is a window of dose, 0.005 to 0.2 Gy, below which and above which the phenomenon was not observed. Such a narrow window has also been observed in experiments with radiomimetic compounds such as bleomycin, which, like x rays, induces double-strand breaks;

(b) the induction of the repair mechanism takes place between 4 and 6 hours after exposure to the conditioning dose and remains effective for three cell cycles [S13]:
ANNEX B: ADAPTIVE RESPONSES TO RADIATION IN CELLS AND ORGANISMS

18. The question why lymphocytes from some individuals do not respond remains unresolved. In fact, the lymphocytes in some cultures exposed to 0.02 Gy from x rays reacted synergistically to subsequent mutagenic treatment [O3]. The cells in this experiment were fixed 2-4 hours after the challenge dose, in contrast to those in other experiments, where fixation times were confined to 6 hours or more after the challenge dose. The unpredictable nature of the response has been confirmed using differences in micronuclei frequency as an end-point [P4].

Changes in the hydrogen ion concentration of the culture medium can affect the yield of induced chromatid aberrations (Table 6). This finding was confirmed when it was shown that adjusting the hydrogen ion concentration of the culture medium to pH 6.4 just before the challenge dose enhanced the effectiveness of the response [O3]. It was also shown that the response could be induced in cultured lymphocytes from donors who had not previously displayed the adaptive response, by adding compounds to the culture medium that could affect the metabolism of the phytohaemagglutinin-stimulated lymphocytes (e.g. interleukin-2, which stimulates proliferation). It is conceivable that the repair systems induced may react differently according to the culture conditions, a situation that should not be overlooked when considering the consequences of the conditioning dose. Thus, the composition of the culture medium may be crucial.

19. It has been pointed out that measurements of the frequencies of aberrations induced in asynchronous cell populations are likely to be misleading if expressed as a simple average from a single fixation time [S23]. The reason for this lies in the intercellular variability of the cell-cycle transition times. It is known that the intrinsic cellular radiosensitivity varies as the cells pass through the cell cycle, and the time of application of the conditioning or challenge doses of radiation may therefore be crucial. In these circumstances, the aberration score will always reflect the average of a mixture of cells having different radiosensitivities, and any shifts in the mixture ratios will influence the aberration yields. A double-labelling technique (Brd-U replication banding), which permits identification of the cell cycle position occupied by each scored metaphase at the time of the conditioning and challenge doses, has recently been described and may help to solve this problem [A10]. Using this technique, it was shown that a conditioning dose of 0.01 Gy from x rays delivered at a rate of 0.05 Gy min⁻¹, followed 6 hours later by a challenge dose of 1.5 Gy at a rate of 0.0044 Gy min⁻¹, resulted in a transient decrease in the frequency of total aberrations at 6 hours, but not at 9 hours, after challenge. Furthermore, when the cohorts at 3, 6 and 9 hours after the challenge dose were combined, there was no evidence of an adaptive response. This preliminary experiment serves to demonstrate the complex nature of the kinetics of cells in stimulated lymphocyte cultures and the possibility that an adaptive response may occur only in a narrow window of cell cycle when cells are particularly radiosensitive.

20. The experiments described above refer to the application of the conditioning dose in the S phase and a challenge dose from x rays in the G₂ phase of the cell cycle. Studies in which phytohaemagglutinin-stimulated lymphocytes were exposed to the conditioning dose at other stages of the cell cycle have been reported, but different laboratories have had different results, which has yet to be explained. An adaptive response was reported when the G₀ or G₁ phase cells were exposed to a conditioning dose and challenged in the late S or early G₂ phase [C8, K4, K5] and when cells were exposed to a conditioning dose in the G₁ phase and challenged in the G₂ phase [S17, W7]. No response was reported by other laboratories if the conditioning dose was given in the G₀ or the G₁ phase and the challenge dose in the G₂ phase [K4, S13]. These results are presented in Table 7.

21. An adaptive response, indicated by a reduced frequency of chromosome aberrations, has also been demonstrated with the conditioning dose given in vivo. Preliminary results of the cytogenetic monitoring of children living in a region of Ukraine contaminated after the Chernobyl accident indicate that the chromosome aberration yield in lymphocytes to a challenge dose in vitro is less than that in control lymphocytes from a challenge dose alone [P10]. This has to be confirmed by further studies, but there is supporting evidence from in vivo studies in the rabbit [L8]. The response of lymphocytes to both conditioning and challenge doses in vitro had been demonstrated earlier [C8]. In the in vivo study, four adult male rabbits were exposed to gamma-radiation at a dose rate of about 6 mGy h⁻¹ for 9 hours each day for 36 days, giving a daily dose of 0.05 Gy. Blood samples were taken before the in vivo irradiations and further samples were collected at intervals of 6, 15, 18, 24, 30 and 36 days, the cumulative doses being 0.3, 0.75, 0.9, 1.2, 1.5
and 1.8 Gy, respectively. Six cultures were established from each blood sample. Two were analysed for baseline chromosome aberrations; two were exposed to a challenge dose of 1.5 Gy from x rays at a dose rate of 0.44 Gy min⁻¹, and phytohaemagglutinin was added to them immediately thereafter. The remaining two cultures were incubated with the addition of phytohaemagglutinin at the start of culture and exposed to a challenge dose of 1.5 Gy from x rays 48 hours later. The results from the analysis of blood lymphocytes from individual rabbits showed the same trend and are presented as average values in Table 8. They show that an adaptive response can be induced in lymphocytes exposed to 0.05 Gy d⁻¹ in vivo when the challenge dose to the phytohaemagglutinin-stimulated cells is given in vitro in either the G0 or the G2 phase of the cell cycle. This could imply that chronic irradiation of circulating blood lymphocytes induces the synthesis of proteins in sufficient amounts to maintain a continuous and effective reservoir of repair enzymes.

2. Clone-forming ability and genomic stability

22. To study the effects of radiation on clone-forming ability and karyotypic abnormalities in human peripheral blood lymphocytes, cells were exposed to 3 Gy from x rays in vitro and either individual T-cell clones or long-term T-cell cultures were established [H16]. The karyotypes were analysed in G-banded chromosome preparations after proliferation for 9-34 days in vitro.

23. T-cell clonal karyotype abnormalities were found in 24 of 37 (65%) irradiated clones and in 2 of 43 (5%) control clones. Balanced reciprocal translocations and deletions were the predominating types of clonal aberrations. Complex aberrations and unstable karyotypes were found in about half of the irradiated clones. Some of the T-cell clones demonstrated sequential change from normal to aberrant karyotype. Other clones seemed to develop multiple, heterogeneous chromosomal aberrations during growth in vitro.

24. T cells irradiated with x rays and grown in long-term culture displayed karyotype abnormalities in 60%-80% of the cells, and the types of aberrations were similar to those found in the individual irradiated T-cell clones. An increasing number of cells with the same abnormal karyotype was observed when the cultivation time was extended, indicating preferential clonal proliferation.

25. These results demonstrate that a surprisingly high proportion of T cells with stable and often complex irradiation-induced chromosome aberrations are able to proliferate and form expanding cell clones in vitro. Furthermore, they indicate that x-irradiation induces latent chromosome damage and genomic instability in human T lymphocytes. What would be interesting would be to repeat this study by giving a conditioning dose of a few tens of milligray before the challenge dose of 3 Gy and observing if any reduction in proliferating T cells with stable aberration occurred.

3. Cell survival and mutation frequency

26. Cell survival and chromosome aberration yield have been measured in phytohaemagglutinin-stimulated lymphocytes exposed to 0.05 Gy from x rays followed by acute exposure to 2 or 4 Gy, both exposures being in the G1 phase [S17]. In studies on six donors, the yields of chromosome exchanges and deletions were found to be less than in cells receiving the challenge dose only. Lymphocytes from only two of the six donors showed an adaptive response expressed as enhanced cell survival with a challenge dose of 2 Gy and none after a challenge dose of 4 Gy. A chromosome adaptive response, therefore, does not necessarily coincide with a cell survival adaptive response. Reductions in the number of cells with several aberrations (multiply aberrant cells) can be the result of a cytogenetic adaptive response, but if the proportion of non-aberrant cells is not increased, then a survival adaptive response will not be seen.

27. In a subsequent study, a lower challenge dose was used. Lymphocytes from the six donors were exposed to 0.05 Gy from x rays, followed by 1 Gy in the G1 phase [S44]. Under these exposure conditions, most of the aberrant cells would be expected to contain only one chromosome aberration after the challenge dose. Cell survival adaptive responses were seen in four of the six donors, but the decrease in the numbers of singly aberrant cells was not in itself sufficient to account for the increase in cell survival. It was proposed, therefore, that some increase in cell survival could have been due to repair of lesions in cells that were at the level of the gene locus, which would not be recognized by the cytological techniques used to identify aberrations.

28. Cell survival has been measured concurrently with the yield of mutations using 6-thioguanine (TG) selection to detect clones mutated at the X-linked hypoxanthine phosphoribosyl transferase (lpr) locus [S18]. Tritiated thymidine was added during the G0 phase, followed by exposure to 1.5 or 3.0 Gy from x rays in the G1 phase. Cell survival was not affected (Table 9), but tritiated thymidine at concentrations of 3.7 and 37 kBq ml⁻¹ in the culture medium produced a significant decrease in the number of mutations induced after the challenge dose from x rays compared with cells receiving the challenge dose only.

29. In support of this observation, the mutation frequency was reduced by 70%, while cell survival was not affected, when lymphoblastoid cells were exposed to 0.02 Gy from x rays, followed by a dose of 4 Gy in the G1 phase [R8]. This decreased mutation frequency was
considered to be the result of an induced repair system, which was shown to be absent from mutant cells deficient in the _hprr_ locus. Lymphocytes exposed in the G1 phase to a conditioning dose of 0.01 Gy from x rays, followed by a challenge dose of 3 Gy from x rays in the G2 phase, also showed a reduced mutation frequency compared with cells exposed to the challenge dose only (Table 10).

30. Reduced mutation frequency was demonstrated with human HL-60 cells exposed to a conditioning dose of 0.01 Gy from 60Co gamma rays at a dose rate of 0.078 Gy min⁻¹ and then exposed 18 hours later to a challenge dose of 2 Gy [Z6]. After irradiation, the cells were cultured for seven days in a non-selective RPM1-1640 medium to allow phenotypic expression of _hprr_ mutants. The frequency of _hprr_ mutations resulting from the dose of 2 Gy was 26.9 x 10⁻⁵. Treatment with the conditioning dose reduced the mutation frequency to 10.7 x 10⁻⁵.

31. Mutant colonies exposed to the challenge dose showed gene deletions and rearrangements in 15 out of 32 colonies (46%). This compared to 12 out of 46 colonies (26%) first exposed to a conditioning dose. Since gene deletions and rearrangements are associated with unrepaird or error-prone DNA double-strand breaks, it could be concluded from this experiment that a DNA double-strand fidelity repair mechanism had been induced.

32. In contrast, the human lymphoblastoid cell line TK6, which is heterozygous for the thymidine kinase gene (TK<sup>[n]</sup>), has been used in studies of mutation induction at two independent genetic loci. One of these is the _hprr_ locus; the other is the autosomal thymidine kinase (TK) locus. The selective agents 6-thioguanine (TG) and trifluorothymidine (TFT) were used to measure mutation at the _hprr_ and TK loci, respectively. Cell survival and mutation rate were measured after preferential exposure to tritiated water, followed by exposure to x rays at the rate of 0.8 Gy min⁻¹ [T1]. The results of this experiment are illustrated in Figure I.

33. The cells were grown in a medium containing 0.74 MBq ml⁻¹ of tritiated water, the tritium irradiating the cells at a dose rate of about 0.05 Gy d⁻¹. During the overall period of incubation in the presence of tritiated water, the cloning efficiency determined after 10, 20 and 30 days of exposure, remained almost constant, and it was comparable to that found for unirradiated cells. After the challenge dose of up to 1.5 Gy from x rays, the survival curves for TK6 cells, pretreated or not with tritiated water for different lengths of time, were also similar, as shown in the upper plot of Figure I. These results showed that with a low-dose-rate, preferential conditioning exposure from incubation in tritiated water, no adaptive effect on cell survival was detectable. Furthermore, treatment with tritiated water had no significant effect on the induction of mutations. When the mutation frequency was plotted as a function of the accumulated dose, regardless of the radiation source and the modalities of treatment, a linear relationship was found, indicating that the mutagenic effects of protracted exposure to tritiated water and acute exposure to x rays were additive, as can be seen from the lower plot of Figure I.

4. Interaction with chemicals

34. A recent review of experiments involving the activation of bacterial oxidative stress genes provides a useful background for understanding adaptive mechanisms in eukaryotic cells [D5]. One of the mechanisms involved in DNA repair after exposure to low-LET radiation is thought to be similar to that operating after exposure to trace amounts of oxidizing radicals. In confirmation of this hypothesis, exposing lymphocytes to low concentrations of hydrogen peroxide, followed by a dose of 1.5 Gy from x rays, was found to induce the adaptive response (Table 11). Conditioning with a chemical and challenge with radiation is termed cross-adaptation.

35. Other studies (see also paragraph 74) have substantiated this finding, in which the adaptive response was shown to occur in donors whose lymphocytes were treated with 25-75 μM of hydrogen peroxide 24 hours before a challenge dose of 1.5 Gy from x rays [C13]. However, when the cells were repeatedly exposed to hydrogen peroxide at intervals of 24, 30 and 36 hours, the adaptive response was not observed. The authors did not give any explanation for this lack of response to repeated doses [W3].

36. A reduction in micronuclei frequency has been demonstrated in lymphocytes conditioned with hydrogen peroxide [D6]. Lymphocytes were exposed to a 30-minute pulse of hydrogen peroxide (25-250 μM) 24 hours after formation of the cultures and to a challenge dose of 1.5 Gy or 3 Gy from x rays 48 hours later.

37. An adaptive response can be induced in the presence of trace amounts of bleomycin, which is known to produce double-strand breaks during the G2 and M phases of the cell cycle [V2, W8]. Thus, when lymphocytes cultured in the presence of low concentrations of bleomycin (0.01-0.1 μg ml⁻¹) for 48 hours were challenged with a high concentration (1.5 μg ml⁻¹) of bleomycin or with 1.5 Gy from x rays, lower than expected frequencies of chromatid and isochromatid breaks were found. This cross-adaptation was not observed if cells were exposed to methylating agents. In fact, radiation and methyl methane sulphonate act synergistically in the same way as a combination of methylating agents (Table 12). Conditioning with interferon (50 IFU ml⁻¹) has been described [M30]. These experiments lend support to the view that cross-adaptation may operate in lymphocytes to reduce the damage caused by some, but not all, DNA-damaging agents.
5. Repair of specific DNA lesions

38. A study to identify the molecular lesions associated with conditioning doses of several mutagens has recently been reported [S19]. The end-points measured in the lymphocytes from two donors included chromatid and chromosome aberrations and sister chromatid exchanges.

39. To measure chromatid aberrations, the cells were exposed to 0.05 Gy from x rays 24 hours after phytohaemagglutinin stimulation (i.e. in the G1 phase) and challenged with 2 Gy from x rays at 48 hours (i.e. in late S/early G2 phase). To measure chromosome aberrations, the cells were exposed to 0.05 Gy from x rays 12 hours after phytohaemagglutinin stimulation and challenged with 2 Gy from x rays at 18 hours (i.e. both exposures in the G1 phase). Cells from one donor showed an adaptive response when challenged in the late S or early G2 phase, in contrast to cells from the other donor, which showed the adaptive response when challenged in the G1 phase.

40. Three drugs were used to induce sister chromatid exchanges: etoposide (VP16), 1,3-bis(2-chloroethyl)1-nitrosourea (BCNU) and cis-diaminedichlorophosphoramide (cis-platin). Etoposide, a topoisomerase II inhibitor, prevents the creation and rescaling of DNA strand breaks, as opposed to the base modifications caused by cis-platin and inter-strand cross-links by BCNU. The repair of DNA damage at specific sites from these drugs is the result of the synthesis of enzymes involved in excision and post-replication repair or the synthesis of damage-recognition proteins (esterases) that prevent cross-linking.

41. Cells were exposed to 0.05 Gy from x rays at 40 hours after phytohaemagglutinin stimulation and exposed to drugs (0.5 μM VP16, 10 μM BCNU or 0.67 μM cis-platin) 6 hours later for 2 hours. At 48 hours, the drugs were washed out and the cultures treated for 4 hours with 30 μM bromodeoxyuridine (BrdUrd). This technique ensured that only the cohort of cells that spent sufficient time in the S phase during the BrdUrd labelling would be scored. Small but statistically significant reductions in sister chromatid exchanges, consistent with an adaptive response, were observed (Table 13). Both donors responded similarly, showing reductions most often for VP16-induced sister chromatid exchanges. Although significant reductions were also observed for chromatid deletions, analysis of the data showed that they occurred independently of those for sister chromatid exchanges. These results are consistent with the view that damage to specific sites in DNA is repairable following a conditioning dose of x rays.

42. No adaptive response was obtained when cells from 10 donors were exposed to mitomycin C, with and without a prior conditioning dose of 0.01 Gy from x rays in the G0 phase [M5]. This may be relevant and in contradiction to the observation that a statistically significant decreased number of sister chromatid exchanges was found in the lymphocytes of workers who had been occupationally exposed to low doses of radiation and whose blood lymphocytes were presumed to be in the G0 phase while they were chronically irradiated [T2].

6. Summary

43. An adaptive response to low-LET radiation exposure has been demonstrated in mitogen-stimulated human lymphocytes when they are acutely exposed to a conditioning dose within the range 0.005-0.2 Gy prior to a challenge dose of a few gray. The response has been expressed as a reduction in the yield in chromatid or chromosome lesions, typically to about one half the yield expected. The adaptive response has been demonstrated when both the conditioning and challenge doses are applied at late stages (S/G2 phases) of the cell cycle. However, there is disagreement as to whether or not the adaptive response occurs if the conditioning dose is applied in the resting or early stages (G0/G1) of the cell cycle. This important point needs to be clarified, since it has implications for the circumstances in which cells are chronically irradiated in vivo.

44. The cellular response is transient, lasting for about three cell cycles in culture. Since radiation-induced double-strand breaks are repaired, this could imply the production of specific repair enzymes in addition to those involved in the process of repair of damage in cells occurring during normal metabolism.

45. There appears to be individual donor variation, with no evidence of an adaptive response in the lymphocytes of some blood samples tested even though the culture procedures are identical to those producing a response. Why this is so is not known. Several explanations have been proposed. One possibility is that the adaptive response requires the maintenance of a narrow range of pH and the presence of specific growth-stimulating factors in the culture medium. Another possibility is that the adaptive response occurs at a precise time in the cell cycle, so that cells outside this phase do not respond. A third possibility is that in vivo factors such as the nutritional status or the immunocompetence of the living organism may influence the cellular response.

B. EFFECTS IN MOUSE CELLS

1. Splenic lymphocytes

46. The results of different studies with mouse lymphocytes have been contradictory. In one experiment, mice of the C57Bl/6 strain received a whole-body dose of 0.05 Gy from gamma rays, at the rate of 1.25 mGy min⁻¹.
on four consecutive days [W9]. Groups of mice were killed at intervals up to 26 days thereafter. Lymphocytes isolated from the spleens of sham-irradiated and irradiated mice were exposed in vitro to UV-radiation to induce unscheduled DNA synthesis or to mitomycin-C to induce sister chromatid exchanges. The results showed a higher rate of unscheduled DNA synthesis and lower sister chromatid exchange frequencies in the irradiated mice than in the sham-irradiated controls. Irradiation in vivo with low doses of gamma rays was consistent with an increase in the rate of DNA repair, which is effective for approximately 12 days. The results support those published by Tuschi et al. [T2, T3] and Liu et al. [L9], who demonstrated that it is possible to induce the adaptive response in vivo.

47. However, the adaptive response was not observed when lymphocytes obtained from the spleens of female mice of the Heiligenberger strain were exposed in vitro, either to 0.05 Gy from x rays at 24 hours or 32 hours after phytohaemagglutinin-stimulation, followed by a challenge dose of 2 Gy at 40 hours; or to 0.1 Gy at 32 hours or 42 hours with the challenge dose at 48 hours [W5, W23]. A reduction in the number of chromosome aberrations as a result of exposure to conditioning doses was seen in the lymphocytes from only 1 of 14 mice tested. Because of the high variability of the radiation-induced break frequencies in the lymphocytes of the different donors, the authors concluded that this one positive result was due to chance and was not a genuine adaptive response.

