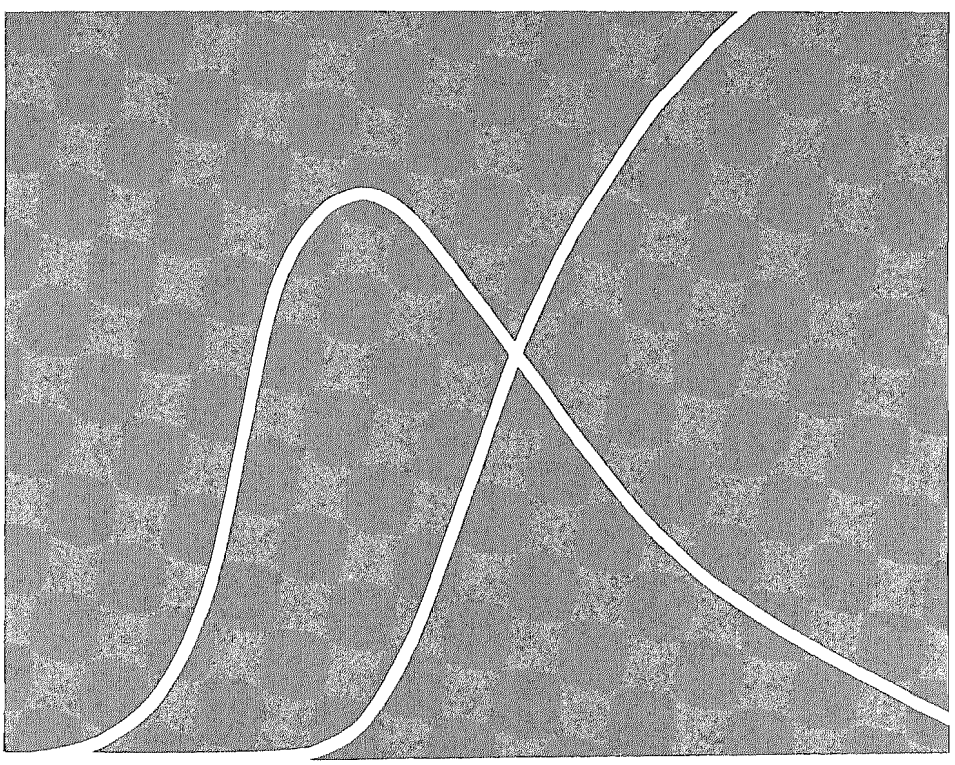
IONIZING RADIATION: LEVELS AND EFFECTS

A report of the United Nations Scientific Committee on the Effects of Atomic Radiation to the General Assembly, with annexes

VOLUME II: EFFECTS



UNITED NATIONS

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UNITED NATIONS
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NOTE

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ABBREVIATIONS

ILO	International Labour Organisation
FAO	Food and Agriculture Organization
WHO	World Health Organization
WMO	World Meteorological Organization
IAEA	International Atomic Energy Agency
ICRP	International Commission on Radiological Protection
ICRU	International Commission on Radiological Units and Measurements

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ABCC	Atomic Bomb Casualty Commission
AEC	Atomic Energy Commission
JNIH	Japanese Institute of Health

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AGR	Advanced gas-cooled graphite-moderated reactor
ATB	At the time of bombing
BWR	Boiling light-water cooled and moderated reactor
CMD	*Per caput* mean marrow dose
DNA	Deoxyribonucleic acid
ECBI	Extracorporeal blood irradiation
FBR	Fast breeder reactor
GCR	Gas-cooled reactor
GSD	Genetically-significant dose
HVT	Half-value thickness
ICD	International classification of diseases
LET	Linear energy transfer
LWR	Light-water reactor
NIC	Not in city at the time of bombing
OMR	Organic moderated and cooled reactor
PHWR	Pressurized heavy-water moderated and cooled reactor
PPD	Purified protein derivative
PWR	Pressurized light-water moderated and cooled reactor
RBE	Relative biological effectiveness
RNA	Ribonucleic acid
SST	Supersonic transport
WL	Working level
WLM	Working level month

ANNEXES (continued)

Effects

ANNEX E

GENETIC EFFECTS OF IONIZING RADIATION

CONTENTS

phase oöcytes in the first meiotic division whereas the lowest ones are encountered in the dictyate oöcytes in mammals and in oögonia in insects (mammalian oögonia have not yet been studied from this point of view); (*d*) the time at which death due to dominant lethality occurs varies in different species; and (*e*) dominant lethals induced in mouse spermatogonia may be due to the unbalanced products of translocations which can successfully pass through the post-meiotic stages of spermatogenesis and be transmitted to the immediate offspring. Studies carried out during the past few years fully support these conclusions.