48. To determine if this lack of an adaptive response was unique to the Heiligenberger strain, spleen lymphocytes were collected from C57Bl/6 female mice in which, as discussed above, adaptive response to UV-radiation-induced, unscheduled DNA synthesis and mitomycin-C-induced sister chromatid exchanges had been observed [W5]. A dose of 0.1 Gy from x rays was given after 32 hours, followed by a challenge dose of 1.5 Gy after 48 hours of culture. Initial results indicated the presence of an adaptive response in some of the C57Bl/6 mice. However, subsequent analysis of the aberration scores of parallel lymphocyte cultures revealed a high intra-individual variability. The authors concluded that the results were a reflection of this variability rather than of any induced adaptive response [W27].

49. Experiments have been reported in which colony-forming units (CFU-S) cells were exposed to low doses of radiation in the range 0.03-0.05 Gy [S20, S32]. The adaptive response was observed from 4 hours until 28 days after each challenge dose and was more pronounced after high-dose-rate exposure. The response could be potentiated by injecting the mice with 50 μg of polynucleotide Poly I-Poly C two days before the challenge dose.

2. Bone marrow cells

50. Male Kunming mice were exposed to a whole-body conditioning dose of 0.1 Gy from x rays, followed 2.5-3 hours later by a challenge dose of 0.75 Gy from x rays [C8, L10]. The combined exposure to the conditioning and challenge doses resulted in a smaller number of chromatid aberrations in bone marrow cells than in cells from animals receiving the challenge dose only. These results are given in Table 14. The whole-body exposure of female C57Bl/6 mice to a conditioning dose of 0.002-0.5 Gy from x rays, followed by a challenge dose of 0.65 Gy within 3 hours, also resulted in an adaptive response at all conditioning doses (Table 14). A similar adaptive response was observed when the animals were exposed to these low conditioning doses and then to a high dose of mitomycin C (0.5-50 mg kg⁻¹) instead of the challenge dose from x rays [Y2].

51. In a sequel to this experiment, mice were exposed to a range of whole-body doses from 60Co gamma-radiation at a rate of 0.09 Gy min⁻¹ and irradiated 3 hours later with a challenge dose of 1.5 Gy from x rays [J2]. Significantly lower chromosome aberration frequencies were observed in bone marrow cells after conditioning doses of 0.05, 0.10 and 0.20 Gy, but not 0.50 Gy, compared with animals receiving the challenge dose only. The protracted whole-body exposure of male mice to 60Co gamma-radiation at the rate of 0.014, 0.025, 0.06 or 0.23 Gy h⁻¹, followed by a challenge dose of 0.9 Gy from x rays within 3 hours after protracted exposure had ceased, also resulted in adaptive responses [Y2].

52. Using a different end-point, male white SHK mice were given whole-body exposures to 137Cs gamma-radiation at the rate of 1.3 mGy h⁻¹ over periods up to 80 days and then exposed to a challenge dose of 1 Gy from x rays within a few hours after the protracted exposure ceased [G2]. The frequency of micronuclei in polychromatic erythrocytes in chronically irradiated mice exposed to the challenge dose was about one third of that observed in mice receiving the challenge dose only. It was also shown that chronic exposure before the challenge dose resulted in a marked decrease in single-strand breaks and an increase in DNA polymerase activity in splenic and liver cells, consistent with the availability of a reservoir of repair enzymes during chronic irradiation.

53. In another experiment, 9-12-week-old male mice of the Swiss albino strain were exposed in vivo to 60Co gamma rays [F23]. The conditioning doses of either 0.025 or 0.05 Gy were given at a dose rate of 1.67 Gy min⁻¹. The challenge dose of 1 Gy was given at 2, 7.5, 13, 18.5 or 24 hours after the conditioning doses. At a time interval of 2 hours, both conditioning doses reduced the frequency of micronuclei in polychromatic erythrocytes and of chromosome aberrations in the bone marrow cells. After exposure to 0.025 Gy, the adaptive response remained for
24 hours. After exposure to 0.05 Gy, however, the adaptive response was not present when the challenge dose was given 13 or more hours later.

54. These experiments indicate that the adaptive response can be induced in bone marrow cells in some strains of mice after acute or chronic exposure to low-LET radiation in vivo, provided that the challenge dose is given within a few hours after the exposure to low doses has ceased. The results contrast, however, with those of Jacobsen-Kram and Williams [J1], who were unable to elicit an adaptive response in the bone marrow cells from their strain of mice irradiated in vivo. However, in the latter experiments, the challenge dose was given 24 hours after the conditioning dose, a time span possibly too long for the DNA repair enzymes to remain effective in rapidly dividing bone marrow cells.

3. Spermatocytes

55. Male Kunming mice were exposed to a whole-body dose of 0.01 Gy from x-rays, followed 2.5-3 hours later with a challenge dose of 0.75 Gy from x-rays [C8]. The number of chromatid aberrations in the spermatocytes of conditioned mice was less than in the spermatocytes of mice receiving the challenge dose only (Table 14).

56. In another experiment involving the whole-body irradiation of male Kunming mice, the adaptive response was shown by reduced chromosome damage and dominant lethal mutations [C17]. A conditioning dose of 0.05-0.2 Gy resulted in a statistically significant reduction (p < 0.01) of chromatid and isochromatid breaks in spermatocytes and in reciprocal translocations in spermatogonia, compared with cells from animals receiving only the 1.5-2 Gy challenge dose.

57. Cross-adaptation has been shown using x-rays and low concentrations of mitomycin C, hydrogen peroxide and cyclophosphamide as the conditioning dose [M27]. Male Kunming mice were exposed to a conditioning dose of 0.05 Gy from x-rays; 3 hours later, 0.1-0.5 mg ml\(^{-1}\) of mitomycin C or 0.1-1 M of hydrogen peroxide was injected intraperitoneally or directly into the testis. Twenty-four hours later, the mice were exposed to a challenge dose of 1.5 Gy from x-rays. The frequency of aberrations in primary spermatocytes was markedly reduced with the use of mitomycin C or hydrogen peroxide. In contrast, cyclophosphamide in the range 0.05-0.5 mg ml\(^{-1}\) acted synergistically with the conditioning dose of x-rays.

4. Mammary carcinoma cells

58. Exposing cultured mouse mammary carcinoma (SR-1) cells to a dose of 0.01 Gy from \(^{60}\)Co gamma rays, followed by a dose of 3 Gy from gamma rays 18-24 hours later, resulted in a decreased frequency of induction of mutations at the hpr locus [Z5]. When cells were exposed to bleomycin (5-10 \(\mu\)g ml\(^{-1}\)) for 12 hours instead of 3 Gy from gamma rays, a similar reduction in mutagenic response was observed. Since bleomycin acts to produce double-strand breaks, it was presumed that the reduction in the frequency of radiation-induced mutations was also attributable to the repair of double-strand breaks.

5. Pre-implantation embryos

59. Mouse embryos of the Heiligenberger strain were exposed to a conditioning dose of 0.05 Gy from x-rays at times corresponding to the late G2/M phase of the four-cell stage or the G1/S phase of the eight-cell stage embryos [M6, W5]. A challenge dose of 1.5 Gy from x-rays was applied 6 hours later, and cells were arrested in metaphase immediately thereafter. The interval of 6 hours between the conditioning and challenge doses was chosen because it was found to be the appropriate time for the expression of the adaptive response in both human lymphocytes and in cultured Chinese hamster fibroblast cells [I5]. The results of these experiments are summarized in Table 15. The yields of chromosomal break frequencies and the percentages of aberrant cells give no indication of an adaptive response compared with cells receiving the challenge dose only.

60. It has been reported that rat mammary gland cells irradiated in vivo may have a higher repair capacity than cells irradiated in vitro, a phenomenon called in situ repair [G4]. To examine the possible influence of in situ repair on the adaptive response in embryos, the conditioning dose was applied in vivo and the embryos were left in situ until shortly before the challenge dose [W5]. One group of embryos given a conditioning dose of 0.05 Gy was irradiated in vivo with 2 Gy from x-rays at 50 hours after conception and isolated 4 hours later. Another group was isolated at 48 hours after conception and irradiated with 2 Gy from x-rays in vitro at 50 hours after conception. Colchicine was added at 55 hours and the metaphases of the embryos in the 8-16-cell stage were harvested at 62 hours after conception. The results of the in situ repair experiments, given in Table 15, indicate that in situ repair was not associated with an adaptive response. They suggest that the mouse embryos are either in situ repair-deficient or that the optimal conditions for the induction of an adaptive response have not been achieved.

61. Wojcik et al. [W5] pointed out that for an adaptive response to occur in pre-implantation mouse embryos, they must be able to perform DNA repair. Unscheduled DNA synthesis does occur in both
pronuclei of the one-cell embryo as well as in cells of the later developmental stages. Until the late two-cell stage, however, there is no gene expression in the embryo, and all proteins required are synthesized constitutively from mRNA inherited from the oocyte [13]. At the late two-cell stage, the embryonic genes are switched on and much of the maternally inherited mRNA is destroyed. There is, however, evidence that despite the transcriptionally active genome, some genes inductive in somatic cells do not respond inductively to the changing environment in embryos of the preblastocyst stages. It is not clear whether induced expression of repair genes in the embryo is necessarily required for an adaptive response to radiation. However, the negative outcome of the above experiment could be due to a general inability of the preblastocyst embryo to adapt to changes in its environment.

62. To investigate the points further, pre-implantation embryos were exposed to a conditioning dose of 0.03-0.1 Gy, with a challenge dose 6-24 hours later [M6]. Table 16 gives the results of an experiment in which two-cell embryos were exposed to 0.05 Gy in the early G2 phase and to 2 Gy when the embryos were in the late G2 phase of the same cycle. None of the endpoints measured indicates a statistically significant effect as a result of the conditioning dose.

63. It is recognized that up to the early two-cell stage, the absence of an adaptive response in early embryonic development could depend on specific traits of this system. Starting with the blastocyst, however, there is no reason why genes coding repair enzymes should not respond to signals calling for additional enzyme synthesis. A similar experiment to the one described for the embryo in the two-cell stage was therefore carried out using blastocysts in which the embryonic genome was active. No statistically significant difference was seen between the effects with and without the conditioning dose [M6].

64. It may be concluded that an adaptive response cannot be induced in pre-implantation embryos, at least with regard to the end-points measured, or that the conditions are entirely different from those determined in other systems, in particular in human lymphocytes.

65. The response of fetal tissue has also been examined. Pregnant Sprague-Dawley-derived rats were exposed to 0.02 Gy from 137Cs gamma-radiation at a rate of 0.4 Gy min⁻¹ at various times on day 15 of gestation, prior to receiving a challenge dose of 0.5 Gy. Fetuses were examined 6 hours and 24 hours after the challenge dose for changes in the developing cerebral cortex [H20]. There was no evidence of a cellular adaptive response under these conditions of exposure, but the authors pointed out that the different conditions of exposure need to be examined before the absence of an adaptive response can be conclusively stated.

C. EFFECTS IN FIBROBLASTS FROM VARIOUS SPECIES

1. Human embryonic and skin fibroblasts

(a) Life-span and mutation frequency

66. The effect on the life-span of human embryo fibroblast cells of chronic exposure to 60Co gamma-radiation delivered at a dose rate of about 0.001 Gy h⁻¹ for 10 hours per day has been investigated [S25]. The average life-span, which was measured by the number of mean population doublings, was 1.2-1.6 times longer in irradiated than in unirradiated cells. The number of chromosomes in the unirradiated cells remained constant throughout their life-span. Conversely, the irradiated cells showed numerical abnormalities with increasing time. These results indicated that the life-span of chronically irradiated cells at low dose rates was prolonged, but that the cells showed chromosomal changes consistent with abnormal phenotypes.

67. In another study [W10, W11] to determine the effect on the growth ability of human embryo fibroblast cells in vitro, the expression of abnormal phenotypes was measured after fractionated low-dose gamma-radiation. Cells were assayed for cell survival by their colony-forming ability, for mutation at the hyp locus and for transformation by foci formation. After a dose of about 2 Gy had been accumulated, the mean population doubling time was 1.3-1.6 times that of the controls. Although transformed foci were not observed until the cells had accumulated about 1 Gy, the numbers of cells with abnormal phenotypes increased thereafter with increasing dose. No cells, however, showed unlimited life-span in vitro.

68. The mechanism responsible for the increased growth potential of embryonic fibroblasts after fractionated low-dose gamma-radiation in vitro remains obscure. Although the data suggest that some damage is repaired during these exposures, it cannot be assumed that all of the damage resulting in the transformation of cells is repaired. The prolonged life-span may allow additional time for the expression of an otherwise unexpressed lesion, perhaps associated with the development of additional karyotypic changes and aneuploidy. This process might be very rare among human diploid fibroblasts grown in vitro [K6, K7] and might be specifically related to the immortalization of human cells. The mechanism leading to the prolongation of their life-span remains to be shown, but calcium ion may act as a signal transducer during cell cycling [I10].
(b) Cell survival and clone-forming ability

69. The human skin fibroblast cell line (AG1522) has been used to determine the effects of low-dose gamma-radiation, followed by a challenge dose of x rays [A2]. Cell survival, colony growth rate and micronuclei formation were measured to assess evidence of adaptive response. Protracted exposure of plateau-phase cells to gamma rays, delivered at a dose rate of 0.003 Gy min\(^{-1}\) over a period of 24 hours, reduced the effects of a challenge dose of 4.25 Gy from x rays given immediately after the chronic exposure. Figure II shows that up to a twofold improvement in cell survival and a twofold reduction in micronuclei formation were observed, compared with the results obtained when cells were exposed to the challenge dose only. Furthermore, after 7 days, the size of colonies from cells surviving the combined exposures was about four times as great as the size of colonies from cells given a challenge dose only.

70. The stimulation of clonogenicity at doses below 0.4 Gy from gamma rays has been observed in cultures of human skin fibroblasts (strain GM 2185). The results, illustrated in Figure III, are compatible with the hypothesis that cells that do not form clones in the absence of radiation are stimulated to do so by low doses of radiation; that is, additional colony-forming cells are recruited from formerly non-clonogenic cells. This enhanced clonogenicity was not observed in fibroblasts (A-T strain GM 2531) in which DNA repair was deficient, although the numbers of non-clonogenic cells are similar to those observed in the normal fibroblast strain. It can be implied from these results that enhanced clonogenicity is dependent on DNA repair competence, but other mechanisms could be involved [G5].

2. Chinese hamster cells

(a) Micronuclei formation frequency

71. Proliferating Chinese hamster cells, cloned from the V79-B310H cell line, were exposed to beta- or gamma-radiation from tritiated thymidine or tritiated water, followed by exposure to 1 Gy from \(^{60}\)Co gamma rays [15, 17, 111]. An adaptive response, expressed as a reduction in micronuclei frequency, was observed. The adaptive response was inhibited by 3-aminobenzamide (3AB) and was not observed after one cell division following the conditioning dose. The optimal range of the conditioning dose was estimated to be between 0.001 and 0.1 Gy on the basis of the amount of tritium incorporated into DNA. When tritiated thymidine was administered at lower or higher concentrations, a reduction in micronuclei induction was not observed.

72. Acute exposure to 0.01 or 0.05 Gy from gamma-radiation also induced an adaptive response to a challenge dose of 1 Gy, but exposure to high-LET radiation did not. This dependency on the type of radiation might reflect the quality and quantity of chromosomal lesions that trigger the adaptive response. The adaptive response did not fully develop until 4 hours after the challenge dose and was not observed if the time interval between the conditioning dose and the challenge dose was extended to 6 hours. The adaptive response can be attributed to the induction of a mechanism that repairs DNA damage.

(b) Cross-adaptation

73. Chinese hamster V79 cells exposed to conditioning doses (0.01-0.05 Gy) from gamma-radiation showed cross-adaptation to challenge doses of UV-B-radiation (97.5-195 J m\(^{-2}\)) and mitomycin C (25-50 \(\mu\)g ml\(^{-1}\)) but not to ethyl methane sulphonate (EMS) (100 \(\mu\)g ml\(^{-1}\)) or cis-platin (1 \(\mu\)g ml\(^{-1}\)), as evidenced by a reduction in the number of sister chromatid exchanges. This could imply that the adaptive response observed after radiation could be coupled to the repair network that cope with chromatin lesions induced by mitomycin C and UV-B [17]. The results observed after exposure to cis-platin were contrary to those observed when human lymphocytes were exposed to this agent.

74. The effects of small amounts of hydrogen peroxide on the killing and mutation of Chinese hamster V79 cells by different agents is supportive of an adaptive response from damage due to oxidative free radicals [G11, S22]. It has been shown that low, non-toxic concentrations (e.g., 0.9 \(\mu\)g ml\(^{-1}\)) of hydrogen peroxide render V79 cells more resistant to subsequent killing by hydrogen peroxide (3-15 \(\mu\)g ml\(^{-1}\)), gamma rays (1-6 Gy) and N-methyl-N-nitro-N-nitrosoguanidine (0.5-2.0 \(\mu\)g ml\(^{-1}\)). However, such pretreatment with hydrogen peroxide increased the mutation yield by N-methyl-N-nitro-N-nitrosoguanidine or gamma rays, suggesting error proneness of the induced repair activity. Cycloheximide or benzamide prevented the induction of repair, and they also suppressed the increase in mutation yield.

75. The treatment of Chinese hamster V79 cells or \(H\), rat hepatoma cells with low concentrations of hydrogen peroxide (1-5 \(\mu\)M) also resulted in an adaptive response, expressed as increased survival when the cells were exposed to high doses of hydrogen peroxide (0.1-1.5 mM) or to a challenge dose from gamma-radiation (up to 8 Gy) [L20]. This adaptive response was observed in both exponentially growing cells and plateau-phase cells, but there was a reduced hprt mutation frequency.

3. Mouse embryo cells

76. C3H10T\(1/2\) plateau-phase mouse embryo cells were conditioned with doses of 0.1, 0.65 or 1.5 Gy from \(^{60}\)Co gamma rays at a rate of 0.0025 Gy min\(^{-1}\). Three and a
half hours later they were exposed to a dose of 4 Gy from gamma rays. The conditioning dose did not affect clonogenic survival, but it led to a reduction in micronucleus frequency in binucleate cells and to a twofold reduction in transformation frequency per viable cell when cells were subsequently exposed to 4 Gy from gamma rays. The data suggest that a conditioning dose of low-LET radiation induces an adaptive response in C3H10T1/2 cells, resulting in enhanced DNA double-strand break repair when the cells are exposed to the challenge dose. This enhanced repair appears to be error-free, since the cells are less susceptible to radiation-induced neoplastic transformation [A17].

4. Derived human epithelial cell line
77. Adaptive response has been described in experiments involving Hela cells [C21]. The cells were exposed to a conditioning dose of 0.03 Gy, followed by a challenge dose of 2 or 3 Gy. A decrease in the number of induced micronuclei occurred within 4 hours of the conditioning dose and lasted for three cell cycles. If the conditioning dose was increased to 0.4 Gy, the adaptive response disappeared and the cells subsequently showed increased radiosensitivity.

D. ADAPTIVE RESPONSE TO CHEMICAL MUTAGENS
78. An adaptive response in human keratinocytes exposed to low doses of the mutagen N-methyl N'-nitro-N-nitrosoguanidine has been described [K8]. Growing and confluent human keratinocytes (HaCaT cell line) were exposed to different concentrations of N-methyl-N'-nitro-N-nitrosoguanidine for one hour, and the number of single-strand DNA breaks was determined by measuring nucleoid sedimentation through neutral sucrose gradients. Strand breaks cause the supercoiled DNA structure of the nucleoids to relax, leading to a reduction in the sedimentation rate. When the growing cells were treated with low doses of N-methyl-N'-nitro-N-nitrosoguanidine, the nucleoids were found to sediment faster than in cells in the confluent phase. Similar shifts have been reported following mitogen activation of human lymphocytes [I4] and mouse splenic lymphocytes [G6]. This effect was attributed to the rejoining of DNA single-strand breaks present in confluent cells.