3. Recent experiments have measured dominant lethality using the pre-natal method,[1] because of its greater reliability compared to the method based on litter size. However, in investigations designed for other purposes, reduction in litter size has been used to estimate the proportion of deaths due to induced dominant lethals (34, 71, 543).

1. *Spermatogonia*

4. The induction of dominant lethals in mouse spermatogonia has been studied earlier by several investigators (37, 245, 246, 288, 396, 507). Recently, Schröder (474) studied this problem using an x-ray exposure of 600 roentgens. The frequencies of dominant lethals varied over a wide range but most of the differences were not significant. The induced frequency of pre-implantation losses can be estimated to be between 2.0 and 8.0 per cent and that of post-implantation losses between 5.0 and 14.0 per cent. The latter is much higher than the frequency of 2.0 per cent recorded by Sheridan (507) after an exposure of 550 roentgens. The over-all frequency of induction of dominant lethals obtained by Schröder is about 10.0 per cent.

5. Pomerantseva and Ramaia (622) found that the frequency of post-implantation losses observed after irradiation of mouse spermatogonia remained at about the same level after x-ray exposures ranging from 400 to 1,200 roentgens. The frequency at 400 roentgens was estimated to be about 4.0 per cent or 1.0 10^{-4} per roentgen. Ehling's recent results (126) show that the frequencies of induced post-implantation losses are 3.0, 6.5 and 5.5 per cent, respectively, after 200, 400 and 800 roentgens (^{137}Cs gamma rays) to spermatogonia. The lack of increase in frequency above 400 roentgens is in line with the observations of Pomerantseva and Ramaia (622).

6. Litter-size data were used by Batchelor, Phillips and Searle (34) to evaluate the incidence of dominant lethality in a study designed mainly to estimate the RBE of neutrons relative to gamma rays. A neutron dose of 214 rads (plus 93 rad gamma contamination) and a gamma-ray dose of 606 rads (plus 2.5 rad neutron contamination) were delivered, in both cases, over a 12-week period. It was found that the mean litter sizes with neutron- and gamma-irradiation were 5.95 and 6.22, respectively; the difference of 0.27 is significant and represents a 4.3 per cent reduction with neutrons (1,806 pairs of litters compared; the total number of animals born was 10,751 in the neutron series and 11,237 in the gamma series).

7. Chambers (71) x-irradiated rat spermatogonia at exposures of 600 roentgens (single, testicular irradiation) or 450 roentgens (in three fractions of 100, 150 and 200 R at 10, 12 and 14 weeks of age; whole-body). Using F_1 litter size at one day of age as the criterion of dominant lethal damage, he estimated the rate of induction to be in the range between (1.2 ± 1.5) 10^{-4} and (3.3 ± 2.9) 10^{-4} per gamete per roentgen.

8. In experiments involving spermatogonial x-irradiation of rat populations (for details see paragraph 216) Taylor and Chapman (543) also used litter size as a measure of dominant lethal damage and estimated the rate to be between (1.4 ± 0.6) 10^{-4} and (0.9 ± 0.9) 10^{-4} per gamete per roentgen. The authors point out that these values are in good agreement with the average estimate for mouse spermatogonia which is 1.1 10^{-4} per gamete per roentgen (217, 246, 270, 276, 288, 454).