79. The ADP-ribosylation system of chromatin responds to radiation-induced damage by processing ADP-ribosyl residues through a complex series of synthetic and catabolic reactions. The key component of this multi-enzyme system is poly(ADP-ribose) polymerase, a zinc-containing protein that specifically binds to single- and double-stranded DNA breaks. Binding activates different catalytic reactions that lead to the synthesis of polymers covalently bound to the polymerase [N3]. These polymers then remove histones from the DNA, thereby allowing access to other proteins, e.g. DNA helicase A and topoisomerase I, to encourage DNA excision repair. Inhibitors of poly(ADP-ribose) polymerase suppress the adaptive response in mammalian cells.

80. Further evaluation [B9], however, has shown that differences in the nucleoid sedimentation rate might also be explained by changes in the amount of RNA and proteins, which affect the sedimentation velocity of the nucleoids. To test this hypothesis, keratinocytes were exposed to 0.005 μM of N-methyl-N'-nitro-N-nitrosoguanidine for 1 hour, followed by a challenge dose of 5 μM of N-methyl-N'-nitro-N-nitrosoguanidine 6 hours later. The results can be interpreted as reflecting fewer DNA breaks in the pretreated cells than in cells exposed to the challenge dose only. The presence of 2 mM of 3-aminobenzamide blocked this response. Repair in the presence of low doses of N-methyl-N'-nitro-N-nitrosoguanidine is consistent with an adaptive response of the cells to the mutagen. However, a synergistic rather than an adaptive response was observed in human lymphocytes pretreated with N-methyl-N'-nitro-N-nitrosoguanidine, followed by a challenge treatment with methyl methane sulphonate [W4].

81. Studies with hydrogen peroxide (0.1 μM conditioning dose, 100 μM challenge dose) and bleomycin (0.1 ng ml⁻¹ conditioning dose, 100 ng ml⁻¹ challenge dose) are also consistent with the view that exposure to a low dose of these mutagens results either in an overall decrease in the number of single-strand breaks or changes in the nucleoid cage of the DNA. They provide complementary evidence of an adaptive DNA repair process.

F. SUMMARY
82. The adaptive response has been demonstrated in proliferating cultured lymphocytes and fibroblasts. In addition to a reduction in chromosome aberrations, the response has been measured as a reduction in the expected number of sister chromatid exchanges, of induced micronuclei and of specific locus mutations. An increased survival rate and an increased proliferative capacity have been shown to be associated with increased mutation and transformation frequency in some experiments.

83. Bone marrow cells and spermatocytes from mice exposed in vivo to low doses (0.01-0.2 Gy) from x rays a few hours before challenge doses (0.75-2 Gy) to the cells showed reductions in the number of chromosome aberrations compared to cells exposed to the challenge dose alone. No adaptive response was observed in pre-implantation mouse embryo cells, even though these embryonic cells were tested at a stage of development in which they were considered to be capable of synthesizing their own DNA repair enzymes.
84. There is evidence of cross-adaptation between some toxic chemical agents and low-LET radiation. While it is reasonable to assume that some common repair pathways exist depending on the category of damage (for example, damage caused directly by the ionizing events or indirectly by induced hydroxyl radicals), the relation between random radiation-induced DNA damage and specific chemically induced DNA damage needs to be further resolved.

II. MECHANISMS OF ADAPTIVE RESPONSE

85. Studies of cultures of lymphocytes, bone marrow cells, melanoma cells and fibroblasts have provided insight into some aspects of the mechanisms involved in the adaptive response. These include:

(a) the effects of radiation on the up-regulation of genes and their influence on cell cycle kinetics;
(b) the identification of activated genes and their enzyme products specifically involved in radiation-induced DNA repair;
(c) the relationship between radiation-induced repair genes and those activated by other mutagens;
(d) the ability of cells to remove toxic radicals;
(e) the activation of membrane receptors and the release of growth factors;
(f) the effects of radiation on the proliferative response to mitogens.

Other mechanisms may be involved, such as enhanced immunosurveillance, which is discussed in Chapter III.

A. CELL CYCLE CONTROL

86. Research into the mechanisms involved in cell cycling is advancing rapidly [M23, N1, N2, N5, S28]. The division of a cell into a pair of genetically identical progeny depends on the precise timing of a sequence of events. To divide successfully, the cell must have completed DNA replication and repaired any DNA damage to the extent that allows the formation of chromosomes and their correct segregation.

87. Control of cell cycling is influenced by feedback mechanisms that can detect failure to complete the above processes and arrest progress at various stages in the cycle (e.g. progression from the $G_1$ to the S phase and from the $G_2$ to the M phase). Much of the basic knowledge of the mechanisms has been derived from studies with yeast cells and sea urchin eggs. Elucidating the mechanisms in mammalian cells is proving more complex than doing so in primitive cells. It is clear that the understanding is as yet incomplete, but there is sufficient information to allow speculating on the principles involved.

1. Protein synthesis

88. The key components in mammalian cell cycle control are two classes of protein, the kinases and the cyclins, which are synthesized in a well-conserved sequence. Cell-division-cycle (cdc) kinases are believed to act at several check-points, switching cell cycle progression on and off principally by interacting with cyclins. Activation of the kinase-cyclin complexes requires dephosphorylation of tyrosine phosphate, and possibly of threonine phosphate, on the kinase molecule.

89. Passage from the $G_0$ to the $G_1$ phase is thought to be triggered by low cell population density, cell size, the presence of mitogens and the activation of proto-oncogenes. Progression in the $G_1$ phase seems to be regulated by kinases similar to, but not identical with, cdc2 kinase encoded by the p34$^{cd2}$ gene [F20]. Cyclin D1 accumulates during the $G_1$ phase and associates with many cellular proteins, including cdk2, cdk4 and cdk5 kinases [M24, X1].

90. The $G_1$ to S phase transition appears to involve the complexing of cyclin E with cdk2 kinase and of cyclin D with cdk4 kinase. The cdc2-cyclin E complex may be particularly important for the transition from the $G_1$ to the S phase in human cells [K20].

91. A gene encoding a cyclin-like protein has recently been isolated from rat fibroblasts [T12]. It is referred to as cyclin G. Cyclin G mRNA is induced within 3 hours of growth stimulation, that is, during the transition from the $G_1$ to the S phase, and its level remains elevated with no apparent cell cycle dependency, indicating its close association with growth stimuli but not with the cell cycle. The kinase-cyclin G complex remains inactive until dephosphorylation of the kinase occurs.

92. A cdc2 kinase-cyclin A complex regulates S phase progression [G9, P11]. Essential accessory growth factors during the S phase include platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF-1).

93. On passing through the $G_2$ phase, proteins that regulate the spindle assembly, chromosome condensation and nuclear envelope breakdown are synthesized. If the spindle assembly is not completed, then the cell is arrested in the M phase. A cdc2 kinase-cyclin B complex controls the transition from the $G_2$ to the M phase [G9, P11].

94. Cyclins A and B are rapidly degraded at the end of mitosis. The process induces the synthesis of enzymes that conjugate ubiquitin to the cyclins and thereby targets them for degradation by proteolytic enzymes. Degrading the cyclins negates the activity of the kinase-cyclin complexes, and the cells proceed to interphase.
95. The effect of radiation on the levels of cyclin present at different stages of the cell cycle has been studied. In normal cell cycling of unirradiated cells, the levels of cyclin B protein increase rapidly in the G2 and M phases and decrease at the end of mitosis. If the cells are irradiated in the G2 phase, cyclin B mRNA is readily detectable, although at slightly lower levels than in the unirradiated controls. However, cyclin B protein is markedly decreased in amount, as can be seen in Figure IV, and this is associated with a delay in the completion of the G2 phase, which is associated with diminished levels of activated kinase-cyclin complex. This finding has been confirmed in experiments with Chinese hamster cells [L13].

2. Tumour suppressor genes

96. As the role of tumour suppressor genes was discussed in the UNSCEAR 1993 Report [U1] Annex E, only the points relevant to their role in cell cycling are referred to in this Annex. Current evidence indicates that the Rb tumour suppressor gene product plays multiple roles in the control of the cell cycle, not only in regulating the response to early mitogenic signals to the cell but also in mediating the transitional phases of the cycle itself. The fundamental mechanism by which this is achieved is the repression of cell growth and division by the Rb binding of regulatory nuclear proteins, such as E2F and Myc, which drive proliferative responses. Mutational loss or inactivation of the Rb gene in an appropriate target cell may therefore be viewed as a principal means of relaxing these controls.

97. The phosphoprotein product of the p53 tumour suppressor gene is also suspected of playing a role in cell cycle regulation [L22]. It is thought to function as an inhibitor of cell replication by delaying entry into the S phase of the cell cycle through influencing the assembly of the late G1 protein complexes that initiate DNA replication. The inhibition of DNA synthesis is therefore an active physiological process, and loss of the p53 gene results in loss of this control.

98. Another possible mechanism of action is that p53 protein, by virtue of its DNA-binding properties, may act as a transcriptional factor influencing critical gene expression controlling a cyclin-dependent protein kinase inhibitor (CKI). This is a p21 protein that can bind to and inhibit a wide variety of cyclin-dependent kinases [N5]. A simple hypothesis for cell cycle progression has been proposed: cyclin-dependent protein kinases build up at the G1 phase and the G2/M transition owing to the presence of cyclin-dependent protein kinase inhibitors. Surplus cyclins then trigger the inactivation of cyclin-dependent protein kinase inhibitors, and the cell proceeds through the cycle. Inducible DNA damage may also cause the build-up of cyclin-dependent protein kinase inhibitors, which may be reversible or irreversible.

99. Proto-oncogenes, originally isolated as functional genes supporting the proliferation of tumour cells, encode proteins that are involved in normal cellular proliferation. Some of these proto-oncogenes are therefore concerned with cell cycling. They are involved in signal transduction from the cell surface to the nucleus, thereby integrating growth signals so as to increase the biosynthesis of DNA. These oncogenes include growth factors (e.g. c-sis encoding platelet growth factor), membrane binding receptors (e.g. c-fms encoding macrophage-colony stimulating factor receptor), signal mediators by subsequent phosphorylation reaction (e.g. c-raf encoding protein, which can be phosphorylated during signal transduction), transcriptional activators (e.g. c-jun and c-fos encoding AP-1 transcriptional activator protein) and replication-related proteins (e.g. c-myc encoding nuclear protein). Some oncogenes possess DNA-binding activity after phosphorylation.

100. As examples, the transition from the G0 to the G1 phase has been associated with the increased expression of c-fos, c-jun and c-myc and EGR-1 proto-oncogenes that become activated within minutes of a growth stimulus. All of the products are directly bound with DNA to activate transcription of the many genes necessary for entry into the growth cycle. The c-fos gene transiently expresses prior to differentiation in a wide variety of premature blood cells. After differentiation, several types of cell further express different oncogenes, such as src [G7], c-sis [P5] and c-fms [S29], depending on the type of cell. Progression from the G1 to the S phase is thought to involve the up-regulation of the ras family genes.

B. GENE ACTIVATION

101. The disruption of DNA structure is a consequence of exposure to many physical, chemical and biological toxins. To a lesser degree, as described earlier, it is also a consequence of the changes that can take place during normal metabolism. It is not surprising, therefore, that cells have evolved a complex system of defence against circumstances that might irreversibly damage them. A major role is played by the activation of genes and gene products that initiate DNA repair processes.

102. Evidence for the activation of genes associated with growth control and DNA repair came initially from studies in prokaryotes. These studies provided an insight into the mechanisms operating in eukaryotic cells. For example, genotoxic stress in the bacterium Escherichia coli induces responses in which regulatory genes (regulons) participate. These include the lexA/recA-mediated SOS response [L3, M1, P9, R1, S4, W2, W24], the adaptive response to alkylating agents [B1, K1, K2, K3, M2, O1, S4, S5, S6], the oxyR-mediated hydrogen peroxide response, the sodK3-mediated superoxide response, and the activation of heat shock protein (HSP) genes [D1, D5, F18, G1, S7, W8, W24, Z4].
103. The response of mammalian cells to mutagens, including radiation, is complex, but it is known that many of the genes involved in normal cell cycling are activated. These include genes responsible for growth stimulation, growth control and differentiation [R9]. Genes associated with growth control and activated by radiation exposure were discussed in a recent review article [F19]. Similar types of genes are activated by some alkylating agents and hydrogen peroxide.

1. Cell growth arrest

104. An immediate reaction in proliferating cells exposed to a mutagen is delayed progression through cell division, and a number of genes have been identified that inhibit cell cycle kinetics, among them the growth arrest and DNA-damage-inducible (DDI) genes [L3]. Delayed progression through cell division is accompanied by an increase in the rate of transcription of genes that encode for the production of enzymes to repair the DNA damage caused by the mutagen [W1]. The different types of repair enzymes produced in response to different types of genotoxic stress are probably interrelated in the sense that they are the products of similar regulons [S3].

105. Cell cycle delay is a primary response to DNA damage that represents active processes mediated by certain genes, such as those involved in the expression of the cyclins, p53 tumour suppressor gene, ras oncogenes, and the gadd (growth arrest and DNA-damage), gas (growth-arrest-specific), spr (small proline-rich), MyD (myeloid differentiation) and c/EBP growth arrest genes [F19]. Genotoxic stress has the puzzling effect of conditioning genes associated with both growth stimulatory and inhibitory responses, but the main effect is inhibitory. Many transcription factors and genes activated soon after exposure to radiation are associated with both responses. Many of the genes involved in signal transduction have been implicated in both the initiation and progression stages of carcinogenesis, and the same genes are often induced by tumour promoters and DNA-damaging agents [C15, D5].

106. A summary of the DNA damage-induced (DDI) genes found by various investigators to be induced within a few hours of exposure is given in Table 17. The complex multi-gene reaction after irradiation makes it difficult to characterize the molecular mechanisms of any particular group of genes. However, most of the DDI genes listed in Table 17 are probably involved immediately after DNA damage.

107. Experiments with cultured normal bone marrow progenitor cells and with myeloblastic (ML-1) leukaemic cells have shown that the levels of p53 tumour suppressor gene protein transiently increase while the rate of DNA synthesis decreases after DNA damage, apparently occurring via a post-transcriptional mechanism [K21]. Since cells with wild-type (wt) p53 genes exhibited transient arrest in both the G1 and the G2 phases after gamma-irradiation, while cells with absent or mutant p53 genes arrested only in the G1 phase. It was concluded that wt p53 genes played a role in G1 phase arrest.

108. This observation was further supported by experiments showing that the transfection of wt p53 genes into malignant cells lacking endogenous p53 genes partially restored the G1 phase arrest after gamma-irradiation and that overexpression of a transfected mutant p53 gene in tumour cells with wild-type endogenous p53 genes abrogated the G1 phase arrest after irradiation [K22].

109. Because the tumour cell lines used for the transfection experiments had multiple genetic abnormalities, the experiments were repeated in irradiated normal murine embryonic fibroblasts in which the p53 genes had been disrupted by homologous recombination [K23]. Under these conditions, the loss of both p53 alleles in otherwise normal fibroblasts led to the loss of G1 phase arrest.

110. Further studies showed that irradiated cells from patients suffering from ataxia-telangiectasia were unable to induce the gadd45 gene. Finally it was shown that wild-type but not mutant p53 gene products bind strongly to a conserved element in the gadd45 gene. It was concluded that in normal mammalian cells, p53 and gadd45 genes participate in a signal transduction pathway that controls cell cycle arrest in the G1 phase following DNA damage and that this check-point pathway is defective in ataxia-telangiectasia patients.

111. The effect of radiation on the expression of two DNA-damage-induced genes, designated gadd45 and gadd153, has been examined in cultured human lymphoblasts [P6]. These genes had previously been shown to be strongly induced by UV-radiation and alkylating agents in human and hamster cells. It was found that the gadd45 gene, but not the gadd153 gene, was also strongly induced by x rays. The level of gadd45 mRNA increased rapidly after x-ray exposure at doses as low as 2 Gy (Figure V). After 20 Gy, gadd45 induction, as measured by increased amounts of mRNA, was similar to that produced by the most effective dose of the alkylating agent methyl methane sulphonate. No induction was seen after treatment with 12-0-tetra-decanoylphorbol-13-acetate, a known activator of protein kinase C. Therefore, gadd45 represents an x-ray-responsive gene whose induction is not mediated by protein kinase C. However, induction was blocked by the protein kinase inhibitor H7, so that induction is likely to be mediated by some other kinase.

2. Radiation-induced gene expression

112. The effects on gene expression of low doses of low- and high-LET radiations have been studied in cultures of
Syrian hamster embryo (SHE) cells [W16]. These fibroblasts are normal diploid cells that can be transformed into neoplastic cells by radiation. Genes coding virus-like 30 S elements, c-fos and β-protein kinase C have been shown to be activated by exposure to x rays (0.75 Gy) or gamma rays (0.9 Gy) but not by exposure to fission neutrons. Further studies [W17] have revealed that the induction of c-fos mRNA occurred within 3 hours of exposure, and a protein-binding site has been identified that mediates transcriptional response of the c-fos gene to serum factors [T5].

113. Of particular interest was the response to radiation of members of the protein kinase C (PKC) gene family, which has been shown to play an important role in tumour promotion and in the regulation of cell growth. The results of these experiments showed that exposure to gamma rays can induce increased expression of PKC mRNA within 1 hour of radiation exposure (Table 18). However, PKC inhibitors prevented the expression of PKC in Chinese hamster V79 cells [111]. Dose effects were evident, with increased accumulation of PKC mRNA at higher doses. Levels of expression of PKC mRNA were increased sixfold over irradiated controls after exposure to 0.75 Gy from x rays (Figure VI).

114. The induction of PKC mRNA occurred at a time when total cellular transcription was reduced following irradiation. The activation of PKC directly stimulates transcription of proto-oncogenes c-fos and c-jun, which are typical early immediate genes. Cellular levels of c-fos and c-jun mRNA also increase transiently after irradiation [I9, S48], and it has been reported that these oncogenes are induced via the activation of PKC after irradiation [H12]. These results suggest that supplementation of PKC after activation of the PKC gene is necessary for prolonged expression of fos/jun genes, since depletion of PKC and down-regulation of fos/jun mRNA occur after their activation. On the other hand, several cytokine genes such as interleukin (IL-β) and tumour necrosis factor (TNF-α) are found to be continuously expressed by irradiation (Table 17). IL-β is known as a radioprotector because the survival rate after a sublethal dose of radiation is increased by the administration of IL-β [N6]. Since there is a potential AP-1 (transcriptional activated protein, complex of fos and jun protein) binding site at the 5-upstream region of these genes, it is considered that the genes are continuously stimulated by regulators containing the products of the early immediate genes as a later response against radiation damage at a whole-body level [W25]. However, transient expression of the IL-β gene is also observed together with transient expression of fos/jun early immediate genes within 1 hour of irradiation [19]. This suggests the activation of early protective mechanisms as a response to whole-body irradiation.

115. The effects of neutrons and gamma rays on the expression of genes encoding the nucleus-associated H4-histone, c-jun, c-myc, Rb and p53 proteins have also been reported [W18]. Syrian hamster embryo cells were irradiated at various doses and dose rates. After incubation of the cell cultures for 1 hour following radiation exposure, the induction of transcripts for c-jun and H4-histone was shown to occur following gamma-ray exposure (Table 19) but not following neutron exposure. The expression of p53 protein was unaffected by either gamma-ray or neutron exposure. The increase in the relative amounts of Rb mRNA was marginal, and the expression of c-myc mRNA was repressed following exposure to gamma rays and was unaffected following exposure to neutrons.

116. These experiments provide support for the hypothesis that radiation induces different cellular responses to radiation-induced damage, be it DNA damage, oxidative damage, protein denaturation or some other intracellular event. Recent experiments implicating oxidative damage as the inducing agent for c-fos, c-jun, c-myc and other genes induced following DNA damage would suggest that gamma-ray induction of these genes may involve oxidative damage as the modulating agent.

117. The activation of oncogenes, including c-raf, c-myc, v-src, Ki-ras, c-H-ras, v-H-ras, N-ras, v-k-ras and v-fms genes, has been correlated with increased clonogenic survival over the dose range 1-6 Gy [M25, S45]. The adaptive response appeared to be a specific consequence of the ras gene mutation rather than transformation, since revertant cells that contained functional ras genes retained their clonogenic survival properties.

118. The effect of oncogene expression on the sensitivity to gamma-radiation of the haematopoietic (32DC13) cell line has been measured [F21]. It was shown that these haematopoietic progenitor cells transfected with the oncogenes v-erb-B, v- abl or v-src also showed increased clonogenic survival when exposed at dose rates of 0.05 Gy min⁻¹ over the dose range 1-10 Gy. Exposure of NIH3T3 fibroblast cells transfected with the oncogenes v- abl, v-fms or H-ras also showed increased clonogenic survival.