(a) *Relationship between induced dominant lethals and translocations*

9. In the 1966 report, it was suggested that a major fraction of dominant lethality induced by spermatogonial x-irradiation might in fact be due to the unbalanced products of translocations. The correctness of this surmise has now been strengthened by the results of the study of Ford *et al.* (139). Male mice received an x-ray exposure of 1,200 roentgens in two equal fractions separated by eight weeks. In the pilot experiment, spermatocytes derived from irradiated spermatogonia were directly examined for the presence of translocations, and, in the main experiment, the irradiated mice were first allowed to produce a large number of progeny and later sacrificed for making cytological preparations. Frequencies of spermatocytes with various numbers and types of multivalents were used to estimate the proportion of sperm with normal, balanced-translocated and unbalanced haploid genomes and hence the expected frequencies of zygotes with abnormal karyotypes. The results are summarized in table 1.

10. It can be seen that the *expected* frequencies of dominant lethals and of semi-steriles are twice as large as those observed in F_1 sons and in other genetic experiments with the same radiation exposure (288, 477). The discrepancy between the expected frequencies of semi-steriles and dominant lethals and the frequencies actually observed is assumed to originate from a selective process operating on translocation-carrying diploid (rather than on haploid) genomes between meiotic metaphase and fertilization (139).

11. Lyon *et al.* (288) observed a frequency of semi-steriles that implied an associated dominant lethality of 6.6 per cent. The observed frequency of dominant lethals being 10.6 per cent, the authors attributed the 4 per cent excess to "primary" dominant lethality not dependent on segregation as such. It is now evident that all the dominant lethals observed genetically can be accounted for by the segregation of unbalanced haploid genomes from spermatocytes with translocation multivalents. Nonetheless, the possibility of some "primary" dominant lethality is not excluded, although the fact that less than 1 per cent of spermatocytes exhibit chromosomal changes other than multivalent associations indicates that only a very small proportion of dominant lethals can be attributed to

[1] Females are dissected at suitable stages of pregnancy (12-18 days for the mouse, 14-31 days for the guinea-pig, 13-19 days for the rabbit and 9-15 days for the hamster) and the numbers of *corpora lutea* and of dead and living implanted embryos are counted. It is thus possible to estimate the proportion of pre-natal deaths that occur before or after implantation.

other forms of gross chromosomal changes induced in pre-meiotic cells.

(b) *Fractionation*

12. The complete results of the fractionation experiment of Sheridan (510) (reported at a preliminary stage in the 1966 report) show that the frequency of post-implantation losses with a single x-ray exposure of 275 roentgens to mouse spermatogonia is 3.3 per cent whereas that with the same exposure delivered in 55 daily fractions is 0.3 per cent. The difference is clearly significant. These results would be expected if dose fractionation reduced the frequency of induction of translocations. Evidence showing that this is indeed the case is presented in paragraphs 72, 79 and 80.

13. Lyon and Morris (283) obtained a nearly three-fold increase in the frequency of dominant lethals when the spermatogonia received an x-ray dose of 1,000 rads in two equal fractions separated by 24 hours, instead of a single dose (18.2 per cent with fractionation *versus* 6.6 per cent for the single dose). The frequencies of post-implantation losses alone were 14.0 and 2.0 per cent with fractionated and single doses, respectively. In the same study, the yield of translocations, specific-locus and dominant visible mutations were also found to be enhanced by fractionation (paragraphs 69, 158, 197).

2. *Post-meiotic stages*

(a) *Dose-response relationships*

14. Léonard (247) and Léonard and Deknudt (251) observed that the relationship between x-ray exposure and yield of dominant lethals in mouse spermatozoa was linear over a wide range of exposures. The estimated rates of induction of dominant lethals are 1.5 10^{-3} per roentgen (10-100 R; 10 levels) and 1.1 10^{-3} per roentgen (100-6,000 R; 15 levels). In the range from 10 to 100 roentgens, the frequency of pre-implantation losses is low (1-3 per cent) whereas that of post-implantation losses shows a steady increase with increasing exposures. Above 100 roentgens, the frequencies of both pre- and post-implantation losses increase with increasing exposures. In addition, the percentage of pregnant females and the number of implants per female are reduced with exposures from 100 to 6,000 roentgens.