119. In a more recent experiment, rat embryo cells from the Fischer strain of rats and derived transfectants containing the Ha-ras oncogenes were irradiated with 60Co gamma rays at dose rates between 0.018 and 0.72 Gy min⁻¹ [O6]. The oncogene-containing cells exhibited higher survival levels at all doses compared with the non-transfected cells.

120. In contrast, the measurement of colony-forming ability following exposure to gamma-radiation was made on transformed human embryo retinoblast cell lines containing mutant ras genes [G10]. No correlation was found between transformation with activated ras, adenovirus or SV-40 genes and increased radiation-
resistance. Nor was there any correlation between clonogenic survival and the level of expression of ras21, but two of the three ras transformants that were least sensitive to gamma radiation were from cell lines expressing the highest levels of ras21 polypeptide, which plays a pivotal role in signal transduction and possibly DNA repair. Notwithstanding the negative findings in some laboratories, it is generally accepted that radiation-induced oncogene activation can induce increased cell survival in some circumstances [W25].

121. A human XRCC1 (x-ray repair cross complementing) gene has been isolated that affects the sensitivity of cells to radiation [C16, T13]. The Chinese hamster ovary cell mutant, EM9, exhibits extraordinarily high sister chromatid exchanges and is unable to effectively repair DNA breaks caused by radiation and certain alkylating agents. Introduction of the human XRCC1 gene corrects the EM9 DNA-repair defect and is the first human gene to be cloned that has an established role in DNA strand-break repair. The gene is 33 kb in length and encodes a 2.2 kb transcript and a corresponding putative protein containing 633 amino acids. Constructs in which the open reading frame of the XRCC1 gene were transcribed from the SV40 promoter, or the genomic promoter native to XRCC1 gene, were compared with regard to their ability to correct the sister chromatid exchange defect in the EM9 mutant. These transfectants displayed significantly fewer sister chromatid exchanges than other transfectants. The results suggest that overexpression of the minigene from the SV40 promoter may increase the repair capacity of EM9 mutant cells relative to that of wild-type cells.

3. Induced protein products and DNA repair

(a) Human melanoma cells

122. Induced gene products synthesized in response to low doses of radiation in human melanoma (U1-Mel strain) cells and in a variety of other human normal and cancer-prone cells have been identified using two-dimensional gel electrophoresis [B12]. U1-Mel cells were chosen since they have a high capacity for potentially lethal damage repair. Eight proteins were induced by radiation, and two proteins were repressed. They were not found after heat shock treatment or exposure to UV-radiation or certain alkylating agents. The expression of one protein termed XIP269 (to indicate an x-ray-induced protein of approximately 269 kDa) at a dose of 0.05 Gy correlated very well with potentially lethal damage repair capacity. This protein was found to be down-regulated by exposure to caffeine or cycloheximide under conditions in which both potentially lethal damage repair and subsequent adaptive responses, expressed as cell survival, were prevented [H3].

123. In addition to characterizing x-ray induced proteins, the levels of x-ray-inducible genes have been measured in cDNA clones isolated by differential hybridization. Some of these genes were increased to over 20 times the background levels by as little as 0.05–0.2 Gy; four have been identified as T-diphosphorase, tissue-type plasminogen activator [B13, B14, H3], thymidine kinase and the proto-oncogene c-fps/er.

124. The first phase of potentially lethal damage repair occurs very quickly (2–20 minutes), presumably to increase the chances of survival of irradiated cells. It is associated with a rapid rescaling of single- and, at a later stage, double-stranded DNA lesions that are either created initially by x rays or produced as a result of the repair of various types of base damage. The second slower phase of potentially lethal damage repair proceeds over a period of a few hours following irradiation, during which attempts are made to repair the remaining double-stranded DNA breaks. This second phase of repair closely corresponds to the restructuring of gross chromosomal damage and can be partially blocked in some human cells by inhibiting protein synthesis [Y3]. The rapid repair of potentially lethal damage may be due to the immediate availability of constitutively synthesized repair enzymes such as DNA ligases, topoisomerases or polymerases [B15]. In contrast, it is proposed that the slow phase of potentially lethal damage repair requires the induction on demand of specific genes and gene products. These slow-phase, potentially lethal damage repair responses may be further enhanced if the genes are stimulated with low doses of radiation before a high challenge dose is given.

125. In a further experiment, confluence-arrested human normal (GM2936B and GM2907A) and neoplastic (U1-Mel, Hep-2 and HTB-152) cells were tested for evidence of adaptive survival recovery responses [B26, M9]. Cells were exposed to 0.05 Gy each day for four days at a rate of 1.13 Gy min⁻¹ and then challenged with a dose of x rays giving a 20% cell survival. Only Hep-2 and U1-Mel cells pretreated with 0.05 Gy showed an improvement in survival after 4.5 Gy, compared with untreated cells. Two genes, XIP5 (human growth hormone-related) and a gene transcript related to XIP12 (human angiogenin-related), showed increased expression over time in these cells. Levels of cyclin A and, to a lesser extent, cyclin B increased in the pretreated cells only after the high challenge dose and were not expressed during exposure to the conditioning dose or in cells receiving only the challenge dose. A slight increase in glutathione S transferase mRNA was noted after the primary dose, but p53 suppressor gene and 10 XIP genes were not activated.

126. Under the conditions of this experiment, U1-Mel cells did not progress into the S phase as measured by the uptake of tritiated thymidine into DNA. The induction of cyclin A under these conditions may thus indicate an involvement of cyclin A in specifically stimulating DNA
repair. Based upon these preliminary data, a model has been proposed in which an adaptive response to low doses of radiation may be achieved in mammalian cells [M9]. Initially cells are assumed to be in the G0 phase or at some point in the G1 phase. Upon repeated exposure to low doses of radiation, for example 0.05 Gy, cells progress to and pause at or near the G2 phase or the beginning of the S phase of the cell cycle. Gene transcripts that build up slowly in response to conditioning doses then produce proteins (e.g. cyclin A) that regulate or control the transcripts that appear following a challenge dose. At that point, cells are poised to stimulate various DNA repair systems that are not inducible in the initial resting state.

127. In another experiment, human melanoma (G361 strain) cells were exposed to gamma radiation [O4]. Eleven induced proteins were extracted from cells with molecular weights between 43 and 98 kDa, while in P39 strain cells, 21 induced proteins were extracted after exposing the cells to 3 Gy. Their molecular weights ranged from 32 to 98 kDa, and four of these, with molecular weights of 57, 58, 77 and 88 kDa, were considered to be specific DNA repair enzymes. These proteins are of a lower molecular weight than those isolated and characterized by Boothman et al. [B12].

(b) **Human lymphocytes**

128. Using two-dimensional gel electrophoresis, a specific group of proteins was detected from human lymphocytes exposed to a conditioning dose of 0.01 Gy from x rays [W6, W8]. Cellular extracts from unirradiated lymphocytes and from other cell types were separated by electrophoresis and then exposed to a mixture of 35S-labelled nick-translated and non-radioactive plasmid pCH110 on nitrocellulose membranes. Several bands that bind to the nick-translated DNA were detected, the protein binding occurring as early as 1 hour after irradiation and reaching its maximum by 6 hours. This binding was diminished by a prior proteinase K treatment of the extracts, indicating that the bands are related to proteins present in the extracts. The cellular extracts contained three proteins (of molecular weights 105 kDa, 35 kDa and 14-18 kDa) that reproducibly bound to the labelled DNA. The binding of the DNA probe to the 30-35 kDa and 14-18 kDa bands was twice as great as that found in unirradiated cells, and the 35 kDa band regularly separated into two bands.

129. The 30-35 kDa band proteins have been substantially purified by affinity chromatography. If enough of the proteins can be obtained, it should be possible to see if their introduction into cells will lead to a reduction in the yield of chromosomal aberrations induced by a dose of 1.5 Gy from x rays, even though the cells are not pre-exposed to the conditioning dose of 0.01 Gy. An alternative approach could be to introduce engineered genes into cells and look for the adaptive response. It is likely that the binding proteins are single-chain molecules and that the binding site does not require a conformation dictated by internal disulfides; and it is unlikely that the 14-18 kDa band protein is a subunit of the larger 34 kDa band protein. Further studies should confirm this.

130. The existence of a specific DNA-binding protein in the nuclei of human lymphoblast cells exposed to radiation, which was not detected in nuclear extracts from unperturbed cells, has been reported [S33]. The effects of this binding protein were shown to be dose-dependent and transient, reaching a maximum 1 hour after irradiation and disappearing from the nuclei by 9 hours. The protein was induced in cells by a mechanism not requiring de novo protein synthesis, and the response was specific for radiation and radiomimetic agents; neither UV-radiation nor heat shock induced a response. The DNA-binding protein was present in the cytoplasm of unirradiated cells, apparently being translocated to the nucleus only after radiation exposure. Analysis demonstrated that the nuclear and cytoplasmic proteins were approximately the same size, that is, 43 kDa.

131. Similar experiments with irradiated lymphocytes from humans, mice and rabbits have been reported [L28]. Human lymphocytes in vitro and mice in vivo were irradiated with 200 kV x rays at a rate of 0.0125 Gy min⁻¹. Rabbits were exposed in vivo to 60Co gamma-radiation at a dose rate of 0.0056 Gy h⁻¹ for 9 hours, resulting in an accumulated dose of 0.05 Gy. Extracts of cells or separated cytosolic and nuclear fractions were subjected to two-dimensional electrophoresis. Four protein spots not present in the unirradiated cells appeared in the extracts of human lymphocytes 4 hours after in vitro exposure to 0.05 Gy, with molecular weights of 25, 167, 168 and 174 kDa. Nine spots were detected in the cytosolic extract from mouse lymphocytes 4 hours after in vivo exposure to 0.075 Gy with molecular weights of 51, 69-70, 145 and 160-179 kDa, and four spots were found in the nuclear extract with molecular weights of 70, 90, 230 and 247 kDa. Five spots were detected in the extract from rabbit lymphocytes with molecular weights of 105, 135, 138, 145 and 174 kDa. When compared to the protein spots identified after exposure of cells to mitomycin C and heat (41°C), it was found that the proteins induced by these treatments showed many aspects in common, although there were some differences in their electrophoretic mobility.

132. Crude extracts of splenic lymphocytes from irradiated and sham-irradiated mice were subjected to gel filtration with sephadex G-100 and aliquots tested for biological activity. Both stimulatory and suppressive effects were noted when separate fractions were added to normal splenocytes exposed to concanavalin A, the stimulatory effect of one protein fraction being more marked in the eluted fraction from the irradiated mice. These various proteins were found to have molecular
weights below 100 kDa. When the fraction with stimulatory effects was added in 10 ng amounts to lymphocyte cultures, it was found that the addition 42 hours after phytohaemagglutinin stimulation reduced the frequency of chromatid and isochromatid breaks produced by a dose of 1.5 Gy from x rays to a magnitude similar to that observed when a conditioning dose of 0.05 Gy from x rays was given 42 hours after phytohaemagglutinin stimulation.

4. Stress-response proteins

133. There are several examples of genotoxic stresses influencing the expression of proto-oncogenes and their products. Temporary hypoxia or glucose deprivation, for example, induces the expression of several proteins. The increased expression of intracellular proteins termed "oxygen regulated proteins" in Chinese hamster ovary cells has been described [W19]. These proteins are different from the heat shock proteins, but two with molecular weights of 80 kDa and 100 kDa appear to be identical to two proteins that are induced by glucose deficiency [S31].

134. Haemoxigenase is a protein associated with oxidative stress. Increased levels of this 33 kDa protein transcript have been observed in human skin fibroblasts and can be induced by treatment with UV-A-radiation, hydrogen peroxide and sodium arsenite [K9], heat shock [S8] and temporary hypoxia.

135. Changes in expression of protein kinase C can follow exposure to physical or chemical agents. Protein kinase C is often up-regulated in proliferative cells compared with quiescent cells in these circumstances. Moreover, the intracellular distribution of protein kinase C in cells is also affected [A3]. Consistent with this, low doses of UV-A-radiation have been shown to increase protein kinase C activity in cultured mammalian fibroblasts and also to inhibit EGF-binding [M10].

136. While some degree of homology between the effects of various stresses has been reported, there are clear differences. The expression of the c-fos and c-jun genes, both involved in the regulatory mechanisms of cell progression, are increased by heat shock [B16] and radiation [W16]. However, hypoxia followed by re-oxygenation has been shown to elevate only c-fos mRNA. Radiation causes up-regulation of the gadd45 gene, while both UV-radiation and alkylation agents up-regulate mRNA for gadd45 and gadd153. Up-regulation of the β-polymerase gene is an example of chemical agents that cause DNA damage affecting transcription, whereas X-radiation and UV, both potent agents for DNA damage, have no effect [F6].

137. The possibility that low doses of radiation induce an adaptive increase in the antioxidant defence mechanism has also been considered [N2]. The expression of stress proteins was given special attention, in view of the finding that these proteins are present in monocytes whose antioxidant activities have been elevated [C9, P7]. A similar response has been observed in mitogen-stimulated lymphocytes [F7, H4]. Male C57Bl/6 mice, six weeks of age, were exposed to a dose rate of 0.04 Gy d⁻¹ from x rays on five consecutive days each week for four weeks [M19]. Three days after the last exposure, their spleens were assessed for the constitutive and mitogen-stimulated levels of heat shock protein (HSP) 70 mRNA and protein. Glyceraldehyde-3-phosphate dehydrogenase (GAPD), a housekeeping gene, was used as a reference. The results indicate that low doses augment the constitutive levels of HSP70 mRNA and HSP70 protein (Table 20). Thus, the magnitude of the proliferative response of splenocytes to T-cell mitogen stimulation can be directly related to the constitutive and mitogen-stimulated levels of HSP70 mRNA and protein. These results are consistent with the view that splenocytes need to accumulate some minimal constitutive level of HSP70 protein before they can undergo an augmented proliferative response to mitogenic stimulation and that T cells adapt to low doses by augmenting their HSP70 gene expression.

138. It was shown subsequently that the chronic irradiation of mice increases the expression of HSP70 genes in tissues [M11]. The mice were irradiated at a dose rate of 0.03 Gy d⁻¹, and the levels of HSP70 genes were analysed. Increased but transient expression of HSP70 in lung, spleen and intestinal cells was observed, commencing on day 5 of irradiation. In an extension of this experiment, the effect of dose rate was examined. The data indicated that chronic irradiation within the range 0.03-0.06 Gy d⁻¹ can activate the transcription of HSP70 genes and their respective protein products. In this respect, the results were consistent with those of Nogami et al. [N4], who observed the induction of HSP70 protein in resting splenic T cells after chronic irradiation at a dose rate of 0.04 Gy d⁻¹, but not at rates above 0.1 Gy d⁻¹ with a total dose not exceeding 0.2 Gy. However, increases in HSP70 protein were not seen in Chinese hamster ovary cells [A14] unless the dose was in the region of 400 Gy [S46].

139. In support of a common mechanism, the effects of viral and activated cellular oncogenes on the sensitivity of Syrian hamster (Osaka-Kanazawa) cells exposed to gamma-radiation, UV-radiation and heat shock have recently been described [S41]. Greater tolerance to gamma-radiation was conferred by the introduction of v-mos and c-cot genes, which encode for serine/threonine kinase. Cells transfected with v-mos and c-cot genes were also more tolerant to UV-radiation and heat shock. Of the activated ras genes, the N-ras gene developed a cell phenotype resistant to UV-radiation and gamma-radiation. The Ha-ras gene produced a cell type resistant to UV-radiation and heat shock, while introduction of the Ki-ras gene did not affect sensitivity. The v-erb B gene was
found to be involved in the development of resistance to heat shock. Transfection with neo, c-myc and v-fgr genes had little or no effect on survival. The karyotypes of the original cell type and the oncogene-containing cells were compared, and no alterations were seen in the cells carrying the foreign genes. These results suggest that activation of serine/threonine kinase may be involved in common processes occurring after gamma-radiation, UV-radiation and heat shock treatment, and that each oncogene may have a different effect on the development of a resistant phenotype. However, cDNA clones for a variety of DNA-damage-inducible (DDI) transcripts have been isolated that may represent more specific responses to DNA damage [F17].

C. OTHER MECHANISMS

1. Radical detoxification

140. In addition to cell cycle arrest and induced genes and gene products to initiate DNA repair, an alternative mechanism has been proposed to explain the adaptive response [F8, F9, F10, F11, F12, F13, F14, F15, F16, M12, Z1]. It relates to the ability of cells to remove toxic radicals. Radicals are known to be generated in small amounts and detoxified during normal metabolism. The process of detoxification involves the mobilization of enzymes, such as catalase, peroxidase, superoxide dismutase, from the cytosol [S34]. Membrane-bound vitamin E is thought to scavenge the radicals as they are formed; the latter are then detoxified by the cytosolic enzymes. The purpose of this radical detoxification system is to maintain the structural and functional integrity of the cell by preventing damage to cellular constituents such as membrane-bound DNA [S35].

141. Evidence in support of this hypothesis is based on the results of a series of experiments in which male Wistar rats were exposed to x-rays within the dose range 0.05-0.5 Gy. Levels of superoxide dismutase were found to be increased in spleen, thymus and bone marrow 4 hours later, while lipid peroxides were decreased [Y4].

142. It has been reported that the concentration of serum thymidine kinase increases after acute exposure to radiation [H5]. This was associated with a decrease in the concentration of the enzyme in bone marrow cells and a delay in the incorporation of the radioactive thymidine analogue, 5-ido-2-deoxyuridine (525IUDr), into DNA in these cells. The function of thymidine kinase is to prepare extracellular thymidine for its incorporation into DNA. Measuring changes in thymidine kinase activity has proved to be a convenient probe for studying changes in radical detoxification.

143. In one procedure of this kind, female (NMRI strain) mice received whole-body 137Cs gamma-irradiation, and the relative activity of thymidine kinase was measured in bone marrow cells after one or two exposures, each of 0.01 Gy. A dose of 0.01 Gy produced a reduction in enzyme activity that reached a minimum about 4 hours after the irradiation, with full recovery after 6-8 hours. If a second exposure to 0.01 Gy was given within 30 minutes of the first exposure, the decrease in enzyme activity was accelerated, as was the rate of recovery. If a second exposure to 0.01 Gy was given 4 hours after the first, there was no change in enzyme activity, and a second exposure 12 hours after the first resulted in a reduction of enzyme activity similar to that observed after a single dose of 0.01 Gy [F8].

144. These reductions in thymidine kinase activity closely followed the decreased rate of uptake of 125IUDr into bone marrow DNA, and there was a simultaneous increase in the radical scavenger glutathione. These responses were absent in cells exposed to a strong magnetic field, which transiently alters lipid membrane structure. A deficiency of vitamin E, which is known to be a radical scavenger, also prevented the response.

145. The effect of low-dose radiation on 125IUDr incorporation has also been measured in mouse intestines, spleen and thymus [M20]. Young female BALB/c mice were irradiated within the range 0.05-0.23 Gy with x-rays at a dose rate of 0.05-0.2 Gy min-1, followed at various times thereafter by an injection of 125IUDr. The incorporation of 125IUDr was decreased for several hours after a single exposure to x-radiation in a dose-dependent manner in spleen and thymus. At 4 hours after irradiation, for example, the decrease relative to controls was 79% in spleen and 86% in thymus. If the first irradiation was followed 4 hours later by a second irradiation (0.05 Gy or 0.1 Gy, for example) the second irradiation did not enhance the inhibitory effect of the first exposure, thus confirming the observations of Feinendegen et al. [F8].

146. To put these studies into perspective, it has been calculated that a radiation dose sufficient to create an average of one ionizing track per cell would produce enough radicals to delay the rate of DNA synthesis in metabolically active cells [F9]. This time delay was sufficient to ensure the availability of radical scavengers to cope with radicals produced by a second dose of radiation a few hours later.

147. Other calculations reveal that 0.01 Gy of low-LET radiation could produce about 6 nM of oxidative radicals in each cell [K10]. This concentration should be compared with the cellular steady-state concentration of radicals from normal metabolic processes involving oxygen, which is about 1 nM. Therefore, it has to be assumed that a small transient increase in radical concentration above that normally present in the cell is able to cause a measurable activation of normal detoxification mechanisms.
2. Activation of membrane receptors

148. The activity of adenylate cyclase increases markedly in isolated membranes from rat hepatocytes after acute exposure to between 1 and 2 Gy from gamma rays [K17]. This was confirmed by experiments with isolated plasma membranes from rat lung tissue, in which the increased adenylate cyclase activity, induced by a low concentration (0.02 mM) of isoproterenol, was further increased if the membranes were irradiated with gamma-radiation at the rate of 0.36 mGy d\(^{-1}\) [K18, R4]. When mitogen-stimulated human lymphocytes [K19] and Raji cells derived from human lymphoma cells [R5] were grown in the presence of serum growth factors, increased cellular proliferation was observed if the cells were irradiated at dose rates of 0.36 mGy d\(^{-1}\). The conclusion from these studies was that low doses of radiation activated membrane-bound enzymes.

3. Stimulated proliferation of splenocytes

149. The effect of radiation on the in vitro proliferation of thymocytes and splenocytes was measured in rats previously irradiated in vivo [13]. The animals received a whole-body dose of x-radiation in the range 0.01-2 Gy. Cells isolated from the spleen and thymus were then cultivated in the presence of various mitogens, and cell proliferation was evaluated by the rate at which tritiated thymidine was incorporated into DNA.

150. The results showed that the proliferation of splenocytes induced by concanavalin A (Con A) was enhanced by the use of x rays within the dose range 0.01-0.1 Gy, whereas that of thymocytes was not affected, as can be seen in Figure VII. Irradiation with 0.05 Gy also enhanced the proliferative rate of splenocytes stimulated by phytohaemagglutinin (PHA) or lipopolysaccharide (LPS), although their responses were less than that produced by Con A. This enhancement in the mitogen-induced proliferation of splenocytes was observed only within a few hours after irradiation, suggesting that low-dose, whole-body irradiation can induce an adapting effect in splenocytes.

151. This study was followed by a study of mouse splenocytes in which interleukin-1 (IL-1) production was measured [14]. The bioavailability of intracellular IL-1 of splenocytes stimulated with LPS was enhanced following whole-body irradiation with 0.025 Gy from x rays. Furthermore, if splenocytes from an unirradiated mouse were exposed to Con A, the addition of a small amount of serum from an irradiated mouse augmented the proliferative effect of the Con A. This could imply the presence of growth-enhancing substances (e.g. cytokine) in the irradiated serum.

D. SUMMARY

152. Studies to characterize gene expression in relation to the radiation-induced adaptive response are continuing, and specific genes and their protein products induced after acute exposure to doses of radiation in the range of a few tens of milligray to a few gray have been identified. It has been shown that groups of genes coding for transcription factors, nuclear proteins, oncogenes, viruses, membrane receptors, functional proteins and enzymes can be activated during the few hours after acute exposure to radiation. Recent experiments have implicated oxidizing radicals as one activating mechanism.

153. While there is a general consensus that the adaptive response can be considered as a consequence of damage to the DNA molecule, other mechanisms have been proposed. A recent review of the mechanisms of induction of transcription factors by damaging environmental agents provides useful insight into these other mechanisms [H11]. As an example, reactive oxygen intermediates and free radicals may directly influence regulatory proteins, such as the transcription factors, which in turn may condition the cellular adaptive response by inducing the expression of genes. The presence of stress-induced proteins at dose rates of 0.04 Gy d\(^{-1}\) has also been demonstrated. Protein kinase C is a common factor in many of these responses, and since it plays a central role in cellular signal transduction, its activation could represent a general response to molecular damage.

III. EFFECTS ON THE IMMUNE SYSTEM

154. The observations of a radiation-induced adaptive response in mitogen-stimulated lymphocytes and of changes in the immune system after exposure to low doses of radiation could imply an essential role for immunocompetence in the living organism. Understanding the mechanisms of T-cell signalling and how they are affected by low doses of radiation may therefore explain some aspects of the adaptive response.

155. Steady progress is being made in identifying the receptors on T cells that permit specific recognition of the infinite variety of non-self molecules and in determining how the signal from the T-cell antigen surface receptor is transmitted to the cell interior. The functioning of the immunosurveillance system of the organism, its response to radiation and its possible involvement in adaptive response are considered in this Chapter.
A. CELLS OF THE IMMUNE SYSTEM

156. T cells are lymphocytes that develop in the thymus. They are primarily responsible for ensuring cellular immunity and delayed hypersensitivity response. They mediate their acquired immune responses first by activation of specific T cells, then by a phase of clonal expansion and finally by a phase in which some of the lymphocytes become effector cells.

157. There are at least three functionally distinct classes of T cells:
(a) cytotoxic T cells, which kill virus-infected cells and tumour cells directly;
(b) helper T cells (T_H), which amplify responses by secreting a variety of local chemical mediators (interleukins) that stimulate activated T cells to proliferate, help B cells to make antibodies, and activate macrophages;
(c) suppressor T cells (T_S), which inhibit the responses of helper T cells.

Helper and suppressor T cells are the principal regulators of immune responses, and the ratio between the two cell subsets is an important factor in ensuring immunocompetence in the living organism.

1. T-cell ontogeny

158. Most T cells bear an antigen receptor consisting of a heterodimer of transmembrane α and β polypeptide chains. Both chains are required for antigen recognition [D3]. These polypeptide molecules resemble antibodies in structure and interact with antigens that are presented to T cells as proteolytic digestion fragments associated with Class I and Class II major histocompatibility complex proteins on the surface of antigen-presenting cells. The specific binding of antigen histocompatibility complexes on the surface of a target cell to antigen receptors on the surface of the T cell is often not strong enough to mediate a functional interaction between the two cells. Various cell-cell adhesion glycoproteins on T cells help to stabilize such interactions by increasing the overall strength of cell-cell binding. The characteristics of these accessory glycoproteins are summarized in Table 21. Among the best characterized are the CD4 and CD8 glycoproteins, which are expressed on the surface of helper and cytotoxic T cells, respectively.

159. The T-cell receptor is also associated with a group of glycoproteins, collectively referred to as the CD3 complex, which is involved in receptor assembly [C10]. The T-cell receptor CD3-α/β heterodimer is expressed on the surface of the vast majority of mature CD4⁺ or CD8⁺ cells in peripheral human blood lymphocytes and lymphoid organs [A5, H6, M14]. (The presence or absence of accessory proteins on T cells is designated by the superscripts + and −, e.g. CD4⁺CD8⁻). A second group of glycoproteins consisting of γ and δ chains is also CD3-associated and is found mainly in the double-negative (CD4⁺CD8⁻) T-cell population [B17]. According to current understanding, CD4⁺CD8⁻ T cells are thought to be the precursors of thymocytes, which differentiate into CD4⁺CD8⁺ T cells, which in turn differentiate into CD4⁺CD8⁻ or CD4⁺CD8⁺ T cells [S36]. These last two cell types become the antigen-positive mature T cells.

160. Spontaneous loss and alteration in antigen receptor expression in mature human CD4⁺ T cells obtained from healthy donors have been described [K11]. The T-cell receptor CD3 complex plays a central role in antigen recognition and activation of mature cells, so abnormalities in the expression of this complex should be related to the unresponsiveness of T cells to antigen stimulus. Using flow cytometry, variant T cells with loss or alteration of T-cell receptor CD3 expression among CD4⁺ cells were detected and enumerated. Variance was demonstrated by defects in protein expression and partial protein deletion. The variant frequency in peripheral blood increased with age in normal donors and was highly elevated in patients with ataxia telangiectasia. Thus, alterations in antigen-receptor expression may be induced by the spontaneous somatic mutation of T-cell receptor genes, and these alterations could be important factors related to age-dependent, disease-associated T-cell dysfunction and radiation-induced T-cell damage.

161. Over the past decade, knowledge of T-cell renewal, differentiation and maturation in the mouse has been remarkably advanced by the development and use of specific monoclonal antibodies that identify T cells at the various stages of differentiation. It should be noted that a different terminology for identifying glycoproteins is sometimes used in the mouse. Thus, CD4⁺ equates with L3T4⁺, and CD8⁺ equates with lyt2⁺. It is now generally agreed that a small progenitor cell compartment exists in the thymus that expresses neither L3T4 nor lyt2 surface antigens (i.e. it is double-negative). The progeny of these cycling cells become the major cell population in the thymus and express both L3T4 and lyt2 antigens (double-positive). At present, there is insufficient evidence to determine whether the mature thymocytes, which express either L3T4 or lyt2, differentiate from a subset of double-positive cells or whether they derive directly from the double-negative cells. In either case, the majority of double-positive cells are not selected because of inappropriate major histocompatibility complex reactivity. The single-positive functional effector cells are exported to peripheral lymphoid tissue, where, in contrast to their behaviour
in the thymus, they become the predominant cell populations. A model for the various stages in T-cell ontogeny, as defined by L3T4 and lyt2 antigen expression, is schematically presented in Figure VIII.

2. Apoptosis and radiation-induced interphase death

162. The mechanism responsible for producing T cells is unusual in that the vast majority of differentiating cells are destroyed prior to their complete differentiation and release to the peripheral lymphoid tissues. This phenomenon, known as apoptosis, occurs within the thymus and results in the death of most of the developing thymocytes. The process is thought to ensure that differentiating cells with potential for reacting against the host are eliminated, thereby preventing an auto-immune response, and it essentially involves double-positive T cells. One feature of apoptosis is then an alteration in the differentiation rate of developing T cells in the thymus [L15].

163. Some types of lymphocytes die soon after exposure to radiation and before entering the mitotic phase of the cell cycle. The process is referred to as interphase cell death. To determine any similarity between apoptotic death and radiation-induced interphase death, thymocytes were collected from male C57Bl/6 mice between five and six weeks old, separated into CD4+CD8+, CD4+CD8-CD8+ and CD4+CD8+ T-cell subsets and exposed to gamma-radiation [M19]. They were assayed 8 hours later for evidence of cell death by measuring the amount of DNA fragmentation. The results given in Table 22 show that double-positive CD4+CD8+ T cells are extremely sensitive to radiation. With a 50% fragmentation index (FD50) at 0.22 Gy, it would appear that a small but significant fraction of the double-positive T cells die as a result of apoptosis following exposure to low doses of radiation. Since the double-positive T cells constitute the majority of the parenchymal cells in the thymus in an intermediate stage of differentiation, elimination of a fraction of them could conceivably alter the dynamic balance between cells in different stages of differentiation.

164. On this basis, interphase cell death caused by radiation may be considered similar to, if not identical with, apoptosis [D4, W20, W21]. The elimination of cells damaged by radiation ensures that the clonal expansion of renegade T cells is prevented.

165. A biochemical mechanism responsible for radiation-induced interphase cell death in lymphocytes has recently been proposed [Z2]. Within tens of minutes after exposing rat thymocytes to radiation, a sharp increase in mRNA polymerase II was observed. This was followed within 2 hours by an increase in intracellular calcium ion concentration and the appearance of newly synthesized polypeptides. In particular, there was a sharp increase in the phosphorylation of three proteins with molecular weights of 20, 35 and 48 kDa [Z3]. Different stress-related proteins of molecular weights of 48, 70 and 90 kDa were also detected after heat shock or after the addition of glucocorticoids to the culture medium [M16].

3. Signalling processes in thymocytes

166. Some consensus has emerged regarding the nature of the signalling process that couples antigen recognition to changes in lymphocyte behaviour. It is postulated that the activation of T cells requires two signals, one through the CD3 complex, the other from antigen-presenting cells. Antigenic, allogenic, mitogenic or monoclonal antibody stimulation of the T-cell antigen receptor (TCR/CD3 complex) leads to a series of biochemical processes, including the activation of phospholipase C, with subsequent hydrolysis of phosphatidylinositol-4,5-
diphosphate to generate two second messengers, inositol-1,4,5-
triphosphate (IP3) and diacylglycerol (DG) [H7, I12, J5, T14]. These two messengers cause an increase in intracellular calcium ion concentration (Ca++) and the activation of protein kinase, respectively. The two events are considered essential in the expression of the "early immediate" gene, c-fos, to be followed later by the expression of c-myc, gamma interferon, interleukins 1 and 2 (IL-1 and IL-2) and transferrin receptor, which are crucial in the activation and proliferation of T cells [K26]. Activated T cells secrete interleukin-2, which results in division in those cells expressing interleukin-2 receptors. The T cells produce a series of lymphokines that clonally expand activated B cells and cause their maturation into antibody-synthesizing cells [S37, W22]. Signals derived from the T-cell receptor could also regulate the maturation and selection of immature T-cell progenitors in the thymus [V5].

167. The kinases responsible for protein synthesis have not yet been fully identified, but it is known that T cells contain transcripts encoded by at least three members of the src family of protein-tyrosine kinase genes: namely lck, fyn and yes [C11, S38]. The p56lck protein was first identified by virtue of its over-expression in a murine lymphoma cell line. It is the product of the lck proto-oncogene, which is closely related to the c-src gene, and hence is a potential signal transduction element [P8]. Two pieces of evidence suggest the type of signalling event that may be mediated by p56lck. First, expression of lck mRNA
and of p56\(^{ck}\) is altered by stimuli that induce lymphokine release from T cells [M15]. Secondly, p56\(^{ck}\) is associated with CD4 and CD8 glycoproteins [V6]. When p56\(^{ck}\) is brought into proximity with the T-cell antigen recognition unit by CD4 or CD8 glycoproteins, its activity may increase to reflect receptor occupancy.

168. A second membrane-associated protein tyrosine kinase, p59\(^{fyn}\), the product of the \(fyn\) proto-oncogene, is also abundant in T cells [C11]. Using transgenic mice in which a lymphocyte-specific protein-tyrosine kinase isoform, p59\(^{fyn(T)}\), was 20-fold overexpressed in T-lineage cells, it was found that thymocytes from these animals were highly sensitive to stimulation [C12]. Furthermore, the phenotypes produced by overexpression of p59\(^{fyn}\) were different from those produced by overexpression of p56\(^{ck}\). Although p59\(^{fyn}\) may regulate early stages in the T-cell receptor signalling, it cannot by itself confer a mature, responsive phenotype upon otherwise refractory, immature thymocytes. Thus, CD4\(^+\)CD8\(^+\) thymocytes from \(fyn\) transgenic mice exhibit enhanced calcium ion accumulation following activation, but they do not release interleukin-2 or proceed to proliferate. Hence, components of the signalling cascade that are essential for activation are apparently unavailable or inactive in immature thymocytes in these transgenic mice.

### B. RESPONSE IN THE ORGANISM

169. The cell types involved in the immune response exhibit a broad spectrum of radiosensitivity with consequent effects in the organism [A6]. Some populations of lymphocytes are exceedingly radiosensitive, while plasma cells and macrophages are, by comparison, relatively radioresistant. The basis of these differences in radiosensitivity is not well understood, but the effects of radiation on the immune system have been extensively studied. Several reviews have been published that provide comprehensive background information [A6, A7, A8, S39, T6].

1. Effects in animals

170. Irradiation at high doses is known to inhibit the immune response. A dose-response relationship was observed almost three decades ago by Kennedy et al. [K12]. They exposed mice to x rays within the dose range 0.5-7 Gy and 10 days later injected the animals with antigenic sheep red blood cells. Four days after injection of the antigen, they measured the number of splenic plaque-forming cells. The effect of exposure to between 1 and 3 Gy from x rays was to reduce the number of splenic plaque-forming cells to about 30% and 1%, respectively, compared with the numbers of plaque-forming cells observed in sham-irradiated animals. The results are illustrated in Figure IX.

171. More recently, the immediate and long-term effects of radiation on the immune response of specific-pathogen-free mice were described after exposing mice to a dose of 3 or 6 Gy from x rays and measuring the surviving fraction of T-cell subsets [S39]. At high doses (>1 Gy), T\(_H\) and T\(_S\) cells were equally radiosensitive but there was a marked difference in the radiosensitivity of T cells between C3H, BALB/C, C57Bl/6 and B10BR strains of mice. No long-term effect of radiation at these high doses on the immune system was found in terms of accelerated ageing of the immune function in animals exposed as young adults.

172. Other experiments have shown an enhancement of the immune response following whole-body exposure to low doses. As an example, rabbits were exposed to small doses of x rays (about 0.25 Gy) before or after immunization with antigenic sheep red blood cells [T7, T8]. These small doses were able to stimulate the proliferation of plaque-forming cells. This finding has been confirmed in experiments in which human and murine T cells were exposed to low doses [A8, A9, D8, L9, L16, L17, M17, S40, S51, T9].

173. In contrast, four different strains of pathogen-free mice (B10/Sn, B10/SgSn, C3H/HeMsNrs and C57Bl/6Js1c) were exposed to doses of 0.025-0.25 Gy from x rays [K13]. Nine hours later, they were injected with sheep red blood cells; the number of direct plaque-forming cells per spleen was assessed after 4.5 days. There was no evidence of enhancement of the plaque-forming cells in any of the strains within the dose range used. In an extension of this experiment, C57Bl/6j mice were immunized with sheep red blood cells and exposed two days later to higher doses (1.5-3.0 Gy) from x rays. Numbers of indirect and direct plaque-forming cells per spleen were assessed at intervals thereafter. Mice exposed to 1.5 Gy were found to have a significant increase in the number of indirect plaque-forming cells 11 days after the injection of the sheep red blood cells. This was highly correlated with an increase in the CD4\(^+\)/CD8\(^+\) cell ratio during the first three days after radiation exposure, followed by a rapid decline, as shown in Figure X. These results suggest that the ratio of T\(_H\) to T\(_S\) subsets changes in favour of helper cells shortly after exposure, possibly contributing to the observed enhancement of the indirect plaque-forming cell response.

174. Anderson and Lefkovits [A9] proposed that the enhancing effect within the dose range 1-7 Gy could be due to the elimination of the more highly radiosensitive T\(_S\) cells. These investigators questioned,
however, whether the same mechanism operated when
doses below this range were used. Under these
circumstances, they postulated that $T_S$ cells could
proliferate. The enhancing effect on murine splenic
T cells, which is illustrated in Figure XI, is however,
effective over only a narrow range of doses [M18].
The maximum effect occurs at about 0.25 Gy.

175. To determine the fate of proliferating T cells,
animals from a normal C57Bl/6J+ strain of mouse
were chronically exposed to gamma-radiation in the
range 0.005-0.04 Gy d$^{-1}$ during 20 days in a four-
week period [17]. Animals from an immunologically
depressed C57Bl/6J lpr/lpr strain were exposed under
similar conditions. A dose-related proliferation in
thymocytes and splenocytes was noted in both strains
up to cumulative doses of about 0.8 Gy. This is
illustrated in Figure XII. The mitogen-responsive
L3T4$^+$lyt2$^+$ and L3T4$^+$lyt2$^+$ T cells increased with
dose, while the mitogen-unresponsive L3T4$^+$lyt2$^+$ and
L3T4$^+$lyt2$^+$ T cells decreased in both strains relative
to sham-irradiated animals.

176. However, the magnitudes of the changes in
thymic and splenic T cells subsets were different,
according to whether or not the animals were fed on
a calorie-restricted diet. The immunologically
depressed mice were more sensitive to these dietary
changes. It was proposed that these experiments
support the hypothesis that the stress of continuous
low-dose irradiation is consistent with an adaptive
mechanism for cell renewal and maintenance of
mitogen-responsive cells.

177. Two recent reviews [L16, M19] provide the basis
of a hypothesis with which to explain a radiation-
induced T-cell response to low doses of radiation. At
the cellular level, the question as to whether or not
some subsets are more radiosensitive than others
remains unresolved. The evidence points to the need
for an intact thymus, in that thymectomy prevented the
adaptive response [J6]. Mitogen-responsive T cells
appear to be a prime target, and their stimulation is
associated with enhanced expression of the HSP70
gene and its related proteins. There may be selective
destruction of mitogen-unresponsive T cells. The
interpretation is complicated by the evidence that the
metabolic status of irradiated animals can influence the
response and that there is a marked difference in
radiosensitivity between the strains of animals used in
the experiments.

178. Liu et al. [L8, L9, L16] have investigated several
reactions of splenocytes in two strains (C57Bl/6 and
Kunning) of mice irradiated with x rays. The dose-
effect relationships for antigenic (plaque-forming cell)
ability, mixed lymphocyte reaction, mutagenic
(concanavalin A)-stimulation, antibody-dependent cell-
mediated cytotoxicity, natural killer cell activity,
interleukin-2 and interferon secretions, and the
lipopolysaccharide reaction are given in Table 23.
Interestingly, the maximum response in most of these
reactions occurs at 0.075 Gy.

179. Some specific features of the reactions occurring
at maximum response are summarized in Table 24. An
analysis of cell-cycle progression by measuring the
uptake of tritiated thymidine showed an increase in
the number of thymocytes entering the S phase, with a
corresponding decrease in the number of cells in the
G$_0$ phase 3-7 days after exposing mice to 0.075 Gy.
The T$_H$/T$_S$ ratio of thymic lymphocytes was the same
for the first three days, with a lowering of the ratio
after seven days, which the authors claim was due to a
decrease in the number of CD4$^+$/CD8$^+$ cells
(compared with CD4$^+$/CD8$^+$ cells) in the thymus.

180. A parallel examination of the phenotypic changes
of the thymocyte subsets, with flow cytometry using
fluorescence-labelled monoclonal antibodies against
surface antigens, showed a significant increase in the
percentage of the double-negative (CD4$^+$/CD8$^+$) cells
in the thymus after 0.075 Gy, implying an enhanced
renewal of thymocytes. These results are also included
in Table 24. At the same time, the secretion of colony-
stimulating factors by thymocytes was stimulated. It is
known that the colony-stimulating factors secreted by
the thymocytes act on macrophages to facilitate the
production of interleukin-1, which, in turn, serves as
a signal for the maturation of immature thymic
lymphocytes. All these changes occurring in the
thymus following low doses should increase the
supply of mature lymphocytes.

181. Liu et al. [L26] proposed a mechanism to explain
their findings after the whole-body irradiation of mice
at low doses. This is summarized in Figure XIII,
which outlines the changes in signal transduction of
T lymphocytes. It is a process similar to that
postulated for apoptosis and interphase death. How it
might be influenced by neuroendocrine factors is being
investigated. What is known is that exposure to
0.075 Gy causes a decrease in serum corticosterone in
the course of a few weeks accompanied by an increase
in 5-hydroxytryptamine from the hypothalamus and a
decrease in adrenocorticotrophic hormone [L27]. It is
not known how these changes in endocrine function
following low-dose irradiation affect the signal
transduction process in T cells, but it is an area of
research worth pursuing.

2. Effects in humans

182. Several parameters of cellular immune function
have been assessed for 186 persons who survived the
atomic bombings of Hiroshima and Nagasaki but who now reside in the United States [B18, B19, B20]. Persons exposed to doses between 0.01 and 1 Gy were compared with persons exposed to less than 0.01 Gy. Lymphocytes were isolated from the peripheral blood of these individuals and assessed for the following parameters of cellular immunity: (a) mitogenic response to phytohaemagglutinin; (b) mitogenic response to allogenic lymphocytes (mixed lymphocyte response); (c) natural-cell-mediated cytotoxicity; (d) interferon production. In every case, the response of the group exposed to 0.01-1 Gy was greater than that of the group exposed to less than 0.01 Gy, although only the difference for natural cell-mediated cytotoxicity was statistically significant (Figure XIV). It is not possible to say whether the increase in natural-cell-mediated cytotoxicity observed in these survivors of the atomic bombings exposed to very low doses of radiation is a genuine adaptive response that was modulated by post-radiation environmental conditions or a chance finding.

183. In more recent studies carried out among 1,328 survivors of the atomic bombings and now living in Japan, there was an age-related decrease in the total number of T cells (CD5+, CD4+, CD8+) [K14]. Although the differences were not statistically significant, the overall impression is that ageing of the T-cell-related immune system is accelerated in older persons receiving doses in excess of 1 Gy, compared with the control group, who received doses of less than 0.01 Gy.

184. The responsiveness of peripheral blood lymphocytes to allogenic antigens in mixed lymphocyte culture was measured in 139 survivors of the atomic bombings. In contrast to the findings in paragraph 182, the study revealed a significant decrease in mixed lymphocyte culture response with increasing dose of previous radiation exposure. This decline was marked in the survivors who were older than 15 years at the time of the bombings. It suggests a possible relationship between the recovery of T-cell-related function and the thymic function that processes mature T cells for the immune system. Thus it may be that in the persons who were older at the time of the bombings, the thymus function had started to involute, allowing less recovery of T-cell function than in younger survivors, who had adequate processing T-cell activity [A19].

185. As indicated in paragraph 160, the spontaneous loss and alteration of antigen receptor expression in mature CD4 cells has been found in blood cells taken from 127 healthy donors who had not been exposed to other than natural background radiation [K11]. The mean frequency of mutant T cells in males with altered T-cell receptor expression was $2.5 \times 10^4$, equal to about $1.3 \times 10^4$ per single T-cell receptor locus among the CD4 cells, increasing with age.

186. The mean frequency of mutant T lymphocytes was measured in 203 survivors of the atomic bombings, 78 of whom received doses greater than 1.5 Gy and 125 of whom received doses less than 0.005 Gy [K16]. The results were $1.39 \pm 0.63 \times 10^4$ mutants per cell in exposed males compared with $1.20 \pm 0.35 \times 10^4$ in unexposed males and $0.99 \pm 0.39 \times 10^4$ in exposed females compared with $0.89 \pm 0.38 \times 10^4$ in unexposed females. There was no statistical difference in mutant frequency between the two groups, although the frequency was higher in males than females. The smoking habits of the males may have contributed to the higher values.

187. Antibody titres to Epstein-Barr virus antigens were determined in the sera of 372 atomic bomb survivors to evaluate the effect of the previous radiation exposure on immune competence against the latent infection of the virus. The proportion of persons with high titres (≥1:40) of IgG antibodies to the early antigen was significantly elevated in the exposed survivors. Furthermore, the distribution of IgM titres against the viral capsid antigen was significantly affected by radiation dose, with an increased occurrence of titres of 1:5 and 1:10 in the exposed survivors, although the dose effect was only marginally suggestive when persons with rheumatoid factor were eliminated from the analysis. These results suggest that reactivation of Epstein-Barr virus in the latent stage occurs more frequently in the survivors, even though this might not be affected by the radiation dose. Otherwise, there was neither an increased trend in the prevalence of high titres (≥1:640) of IgG antibodies to the viral capsid antigen among the survivors nor a correlation between the radiation exposure and distributions of titres of IgA antibodies to the viral capsid antigen or antibodies to the nuclear antigen associated with anti-Epstein-Barr virus [A11].

188. The effects on the immune system of long-term low-level radiation exposure were measured in two areas in Guandong Province, China [Y8]. The annual effective dose in the area of low background radiation in Enping County was about 2.0 mSv. In the area of high background radiation in Yangjiang County, the annual effective dose was about 5.4 mSv. The annual equivalent doses from external gamma-radiation to the red bone marrow were estimated to be about 0.77 mSv and 2.1 mSv, respectively. Twenty-five healthy male subjects from the high-background-radiation area and 27 subjects from the control (low-background-radiation) area were divided into three age groups, and the frequency of interleukin-2-secreting cells was measured as an index of immune competence in peripheral blood lymphocytes.
189. The results shown in Table 25 indicate that the frequency of interleukin-2-secreting cells was significantly greater in subjects from the high-background-radiation area than in those from the control (low-background-radiation) area. The increased production of interleukin-2 in lymphocytes after low-dose irradiation has also been demonstrated [Z7], consistent with the view that long-term exposure to low doses of radiation may affect the immune system. However, other factors, particularly differences in smoking habits, could also have contributed to the differences.

190. A study of the T-cell subsets in occupationally exposed persons revealed no significant influence of radiation on their profile [T10]. Data were pooled into two groups, one with individual exposures less than 0.5 mSv and another with exposures in excess of 0.5 mSv in the previous three months. The average doses for the two groups were 0.36 ± 0.09 mSv and 1.06 ± 0.77 mSv, respectively. Natural background radiation was estimated to be 0.06 mSv during the period. The results shown in Figure XV indicate no statistically significant difference in the percentage of CD2^+, CD4^+, CD8^- and HNK-1 (natural killer cells). However, as indicated by the data given in Table 26, significant differences were shown as an effect of cigarette smoking.

3. Effects on tumour growth

191. The precise involvement of the immune system in the natural course of cancer remains uncertain. It is known that white cells cultured in vitro in the presence of the cytokine interleukin-2 acquire the capacity to kill tumour cells [S50]. The phenomenon was termed lymphokine-activated killer activity. The use of in vitro techniques to study cytokine kinetics has given some insight into their role in tumour biology. Some cytokines (e.g. gamma-interferon) can render tumour cells resistant to cell-mediated killing, while others (e.g. transforming growth factor β) can enhance the growth of some tumour cells. Thus, cytokines interact to generate immune responses and modulate the outcome of effector mechanisms on target cells.

192. Thus, the response of a cell to a cytokine depends on the context in which the cytokine signal is received. For example, if white cells are mixed with tumour cells in culture, the ability of the white cells to kill the tumour cells in the presence of cytokines can be measured. This is shown in Figure XVI. Interleukin-2 can profoundly increase the cytotoxicity of the white cells. Conversely, if either interleukin-4 or transforming growth factor β is added at the same time as the interleukin-2, the cytotoxicity is significantly reduced. However, if the white cells are first stimulated with interleukin-2 and then interleukin-4 or transforming growth factor β is added, the reduction in cytotoxicity does not occur.

193. Low doses of radiation (0.25-1 Gy) have been used to augment the effect of immunization of animals to reduce tumour growth [A4]. The immunization procedure involved injecting animals with an antigen of non-active tumour cells before exposing them to live tumour cells. The antigen was prepared by treating the ascitic form of a methylcholanthrene-induced fibrosarcoma (Sal cells) with mitomycin and paraformaldehyde. These mitomycin-treated cells were then injected subcutaneously into the A/J strain of mouse and, to assess immunity, the mice were injected subcutaneously with an amount of living Sal cells known to induce subcutaneous tumours. The effect of irradiation upon this immunization process can be measured. Figure XVII shows the effect of whole-body exposure to 0.15 Gy from x rays on the response of A/J mice to mitomycin-treated Sal cells. Immediately after irradiation, the mice were injected with varying numbers of treated Sal cells, as indicated in the Figure. Twenty-one days later, they received a subcutaneous injection of 10^5 viable Sal cells into the left flank, and the size of the growing tumour was measured over a period of 26 days. The non-irradiated control group (solid line) did not receive the mitomycin-treated cells. Injection with 10^5 to 10^6 mitomycin-treated tumour cells resulted in variable degrees of immunity in both sham-irradiated and irradiated groups, expressed in terms of smaller tumour size than in the control group. However, the degree of immunity was almost always greater in the irradiated mice. Low-dose augmentation was less pronounced in recipients of thymus-derived T cells. From these results it was suggested that exposure to low doses of radiation reduces the number of T_s cells, which normally suppress tumour rejection.

194. In a follow-up to these studies, the effect of radiation was shown in an in vitro system in which the effect of irradiating donor spleen cells could be measured [A18]. One procedure (Winn assay) involved injecting viable Sal cells into A/J mice and killing them two days later. The spleens were sham-irradiated or irradiated with 0.15 Gy, and 10^4 spleen cells were mixed with 10^4 Sal cells. This mixture was injected subcutaneously into A/J mice and tumour size was measured over 18 days. Figure XVIII shows the inhibitory effect of the radiation.

195. The effects of low-dose radiation on another type of experimental tumour, the Lewis cell carcinoma, have been measured [L29]. C57B1/6 mice received whole-body x-irradiation giving doses in the range 0.05-0.15 Gy at a dose rate of 0.0125 Gy min^-1. All mice were injected intravenously with 7 10^5 cells of
Lewis lung carcinoma 24 hours after the irradiation and were killed 14 days later. The lungs were removed and the tumour nodules on the lung surface were counted. The mean number of lung tumour nodules was 16 in the 0.05 Gy dose group, 27 in the 0.075 Gy group, 20 in the 0.1 Gy group and 27 in 0.15 Gy group, all significantly lower than the 85 nodules counted in the sham-irradiated group (p < 0.01-0.001). This supports the view that there may be an inhibitory effect of low-dose radiation on the pulmonary dissemination of Lewis lung carcinoma in mice.

196. Miyamoto and Sakamoto [M28] demonstrated the anti-tumour effects of low doses of radiation in mice of the WHT/Ht strain. Mice were exposed to 250 kV x rays in the dose range 0.05-1 Gy at a dose rate of 1.23 Gy min⁻¹. They were then injected with squamous carcinoma cells, and the effect of the radiation on subsequent tumour growth was measured. For example, whole-body irradiation of 0.1 Gy to mice bearing primary leg tumours followed 12-24 hours later by localized tumour irradiation delayed the growth of the tumours more than did localized irradiation alone. The results for various times and amounts of local irradiation are given in Table 27. The in vitro immune responses of splenocytes from tumour-bearing mice exposed to 0.1 Gy are compared to those from sham-irradiated tumour-bearing mice in Figure XIX. The selective depression of T₅ cells may have been responsible for this low-dose effect.

197. Immune response and tumour growth have also been studied in humans. In a study of non-Hodgkin’s lymphoma patients, the effect of whole- or half-body x-irradiation on the various T-cell subsets was measured [T16]. Patients received 0.1-0.15 Gy per fraction, delivered two or three times per week, until the cumulative dose was 1.5 Gy. The changes in the T-cell subsets are shown in Table 28. A statistically significant increase in helper T cells and helper-inducer T cells was found, with no change in the suppressor cells or natural killer cells. The direct anti-tumour effect was evaluated in 10 patients. Two patients showed complete remission, seven partial remission and one, no change. In four patients given half-body irradiation, the tumours showed complete or almost complete remission even though the primary tumours in the tonsils or neck lymph nodes were outside the irradiation field. Anti-tumour activity in the other patients was obscured by chemotherapy received after the irradiation. The changes in T-cell subsets and tumour responses could be consistent with an adaptive feedback control signal that up-regulates stem cells, with preferential proliferation of differentiating T₅ cells. These studies need to be substantiated to eliminate the possibility of spontaneous remission.

C. SUMMARY

198. Studies to characterize the role of the immune response to low doses of radiation are continuing. The mechanisms responsible for T-cell selection during normal differentiation in the thymus and activation of the signalling process that couples antigen recognition to changes in lymphocyte behaviour are gradually being elucidated. A sequential role for phospholipase C, intracellular calcium ion and protein kinase C, followed by transcription of genes such as c-fos, and interleukin-2 production in the activation of T cells has been proposed. Similar sequences of events appear to be associated with apoptosis and in low-dose-radiation-induced interphase death, which causes enhanced DNA replication and proliferation of T cells. Whole-body irradiation of mice results in a dose-related enhancement of the T-cell response to antigenic, allogenic and mitogenic stimuli at doses below about 0.1 Gy.

199. As an alternative explanation to radiation-induced interphase death, experimental studies using mice have shown selective depression of radiosensitive suppressor T cells within the dose range 0.025-0.25 Gy, with an optimum response at 0.075 Gy. This needs to be investigated further as a possible mechanism of the adaptive response.

200. The evidence for changes in the T₅/T₃ ratio of T cells in humans exposed to radiation remains equivocal. Reported changes in the ratio of these cells in the blood of atomic bomb survivors over 40 years after exposure are difficult to interpret, the effects of the long time interval and of changes in the environment and cigarette smoking being confounding and inexplicable factors. A Chinese study of the chronic exposure of a large population to background levels of external radiation of about 5.4 mGy a⁻¹ indicated that levels of interleukin-2-secreting cells were significantly increased in persons from this area compared with persons living in an area of lower radiation background where the annual effective dose from external exposure was about 2.0 mSv; the authors acknowledged, however, that differences in geochemical and other environmental factors provide an equally plausible explanation. A study of workers chronically exposed to low doses of radiation showed no statistical difference in the percentage of helper or suppressor T cells or of natural killer cells in groups with average doses of 0.36 and 1.06 mSv, however significant differences were found in relation to cigarette smoking.

201. A role for the neuroendocrine system in influencing T-cell proliferation after low doses of radiation has recently been proposed. The blood vessels in most lymphoid tissues are innervated with sympathetic nerves, and the lymphoid tissues are under the control of hormonal factors via the hypothalamus. There is evidence to support the view that radiation can activate the neuroendocrine system and enhance the immune response.
202. A cytotoxic effect of irradiated T cells on tumour cells has been demonstrated. It is postulated that low doses of radiation act by modulating antigen-stimulated clonal growth. There is evidence of a down-regulating effect on tumours in animals, but the evidence for a similar effect on human tumours is sparse. There is a need to investigate these effects, which could be of clinical significance.

IV. EXPERIMENTAL STUDIES OF RESPONSE IN MAMMALS

203. The effects of radiation in animals have been studied in numerous experiments. Evidence of possible adaptive responses have sometimes emerged in lifetime studies of animals exposed to acute or chronic low-LET radiation at doses well below those associated with bone marrow failure. Life-span and incidence of neoplastic diseases have been assessed. Other experiments have been done with animals exposed to non-lethal conditioning doses prior to acute exposures to potentially lethal doses. It should be noted that in many of these studies the conditioning doses were well above the range of doses producing the adaptive response in cellular systems.

A. SHORT-TERM SURVIVAL FOLLOWING ACUTE, HIGH-DOSE EXPOSURE

204. Experiments were carried out in the 1950s and 1960s to investigate the hypothesis that stimulating haematopoietic stem cells to proliferate before exposing animals to a potentially lethal dose of radiation could improve their chances of recovering from acute bone marrow failure. A historical review of these experiments was given by Dacquisto and Major [D2].

205. In one of these early experiments, adult female Swiss white mice (Bethesda Naval Medical Research Institute strain) were acutely exposed to a dose of about 1.2 Gy from 250 kV x rays at weekly intervals over a period of three weeks [C18]. Thirty days after the third exposure, the mice were acutely exposed to a challenge dose of about 5.6 Gy, which was known to be the dose approximating the LD<sub>50/30</sub>. Of the animals in this group, 26% died after 28 days, compared with 41% in the control group, which received only the challenge dose.

206. This observation was confirmed by another study in which adult female Swiss white mice (Walter Reed strain) were acutely exposed to a conditioning dose of about 0.4 Gy from x rays, either 10 or 15 days before determining LD<sub>50/30</sub> values [D2]. The LD<sub>50/30</sub> values for the two irradiated groups were about 4.48 ± 0.25 Gy and 4.94 ± 0.25 Gy, respectively, compared with 3.90 ± 0.21 Gy for animals not exposed to the conditioning dose. There was no evidence of splenic or thymic hypertrophy in animals that received the conditioning doses, in contrast to an earlier observation that localized splenic irradiation to a dose of about 0.16 Gy caused transient hypertrophy [P13].

207. Age at exposure was shown to be an important factor influencing survival. Female mice of the BDF<sub>1</sub> (C57Bl/6J x DBA/2J) strain were exposed to 0.8, 2.4 or 3.2 Gy from 250 kV x rays at 90 days of age, and LD<sub>50/30</sub> values were determined at various ages thereafter [S55]. The results, given in Table 29, showed that there was no statistically significant difference in LD<sub>50</sub> values between controls and those pretreated with 0.8 Gy in animals below the age of 550 days. With higher conditioning doses, however, there was residual damage in the animals resulting in lower resistance to a lethal dose (expressed as reduced LD<sub>50</sub> values).

208. As a recent example of these short-term survival studies, two-month-old SPF mice of the C57Bl strain were acutely exposed to a range of doses from x rays between 0.025 and 0.1 Gy [Y9]. Two months later, they were acutely exposed to 7.75 Gy, and 30-day survival was measured. The results, given in Table 30, showed a marginally significant increase in the life-span of animals receiving the lowest conditioning dose and a definite improvement in survival at conditioning doses of 0.05 and 0.1 Gy compared with animals not exposed to a conditioning dose. The improvement in survival rate coincided with an increase in endogenous colony-forming units, consistent with increased proliferation of haematopoietic stem cells following the conditioning doses.

209. In a subsequent experiment, mice were irradiated with x rays to doses of 0.025-0.5 Gy at six weeks of age and exposed two months later to a dose of 7 Gy from x rays [Y7]. A dose of 0.025 Gy was insufficient to affect survival after exposure to the high dose, but pretreatment with 0.05-0.1 Gy produced a significantly increased survival rate. However, the response to pretreatment with 0.05 Gy after two months was strain-specific. It occurred in C57Bl but not in BALB/c mice. Surprisingly, an increase in survival was not observed in animals pretreated with 0.2 Gy if exposure to the challenge dose occurred up to 1.5 months after the conditioning dose; and pretreatment with 0.5 Gy resulted in an increase in survival if the challenge dose was given two weeks later but not after two months. These findings persuaded the authors to postulate different mechanisms involving time-related stimulation of haematopoiesis, although they were not specified.
B. LONG-TERM SURVIVAL FOLLOWING SUB-LETHAL EXPOSURE

210. Studies using rodents and beagle dogs have provided some insight into the long-term effects of acute exposure to low doses and of chronic exposure at low dose rates. They date from the early pioneering work of Russ and Scott [R2, R3].

1. Experiments with rodents

211. The effect of chronic irradiation of mice was described by Lorenz et al. [L14, L19] in an early experiment in which both survival and tumour incidence were measured. Male and female mice of the (C57Bl x A)F1 strain, referred to as LAF1, were exposed from the age of one month for the duration of life to gamma rays from 226Ra delivered at the rate of about 1 mGy for 8 hours each day. Control mice were housed in a room adjacent to the exposure room. Almost a year after the start of the experiment, the control mice developed dermatitis and had to be replaced by mice not directly related to the irradiated mice but from crosses of the same inbred strains.

212. The mean survival times of the irradiated male mice was significantly higher than those of the control mice, but there was no statistical difference between the two groups of females, although there was a tendency for the females to live longer than the males (Table 31). However, the differences in life-span in the males cannot be accepted without reservation, since the replacement control group may have been subject to different environmental conditions and may have possessed genetic characteristics slightly different from those of the original group.

213. Although no single cause of death predominated, pyelonephritis appeared to be a major contributing factor. Its occurrence is frequently associated with dermatitis, particularly among males. There was an increase in the incidence of lymphosarcomas and other tumours of the reticular tissues in both male and female irradiated mice, and mammary carcinoma incidence was also higher in the irradiated females (p < 0.1). The irradiated females showed an increase in ovarian tumours (p < 0.05), and the irradiated males showed an increase in lung tumours (p < 0.05). These results were essentially confirmed in a study in which guinea pigs of the Tumble Brook Farms strain were exposed to 60Co gamma rays from the age of 100 days for the duration of life at a dose rate of about 1 mGy d⁻¹ [R11].

214. Sacher and Grahn [S52], in a study of the survival of mice exposed for the duration of life, also reported an increase in the life-span of animals exposed to 60Co gamma rays at a dose rate of up to about 30 times that of the background radiation. Male and female mice of the LAF1 strain were exposed to a wide range of doses at different dose rates. Non-irradiated animals serving as controls were placed either in a corridor adjacent to the irradiation facility or in an adjoining room. It was subsequently discovered that the control group in the corridor were in fact exposed to stray radiation from the irradiation facility at a rate of between 1.6 and 4.9 mGy a⁻¹. This compared to about 0.14 mGy a⁻¹ in the control group in the adjoining room. The mean survival times are shown in Table 32. For comparison, the results for animals irradiated at the rate of 40 mGy a⁻¹ are given. There was a statistically significant increase in the mean survival times of males but not of females exposed to between 1.6 and 4.9 mGy a⁻¹ compared with those exposed to dose rates of 0.14 mGy a⁻¹.

215. One interpretation of these studies is that any increase in survival for male mice receiving the higher doses of radiation reflects a decreased number of deaths in early life. A possible explanation could be that minimal injury to the haematopoietic system causes a rebound in stem cell proliferation. This regenerative hyperplasia could then create a larger mass of tissues devoted to the defence against intercurrent and potentially lethal infection. This mechanism, however, does not prevent the occurrence of tumours that appear in late life, which are not necessarily the cause of death. Why this response is confined to males cannot be explained.

216. Upton et al. [U12, U13] studied the late effects of low-LET radiation in RF/Un mice. Life-span and the incidence of neoplastic diseases were assessed. The effects of mean accumulated doses up to about 3 Gy at varying dose rates between 0.05 Gy d⁻¹ and 0.8 Gy min⁻¹ are shown in Table 33. Several interesting features emerge from these studies. In males, there was little effect on life-span (mean age at death) at dose rates of 0.8 Gy min⁻¹ until the accumulated dose approached about 1 Gy; there was little effect of dose rate in the range 0.05-0.77 Gy d⁻¹ at an accumulated dose of about 1.5 Gy; and, overall, gamma rays were less effective at low dose rates than at high dose rates at accumulated doses of about 3 Gy. In females, there was no significant change in the mean age at death at dose rates of 0.067 Gy d⁻¹ until the accumulated dose reached about 2 Gy; reducing the dose rate to 0.01 Gy d⁻¹ increased the mean age at death at accumulated doses of about 3 Gy.

217. The mean age at death of animals with neoplasms was also influenced by dose rate. Thus in males, irradiated animals died earlier than controls at dose rates of 0.8 Gy min⁻¹, even at the lowest accumulated dose of 0.25 Gy, but there was no significant difference compared with controls at dose rates of 0.15-0.77 Gy d⁻¹ at an accumulated dose of about 1.5 Gy, with a marginal difference at a dose rate of 0.05 Gy d⁻¹. In females, earlier death from neoplasms occurred at dose rates of 0.067 Gy d⁻¹ after an accumulated dose of about 2 Gy, and there was no difference from controls at doses of
3 Gy, if the dose rate was reduced to 0.01 Gy d⁻¹. In summary, these results support the view that at low dose rates and accumulated doses of a few gray there is no apparent life shortening or early appearance of neoplastic diseases causing death of the irradiated animals compared with unirradiated controls. There is, as well, no support for an apparent increase in life-span or decrease in tumour incidence due to the radiation exposures at the lowest doses.

218. Ulrich and Storer [U14, U15] also described the influence of radiation on life-span and tumour induction in mice of the RFM and BALB/c strains. Specifically, a study was made of the effects produced by ¹³⁷Cs gamma-radiation delivered at 0.4 Gy min⁻¹ or 0.083 Gy d⁻¹, within the dose range 0.1-4 Gy for RFM mice and 0.5-4 Gy for BALB/c mice. At the lower dose rate, the life-shortening effect in BALB/c mice could be described as a linear or linear-quadratic function of dose, although the lowest dose used was 0.5 Gy. The influence of dose at a dose rate at 0.083 Gy d⁻¹ on the incidence of neoplastic disease in female BALB/c mice is shown in Table 34. For RFM mice, thymic lymphomas were the predominant reticular tissue neoplasm, the solid tumours being lung adenomas or endocrine-related tumours. The incidence of tumours was at all doses higher than in the unirradiated controls.

219. Sato et al. [S42, S53] exposed male and female mice of the C57Bl/6j strain to ¹³⁷Cs gamma rays at rates of 0.029-0.38 Gy d⁻¹. Exposures at the rate of 0.029 Gy d⁻¹ started at four weeks of age. The mean life-span of female mice was 628 ± 10 days, with a mean accumulated dose of 16.4 Gy; for male mice, the mean life-span was 723 ± 10 days, with a mean accumulated dose of 18 Gy. The mean life-spans of unirradiated females and males were 693 ± 11 days and 725 ± 15 days, respectively. In the irradiated females, this life shortening was statistically significant in the males, it was not. In a follow-up study, life shortening at the higher dose rates was associated with the occurrence of thymic lymphomas, which occurred more frequently in exposed younger mice [O5]. At the highest dose rate, and at accumulated doses of 39 Gy over a period of 105 days, life shortening was also associated with haemorrhage and infectious diseases owing to depletion of the stem cells of bone marrow.

220. Covelli et al. [C20] studied the effects of x rays on life-span and tumour induction as a function of age at exposure. Male and female mice of the B6C3F₁ strain were acutely exposed to 250 kV x rays, either in utero at 17 days after coitus, at 3 months, or at 19 months of age. The doses varied from 0.3 to 2.1 Gy for in utero irradiation and from 0.5 to 7 Gy at 3 months or 19 months of age. Prenatal irradiation or irradiation at 19 months of age did not result in clearly measurable life shortening (Table 35). There was, however, a trend suggestive of life shortening in animals exposed at 3 months of age at doses in excess of about 2 Gy, albeit the confidence limits were extremely wide. There was a non-significant trend suggesting an increase in life-span in animals exposed at 19 months of age at a dose of 2 Gy. Life shortening was associated with an increased incidence of reticulum cell sarcoma (a non-lymphatic malignant lymphoma that infiltrates the spleen, liver and mesenteric lymph nodes). Primary tumours of the lung and liver, mostly benign adenomas, and subcutaneous fibrosarcomas were also found. However, no difference could be detected in the patterns of total tumour incidence for irradiated and non-irradiated (control) animals.

221. Sasaki and Kasuga [S54] compared the effects of ¹³⁷Cs gamma rays on female mice of the B6C3F₁ strain exposed under specific-pathogen-free conditions until natural death. Mice were irradiated at 17 days in utero, or at birth, or at 15 weeks of age with doses of 1.9, 3.8 or 5.7 Gy. There was a decrease in life-span at 1.9 Gy in all groups, but no statistically significant increase in total tumour incidence between irradiated and control groups was observed (Table 36). Mice in the fetal period were found to be susceptible to pilatory tumours and liver and lung tumours. There was an excess of malignant lymphomas at a dose of 5.7 Gy, and the total time until incidence was less than with controls. The exposure of young adults was associated with the induction of myeloid leukaemias and Harderian gland tumours.

222. Maisin et al. [M29] measured life-span and disease incidence in the C57Bl/10b strain of mice exposed to acute and fractionated doses of ¹³⁷Cs gamma rays. Twelve-week-old mice were exposed to doses ranging from 0.25 to 6 Gy at a dose rate of 0.3 Gy min⁻¹ in a single session or in 10 sessions delivered 24 hours apart, or in 8 sessions, delivered 3 hours apart. The results of an acute exposure are shown in Table 37. There was no indication of an increase in life-span at these doses; decreases in life-span were not statistically evident until the dose approached 1 Gy. A fractionated exposure was less effective in reducing survival time than an acute exposure, and life shortening was not observed in the 8 and 10 fraction protocols until the dose approached 2.3 Gy and 4 Gy, respectively.

223. The causes of death in unirradiated mice could be segregated into late degenerative changes in lung and kidney (glomerulosclerosis (~60%), leukaemias (~21%) and carcinomas (mainly liver adenocarcinoma) and sarcomas (~16%). The incidence of thymomas was low (~1%), and no radiation-induced excess of lymphoma was observed until the doses approached 2.4 Gy, but the tumours did result in death at an earlier age than in unirradiated animals. The incidence of all malignancies was less than in the unirradiated mice after acute doses between 0.25 Gy and 1 Gy, with no significant change in the alpha parameter of the Cox proportional hazard models within this dose range. Fractionation increased the
incidence of all malignancies: thus their incidence was 45% and 52% at 1 Gy and 2 Gy, respectively, for the 8-fraction protocol and 40%, 52% and 36% at 0.25 Gy, 0.5 Gy and 1 Gy, respectively, for the 10-fraction protocol. The authors concluded that these differences between fractionated and acute exposures were small and not well enough established from a statistical viewpoint.

224. The effects of gamma rays and fission neutrons on the life-span of mice has been studied for many years at the Argonne National Laboratory in the United States. Earlier studies of low-LET radiation principally involved $^{60}$Co gamma rays delivered either as acute exposures or continuously [C19, T15]. For the most part, the acute exposures were delivered over a 20-minute period at dose rates ranging from 0.0083 to 0.094 Gy min$^{-1}$. The exposure regime began when the animals were 110 days of age.

225. The results obtained on the mean survival after irradiation of male mice of the B6CF$^1$ strain exposed for 22 hours each day, five days each week have been reported [T15]. The exposures were continued for either 23 or 59 weeks, the accumulated doses ranging from 2.1 to 24.6 Gy. The effects of these exposure conditions are shown in Table 38. There was no indication of an increase in life-span at any dose in this range. Life shortening from deaths due to all causes did not become significant until the total dose approached 4 Gy for a 23-week exposure or 5 Gy for a 59-week exposure. These values were not significantly altered when the analysis was restricted to mice dying from tumours. Above $\sim$4 Gy, the dose-response relationship was linear and inversely dependent on the protonation period. The life-shortening coefficients for the 23-week continuous and 59-week continuous protocols were 0.16 and 0.08 days lost per 0.01 Gy, compared with 0.39 days for an acute single exposure, thus showing a marked dose-rate effect.

226. The effects on life-span of external whole-body exposure of beagle dogs to x or gamma rays has been studied. In one study, dogs were exposed to 0.16 Gy or 0.83 Gy from $^{60}$Co gamma rays at 8, 28 or 55 days in utero or 2 days after birth. In addition, some dogs were exposed to 0.83 Gy at 70 days or 365 days after birth. The status of these studies in 1982 is shown in Table 39. Through 10 years of age, no differences in survival were evident in any of the exposure groups [B23]. Irradiation during the fetal period was, however, associated with abnormalities of skeletal, dental and central nervous system development. Perinatal irradiation resulted in kidney dysplasia and, in animals receiving higher doses, in chronic renal disease. The thymus gland, particularly the thymic epithelium, was found to be highly radiosensitive during fetal development.

227. In another study, dogs were exposed bilaterally to 250 kV x rays, delivered in different numbers of fractions and different fractionation intervals, to total doses of 1-3 Gy. The dogs were exposed between 8 and 15 months of age. Some dogs were bred after exposure, distinguished as parous or nulliparous. The dogs listed in each group shown in Table 40 are those surviving at least 90 days after the irradiation. Two general summaries of the data have been published, one before all the animals were dead [A13], the other emphasizing the effects on life-span and tumour induction [R12].

228. There was no increase in survival time, expressed as median survival after exposure, of any of the irradiated dogs compared with the unirradiated controls. Although life shortening was only marginal in animals given acute or fractionated doses up to 1 Gy, it occurred in some groups of animals given 2 or 4 fractionated doses of between 0.75 and 1.5 Gy total dose and fractionated doses of 3 Gy. The main causes of death were similar in irradiated and unirradiated dogs. The development of non-neoplastic diseases (essentially fibrosis) at an earlier age in irradiated animals at the higher dose explained, in large part, the observed life shortening. However at 1 Gy, the incidence of non-neoplastic diseases, mammary tumours and non-mammary tumours was broadly similar to that observed in the unirradiated group.

229. In a third study, dogs were exposed to $^{60}$Co gamma rays continuously until death or, in a companion experiment, until they had received a predetermined total dose [F1, F22, G13]. Dose rates ranged from 0.003 to 0.054 Gy d$^{-1}$, and the accumulated doses at termination of exposures ranged from 4.5 to 30 Gy. At dose rates greater than 0.019 Gy d$^{-1}$, the responses were dose-rate-dependent and limited primarily to damage of the haematopoietic system (Table 40). At dose rates between 0.003 and 0.008 Gy d$^{-1}$, myeloproliferative diseases ultimately expressed as myelogenous leukaemia were the limiting factor for survival [K25].

C. SUMMARY

230. Observations of adaptive responses in in vira cellular studies cannot be readily extrapolated to postulate adaptive response in the irradiated animal. The different exposure patterns in animal studies (acute versus chronic lifetime exposure) could involve mechanisms different from those involved in the cellular adaptive response to a low conditioning dose followed by a high challenge dose. The complexity of multicellular organisms, including the crucial role of immunosurveillance and endocrine factors in maintaining the healthy state, must be taken into account. The careful management of the animal colonies to avoid infections and stress and the need to recognize strain-specificity of tumours should not be overlooked in future experiments.
231. Stimulating the haematopoietic system to proliferate by exposing the bone marrow in vivo to between about 0.025 and 0.1 Gy has been shown to improve the short-term survival (expressed as LD₁₀/₅₀) of mice subsequently exposed to a potentially lethal dose of radiation. Whether a similar mechanism is responsible for improving the longer term survival of animals chronically exposed at low dose rates is unclear. It is plausible to suggest that improvement in long-term survival could be a result of chronic minimal injury to the bone marrow, causing a rebound in stem cell proliferation and protection against infections, but occurring along with this effect is an increased risk of malignancy due to the proliferation of potentially malignant cells.

232. Experiments in the 1950s indicated that the mean life-span of male, but not female, mice could be increased if the animals were exposed daily to low-LET radiation from a few milligray to several hundred milligray per year above the level of background radiation. A feature of these observations was that the mean increase in life-span reflected a decreased number of deaths in younger irradiated animals. Why the response was confined to males is not apparent.

233. Other experiments related life-span to the incidence of neoplastic diseases. Taken together, the results of these experiments could be interpreted as demonstrating that, compared with the pattern observed in unirradiated controls, there was no significant effect on mean life-span following exposure to accumulated doses up to a few gray, at dose rates between 0.005 and 0.3 Gy d⁻¹, or on the time of appearance of tumours or on tumour incidence among irradiated animals. Why some experiments resulted in an increase in life-span is not easily explained. While the observed increase in life-span could be due to random variability, it is possible that the effect is real. If so, it is important to understand the precise conditions under which life-span is increased.

234. More recent experiments using mice have confirmed that there is no statistically significant change in tumour incidence in irradiated mice compared to unirradiated controls below about 2 Gy, depending on the strain of mouse used. At higher doses there is a dose-related increase in several tumour types causing death and a corresponding decrease in mean life-span.

235. The results of experiments using beagle dogs are in general agreement with those obtained using mice. In addition, they confirmed the increased sensitivity of the fetus and young animals. Observed life shortening at accumulated doses greater than a few gray could be related to the development of non-neoplastic diseases at an earlier age than in unirradiated animals. Irradiated animals were susceptible to the development of myeloproliferative disorders, which were frequently expressed as myelogenous leukaemia at dose rates above about 0.003 Gy d⁻¹ and after accumulated doses of a several gray. Error-prone DNA repair mechanisms were proposed to explain the onset of myeloproliferative disorders.

V. EPIDEMIOLOGICAL STUDIES OF RESPONSE IN HUMANS

236. The effectiveness of a cellular adaptive response in humans, expressed in terms of a reduced rate of spontaneously occurring cancers or of a reduction in the expected numbers of radiation-induced cancers, would be most convincingly demonstrated if it were the outcome of epidemiological studies involving exposure to low doses. The general results of these studies are discussed in Annex A, “Epidemiological studies of radiation carcinogenesis”, most of which indicate steadily increasing cancer incidence with increasing dose. Only those cases in which exposures were only marginally above the natural background level are re-examined in this Chapter. For inherent reasons, the evidence of an increase in the spontaneous incidence of cancer is in these cases equivocal.

237. There are theoretical reasons based solely on the nature of DNA damage and repair to expect that cancer can occur at the lowest doses without a threshold in the response, although this effect would perhaps not be statistically demonstrable. The dose-response relationship has been judged to be linear without threshold for all cancers excluding leukaemia. For leukaemia, the relationship that best fits the data is linear-quadratic and linear at low doses. An increase in total cancer mortality has been statistically demonstrated at doses above –0.2 Gy of low-LET radiation but not at lower doses, except in special circumstances such as cancer in childhood following in utero irradiation and thyroid cancer after acute exposure of the thyroid to low-LET radiation during childhood.

238. Among the limitations on the confidence of low-dose epidemiological studies, by far the most consistent problem has been the lack of statistical power. This has been due to a combination of factors, including insufficient numbers in the irradiated population, inadequate follow-up and doses that are collectively too small to have any chance of providing a dose-response relationship.
ANNEX B: ADAPTIVE RESPONSES TO RADIATION IN CELLS AND ORGANISMS

A. INHABITANTS OF HIGH-BACKGROUND AREAS

239. Examples of background radiation studies in France, Japan, Sweden, the United Kingdom and the United States are reviewed in Annex A, "Epidemiological studies of radiation carcinogenesis". The conclusion from these studies is that there is no demonstrable association between leukaemia and other cancers and background radiation.

240. Updated estimates of the cancer risk for a population of about 80,000 persons continuously exposed to natural background radiation at a dose rate about three times higher than the world average have recently been reported [W29, W30]. This population lives in two areas in Yangjiang County of Guangdong Province, China. A comparison is being made with a population of similar size and age structure in a nearby control area of low background radiation in Emping County. Details of the doses are given in paragraph 188. These populations are stable in that there is little movement into and out of the areas. Their age structure is biased towards younger age groups compared with the rest of China. Ascertaining of cause of death is of a high standard, and the dose measurements, based on several different techniques, are in good agreement. The rates of site-specific cancer mortality and estimates of excess risk in the high-background-radiation area, relative to the control area, are given in Table 41 [W29]. The high incidences of primary liver cancer and nasopharyngeal cancer are peculiar to the whole of Guangdong Province and are probably related to local environmental factors, which include infection with hepatitis B virus and smoking, respectively. These two cancer types comprise the largest number of cancers in the two areas and are highest in the low background area. The totals of all cancers other than leukaemia are not strictly comparable with these cancers included.

241. While there are slight differences in the site-specific cancer mortality rates between the two populations, the overall differences in leukaemia and other cancers combined are not statistically significant, and the results so far do not provide clear evidence of the presence or absence of deleterious effects due to low doses of radiation in the environment. This may not be surprising, considering the difference in cumulative dose between the two areas, based on a 50-year exposure, of about 60 mSv. The problem, then, is the lack of statistical power to detect an effect on risk at such low doses.

242. Thyroid nodularity following continuous low-dose exposure in China was determined in about 1,000 women aged 50-65 years living in the high-background-radiation area and in a similar number of controls living in the low-background control area [W28]. Cumulative doses to the thyroid were estimated to be about 0.14 Gy and 0.05 Gy, respectively. For multiple nodular disease, the prevalences in the high-background-radiation area and in the control area were 9.5% and 9.3%, respectively. For single nodules, the prevalences were 7.5% in the high-background area and 6.6% in the control area (prevalence ratio = 1.13; 95% CI: 0.82-1.55). No differences were found in serum levels of thyroid hormones. Women in the high-background region, however, had significantly lower concentrations of urinary iodine. The prevalence of mild diffuse goitre was higher in the high-background-radiation region, perhaps related to a low dietary intake of iodine. The authors suggested that continuous exposure throughout life to a total dose of about 0.1 Gy in excess of the low background level did not influence the risk of thyroid nodular disease; however, this study did not provide statistically significant results.

B. OCCUPATIONALLY EXPOSED INDIVIDUALS

243. Studies of the effects of radiation following occupational exposures are useful in clarifying the possible relationship between relatively low doses of gamma-radiation and the risk of cancer. One relevant study was of nuclear shipyard workers in the United States [M13]. From a database of almost 700,000 shipyard workers, including about 108,000 nuclear workers, three study groups were selected, consisting of 28,542 nuclear workers with working lifetime doses ≥5 mSv (many of them received doses well in excess of 5 mSv), 10,462 nuclear workers with doses ≥5 mSv and 33,352 non-nuclear workers. The type of work carried out by the three groups was identical, except that the nuclear workers were exposed additionally to 60Co gamma-radiation. The median age of entry into employment for the three groups was similar, that is, about 34 years. The study included exposures received from the beginning of nuclear ship overhauls in the 1960s until the end of 1981. All groups worked in areas where intakes of asbestos were possible.

244. Deaths in each of the groups were classified as due to all causes, leukaemia, lymphatic and haematopoietic cancers, mesothelioma and lung cancer. The results, summarized in Table 42, demonstrate a statistically significant decrease in the standardized mortality ratio for the two groups of nuclear workers for "death from all causes" compared with the non-nuclear workers. This was due in part to a higher incidence in the non-nuclear workers of deaths from diseases other than cancer, which included cardiovascular disease and respiratory, genitourinary and digestive tract disorders. Both groups of nuclear workers had lower death rates from leukaemia and from lymphatic and haematopoietic cancers than the non-nuclear workers, but this was not statistically significant. All three groups of workers had lower death rates from lymphatic and haematopoietic cancers than the general population in the United States. This has been referred to as the healthy worker effect. Mesothelioma was the only
cancer that showed a significantly higher incidence for all groups. The slightly higher, but non-significant, incidence of lung cancer for all three groups compared to the general population of the United States could have been associated with asbestos exposure.

245. Two features of this study need to be emphasized. First, the collective lifetime doses from occupational exposure in the two nuclear worker groups were estimated to be 1,450 man Sv and 26 man Sv, respectively. The estimated collective doses from natural background radiation in the same periods were 1,067 man Sv and 409 man Sv, respectively. The collective dose to the non-nuclear workers from background radiation was estimated to be 1,275 man Sv. It is difficult to draw conclusions about the incidence of diseases that might be associated with low-level occupational radiation exposures when the doses are comparable to, or less than, the doses from natural background radiation. Also, the statistically significant decrease in standardized mortality ratio for deaths from all causes cannot be due to the healthy worker effect alone, since the non-nuclear workers and the nuclear workers were similarly selected for employment and were afforded the same health care thereafter.

246. A study of about 95,000 radiation workers in the United Kingdom has recently been reported. The cohort received a collective lifetime dose of 3,200 man Sv, with an average individual dose of 34 mSv [K15]. Up to 1988, 6,600 workers had died. The standardized mortality ratio for all causes of death after excluding the first 10 years following the start of radiation work was 0.85 (p < 0.001). The standardized mortality ratios for all malignant neoplasms in 23 organs or tissues and for all known, non-violent causes other than malignant neoplasms were 0.86 (p < 0.001) and 0.84 (p < 0.001), respectively. For most other tissue-specific cancers, the standardized mortality ratios were below unity, but they were not statistically significant. The only cancer for which an elevated standardized mortality ratio reached statistical significance was thyroid cancer (SMR = 3.03). It is not unexpected that one organ or tissue showed an increased standardized mortality ratio by chance. The excess relative risks for all cancers and for leukemia, excluding chronic lymphocytic leukemia, were 0.47 (90% CI: 0.12-1.20) and 4.3 (0.4-13.6), respectively. A confounding factor in this study was that cancers could have been associated with exposure to chemical carcinogens.

247. Since the healthy worker effect complicates the interpretation of standardized mortality ratios, greater importance was attached by the authors to internal analysis or to testing for the trend in risk with dose, in which steps are taken to compensate for the factors that lead to the healthy worker effect. Positive trends with dose were found for all malignant neoplasms taken together and for leukaemias, excluding chronic lymphatic leukaemia (Figure XX). The former trend did not reach statistical significance (p = 0.10, one-sided test), while the latter was statistically significant (p = 0.03). Because it was considered possible that cigarette smoking may have influenced the results, Figure XX also shows the relative risk for the malignant neoplasms, excluding both lung cancer and leukaemia. The scatter of the points showing the average relative risk and the wide confidence intervals on these points provide no reliable information on the risks at doses below about 0.2 Gy.

248. Updated internal analyses of mortality in 33,000 workers monitored for six months or longer at the Hanford site in the United States provide no evidence of a correlation between cumulative occupational external dose and mortality from leukaemia and from other cancers [G12]. The relative risks and their confidence intervals are summarized in Table 43. Of 24 tissue-specific cancer categories evaluated, only cancer of the pancreas and Hodgkin's disease showed positive correlations with dose that approached statistical significance (one-tailed p-values of 0.03 and 0.04, respectively). These correlations were interpreted by the authors as probably spurious. A significant correlation (p < 0.05) was obtained at doses above 50 mSv but not at 10 mSv. Earlier analysis of mortality from multiple myeloma had shown a significant excess risk [G14], but the extended analysis of these data now show that the excess relative risk for this form of cancer is no longer statistically significant [P1, R10].

249. A study has been under way since 1980 on the mortality of past and present employees of Atomic Energy of Canada Ltd. [G8]. The study population consists of 13,491 persons, 9,997 males and 3,494 females, for a total of 262,403 person-years at risk until 1985. The number of female deaths (121) was too small for detailed analysis, but the 1,178 deaths in the male population gave a limited basis for study. Mortality patterns in the cohort between 1950 and 1985 were examined by comparing the observed mortality with that expected in the general population for three groups of workers: those with no occupational exposure, those with up to about 50 mSv and those with more than 50 mSv. The number of deaths was fewer than would have been expected on the basis of general population statistics for males in the three groups. The findings were similar for the groups "all cancer deaths" and "all other causes of death". In the occupationally exposed males, elevated standardized mortality ratios were seen for non-Hodgkin's lymphoma and for buccal cavity, rectum, rectosigmoid and prostate cancers. But in the
C. SURVIVORS OF THE ATOMIC BOMBINGS

250. The epidemiological study of the survivors of the atomic bombings in Japan has up to now been the primary source of data from which to estimate the effects of radiation on humans. A dose-response analysis of these data for doses less than 0.5 Sv was recently presented [S30]. The endpoints measured were cancer mortality, cancer incidence and non-cancer mortality. The relative risks with 95% confidence intervals and the dose-response relationships fitted by the authors are illustrated in Figure XXI.

251. For mortality from leukaemia, the relative risks varied among the five dose groups (0.010-0.019, 0.020-0.049, 0.050-0.099, 0.100-0.199, 0.20-0.489 Sv), but they did not differ statistically from the control group, which received less than 0.010 Sv (p > 0.10). The relative risks for the three dose groups less than 0.1 Sv were less than unity but still within the range of what the authors considered to be random variation about a value equal to unity. For leukaemia mortality, a linear-quadratic model fitted marginally better than a linear model (p = 0.07 compared with 0.06).

252. For all cancers other than leukaemia, the relative risks generally increased with dose, as shown by the dose-response curve, although the lowest four dose groups had relative risks not significantly different from unity. The highest dose interval (0.20-0.49 Sv) showed a statistically significant increase in mortality.

253. Mortality from lung cancer and incidence of thyroid cancer by dose group, based on data from the Hiroshima and Nagasaki tumour registries during 1958-1987, were also analysed. A pattern similar to that observed for mortality from all cancers except leukaemia was seen, that is, the relative risk varied among comparison groups with wide confidence limits but did not differ statistically from unity within the lowest dose intervals.

254. For mortality from all diseases other than cancer, a significantly elevated risk was observed at higher doses (estimated threshold dose: 1.5 Sv) for younger survivors of the atomic bombings (age at the time of bombings <40 years). However, the relative risks for the various low-dose groups (<0.5 Sv) did not differ and were close to unity with the exception of one significant point at the dose interval 0.20-0.49 Sv (RR = 0.83). It is interesting to note that the decrease in relative risk at this point corresponds to a significant increase in relative risk for mortality from all cancers except leukaemia.

D. PATIENTS EXAMINED OR TREATED WITH RADIATION

255. There are several examples in clinical practice where low doses have been used for diagnostic purposes. These are x-ray examinations to detect fetal abnormalities, x-ray fluoroscopy to check the efficacy of artificial pneumothorax in the treatment of pulmonary tuberculosis, (e.g., [B2]), x-ray examinations to assess the progress of skeletal development during treatment for scoliosis and [31] diagnostic tests to detect thyroid abnormalities.

256. The available studies [G3, H19, M8, M22, M26, S24] of in utero exposure are discussed in detail in Annex A, "Epidemiological studies of radiation carcinogenesis". The main conclusion is that the low-dose exposure of the fetus in diagnostic examinations is associated with a positive risk of cancer induction, but quantification of the risk is subject to much uncertainty. There is nothing to support the assumption that adaptive processes could be operating after irradiation that could reduce the incidence of radiation-induced cancers.

257. The evidence for radiation-induced breast cancer is discussed in Annex A, "Epidemiological studies of radiation carcinogenesis". Although exposure to radiation at high doses and high dose rates is associated with excess breast cancer, the potential hazard from low-dose, fractionated exposures during early breast development has not been thoroughly evaluated. The failure to detect increased breast cancer in several large studies is surprising, and no satisfactory explanation is forthcoming. However, many of the women were over 35 years of age at exposure.

258. A retrospective study of 35,074 Swedish patients receiving $^{131}$I for suspected thyroid disorders between 1951 and 1969 has been reported [H17, H18, H21]. It is not possible to be precise about the doses delivered to individual thyroids because of differences in $^{131}$I uptake and variations in mass of the thyroids. However, the average amount of $^{131}$I activity administered was 1.92 MBq, delivering an estimated average dose of approximately 0.5 Gy to the thyroid. The data are given in Table 44. Patients given $^{131}$I for reasons other than a suspected tumour were not at increased risk (standardized incidence ratio = 0.62; n = 16). Overall, the data provide no indication that exposure to $^{131}$I for diagnostic purposes
increased the incidence of thyroid cancer in the follow-up period after 10 years. It was concluded by the authors that the observed increase in the 5-9 year period was most likely to be due to a high level of medical surveillance, leading to an increased detection of indolent tumours.

E. SUMMARY

259. The human epidemiological studies following exposures at low doses and low dose rates to low-LET radiation do not at present provide evidence of an adaptive response expressed as a decrease in the prevalence of spontaneously occurring human cancers. This is not surprising in view of the low statistical power of these studies. The results have been interpreted variously as being consistent with the upper bound on the confidence limits of total cancer risk at low doses obtained by extrapolating from high-dose and high-dose-rate data; or as indicating no additional risk at low doses compared with the spontaneously occurring rate. Statistical limitations do not permit a decisive choice at the present time. Caution is necessary when using isolated examples in the epidemiological literature to justify either an increased or a decreased risk at doses of a few hundred milligray, bearing in mind the statistical limitations of the data.

CONCLUSIONS

260. Adaptive response is the collective term used to describe the results of experiments in which a small dose of radiation can condition cells so as to induce repair processes and/or to stimulate proliferation. One consequence of DNA repair might be to reduce the natural incidence of cancer in its various forms or the likelihood of excess cancer being caused by further radiation exposure. A great deal of effort is being directed into characterizing these processes, and in recent years results of research especially at the cellular level have become available.

261. There is convincing evidence that the number of radiation-induced chromosome aberrations and mutations that occur in proliferating mammalian cells after an acute dose of low-LET radiation in the range 1-3 Gy can be reduced by exposing the cells to an acute dose of between a few milligray and a few tens of milligray several hours before the high dose. These experiments involving a low conditioning dose and a high challenge dose were designed to demonstrate the adaptive response as a laboratory phenomenon. They were carried out under clearly defined conditions using mitogen-stimulated lymphocytes, proliferating bone marrow cells, spermatocytes and fibroblasts. The response has not been demonstrated so far in other cell systems or convincingly in cells under conditions of chronic exposure.

262. The evidence that is becoming available indicates that following radiation-induced damage to cells, a number of changes occur. Among these changes are the activation of several classes of genes, including those coding for the synthesis of enzymes involved in the control of cell cycling, proliferation and repair. It is not entirely clear how these changes may specifically improve repair capacity. There is some evidence to indicate that radiation-induced enzymes, which remain to be isolated and characterized, are related to stress-response proteins. There seems to be some similarity in the types of damage induced by radiation and other toxic agents. The adaptive response may therefore be a common feature of cellular response to damage.

263. A multi-step process has been proposed to explain the cellular adaptive response. After acute doses of several hundreds of milligray, cell cycling in proliferating cells may be delayed. The period of delay allows enzymes induced by the radiation to repair damage before the cells proceed through cell cycle and undergo mitosis [Y5, Y6]. The adaptive response at these higher doses may therefore depend on whether or not the cells in cycle are temporarily blocked. There is no direct evidence, however, to suggest that cell cycle delay occurs after acute doses ranging from a few milligray to a few tens of milligray, even though the adaptive response has been observed in this range of doses. The fate of cells exposed to a radiation dose in the resting phase remains to be established.

264. In evaluating the effectiveness of the adaptive response in cells exposed to a conditioning dose of up to a few tens of milligray or to concentrations of toxic agents below that concentration known to produce a toxic reaction, it is important to recognize the unstable nature of DNA in living cells during normal metabolism [H2, L11, L21, V3, V4]. It has been estimated that the DNA molecule within each nucleus undergoes several thousand detectable changes every hour as a result of metabolism [B10, L12, S27, W12, W13, W14, W15]. Despite this high rate of spontaneous molecular change, few stable mutations accumulate in the genome. Thus, cells have evolved efficient processes for the correction of metabolically induced changes.

265. This inherent ability to repair DNA needs to be taken into account in assessing the ability of cells to repair the damage caused by doses of radiation in the wide range of a few milligray per year to a few tens of milligray
delivered in minutes. Just how capable the existing mechanisms are of coping with the additional radiation-induced damage is not readily obvious. But it would be reasonable to assume from the evidence that damage caused by natural background radiation, in which the energy deposition events in a particular nucleus are separated by weeks or months [B21], should be readily repairable by the available metabolically driven mechanisms.

266. However, errors in repair do occur, even during metabolism, such as small base-sequence changes (point mutations), gene deletions or rearrangements, although the overall DNA integrity may be retained [F3, F4, F5]. It needs to be recognized, therefore, that the effectiveness of DNA repair in irradiated mammalian cells is not absolute, some fraction of the cells retaining stable mutations. Thus, the same low conditioning doses that result in an adaptive response are likely also to result in malignant cellular transformations by the mechanisms discussed in Annex E, "Mechanisms of radiation onco-genesis" in the UNSCEAR 1993 Report [U1]. It would seem important to judge the balance between the fidelity of repair, residual damage and malignant transformations and whether indeed these effects interact with each other. The Committee hopes that more data will become available in the near future to address this point.

267. Alternative cellular mechanisms have been proposed to explain the adaptive response. These include the detoxification of reactive radicals, thereby reducing the potential for damage, and the activation of membrane-bound receptors stimulating cell proliferation. Efforts should be made to characterize the possible role of these processes.

268. It remains doubtful whether the immune system plays a significant role in any of the adaptive processes at low doses. In the UNSCEAR 1993 Report [U1], the Committee concluded that the immune system may not play a major role in moderating human radiation onco-genesis, although immune function in certain organs may ensure that some early neoplastic cells are eliminated before they become established. The data in this Annex are not in conflict with this generalized conclusion. Some transient effects on the ratio of subsets of T cells and in accelerating programmed cell death in damaged lymphocytes have been identified. It is interesting in this respect to note that a dose of a few hundreds of milligray can influence tumour growth kinetics, expressed as a transient reduction in tumour size in experimental animals. The evidence for changes in the human immune system long after exposure is not convincing.

269. Animal experiments in the 1950s and 1960s showed that chronic exposure of rodents at doses of up to a few milligray per day from low-LET radiation could result in increased life-span compared to controls exposed to only background radiation. However, some anomalies in these experiments need to be explained. Why was the response confined to male mice, and why was it not observed consistently in pathogen-free mice?

270. More recent experiments with rodents and beagle dogs exposed at various ages to low dose rates of low-LET radiation have generally been unable to demonstrate any statistically significant difference in life-span of irradiated and control groups after accumulated doses of up to about a gray. However, tumour incidence did not increase until the dose was in excess of about a gray, depending on the mouse strain and the susceptibility of the animals to developing spontaneous tumours. In some studies there was a non-statistical trend towards a lower-than-expected incidence of tissue-specific tumours, but in other studies there was a non-statistical trend towards a higher-than-expected incidence at doses of ≥2 Gy. A reduction in life-span or an increase in tumour incidence after fractionated exposures was not of statistical significance until accumulated doses exceeded a few gray.

271. The low statistical power of most human epidemiological cancer surveys with exposures at low doses makes it difficult to reach a decisive conclusion on the existence or absence of an adaptive response. Studies of exposure to higher-than-average levels of natural background radiation have made little contribution so far to estimating the risk of cancer from low-dose-rate, low-LET radiation. Studies of occupational exposures have recently shown more promise of yielding positive results, especially after moderate doses, but in the low-dose region the confidence limits are so broad that the results are still equivocal. The Life Span Study of survivors of the atomic bombings shows no significant excess of total cancer mortality below about 0.2 Gy. All cancers other than leukaemia are in excess but not statistically significant down to a dose range of 0.01-0.05 Gy. Leukaemia shows a deficit at doses less than 0.1 Gy, which again is not statistically significant. At present no conclusion can be drawn about the dose response below 0.2 Gy because of statistical limitations.

272. In conclusion, there is substantial evidence of an adaptive response in selected cellular systems following acute exposure to conditioning doses of low-LET radiation. The precise molecular processes involved in the adaptive response are not well understood at present, but cellular repair is likely to play a role by mechanisms similar to those involved in the generalized stress response. The presence of an adaptive response is not readily evident from the results of experiments in mammalian organisms in terms of reduced tumour induction. Interpretation of these experiments is complicated by variations in the susceptibility of different animal strains to spontaneous tumour induction. The low statistical power of the epidemiology studies also prevents a clear statement on the presence of an adaptive response in humans.
exposed to low doses. It is to be hoped that better understanding of mechanisms of radiation effects obtained in molecular studies might provide a basis upon which to judge the role of adaptive response in the organism. In the meantime, it would be premature to conclude that cellular adaptive responses could convey possible beneficial effects to the organism that would outweigh the detrimental effects of exposures to low doses of low-LET radiation. The Committee recommends that this research be continued in order to clarify the nature and importance of the effects of radiation-induced adaptive response.