15. Pomerantseva and Ramaia (622) observed a linear relationship between x-ray exposure and the frequency of post-implantation losses when mouse spermatozoa were irradiated. The rate of induction is 1.0 10^{-3} per roentgen (100-1,200 R; 6 levels). This figure is almost identical to that of Léonard (paragraph 14), in spite of the fact that Léonard's estimate applies to both pre- and post-implantation losses. A linear relationship (1.5 10^{-3} R^{-1}) was also observed by the same authors after x-irradiation of spermatids (100-900 R) and spermatocytes (100-600 R). The delineation of the stages, however, was not clear-cut.

16. More recent evidence for the linear dose-effect relationship for dominant lethals induced in meiotic and post-meiotic stages of the male mouse comes from the work of Schröder and Hug (476) and of Ehling (126). Ehling, however, uses a different procedure[2] to estimate the frequency of dominant lethals and consequently his figures are not directly comparable to those given by others.

(b) *Stage differences in sensitivity*

17. Sensitivity differences of the spermatogenic stages in the induction of dominant lethals (and of other types of genetic damage) are known to exist between the mouse and other organisms. Recently, Ehling (126) found that after x-irradiation (200 R) of male mice the frequency of dominant lethals in early spermatids was nearly twice that in spermatozoa, late spermatids and spermatocytes. With 400 or 800 roentgens, however, the spermatocytes showed the highest sensitivity, the number of live embryos per female in the irradiated groups being far below that in the controls. For induced post-implantation losses that can be estimated from his data (all exposures) spermatids (sampled between 15 and 22 days after irradiation) show maximal sensitivity.

18. Ehling (126) found that pre-irradiation injection of chloramphenicol led to an enhancement of the frequency of dominant lethals in mouse spermatozoa (exposure: 600 R single; two equal fractions of 400 R separated by 24 hours). This result is similar to what has been observed for sex-linked lethals in *Drosophila* spermatozoa (528). The mechanism of chloramphenicol-mediated enhancement of dominant lethality in mouse spermatozoa is not known.

19. Ehling (127) also observed that treatment of males with mitomycin-C (intraperitoneal injection; 1.75 mg kg^{-1}) prior to irradiation with 200 roentgens (^{137}Cs) resulted in a drastic reduction of the embryonic litter size, the magnitude of the reduction far exceeding those in parallel series treated with either mitomycin-C or gamma rays alone; this synergistic effect was very pronounced in the mating intervals from 27 to 34 days after treatment.

20. In another study Ehling (123) examined the effects of pre-treatment with aminoethylisothiourea (AET) and observed a decrement in dominant lethality in early spermatids; the mean number of embryos per female increased from 1.1 $\pm$ 0.1 in the controls receiving NaCl and an x-ray exposure of 600 roentgens to 2.6 $\pm$ 0.2 in the group receiving AET and 600 roentgens. The radio-protective action of AET was less pronounced after an exposure of 1,000 roentgens.

21. In similar work with 5-methoxytryptamine pre-treatment, Pomerantseva (621) observed a reduction of x-ray induced dominant lethals in spermatids but not in spermatozoa. With cysteamine pre-treatment, decreased yields of dominant lethals were obtained in spermatocytes, spermatids and in spermatozoa (617).

(c) *Species differences*

22. Lyon (281) carried out a study comparing the pattern of sensitivity to dominant lethal induction in the male germ-cell stages of the mouse, guinea-pig, golden hamster and rabbit. Attention was focused primarily on the response of post-meiotic germ cells although some limited information was obtained for the germ-cell stages sampled soon after the period of sterility.

23. The data are presented in table 2 which shows that (*a*) the frequencies of dominant lethals at the dose

[2] Dominant lethal frequency $= 100 - \left(\frac{\text{live embryos per female in the experimental group}}{\text{live embryos per female in controls}}\right) \times 100$

f 500 rads are lower in the guinea-pig and the rabbit han in the mouse; the pattern of relative sensitivity of he germ-cell stages, however, is similar in these three pecies, spermatids (sampled during the third week in he mouse but in the fourth and fifth weeks in the guinea-pig and in the rabbit) being more sensitive than nature spermatozoa (first week). The finding in the present study, that the rabbit is less sensitive than the mouse, is at variance with the results of Shapiro *et al.* (624) who found the opposite; (*b*) after a dose of 200 rads to the hamster, the yield of dominant lethals from mature sperm is nearly as high as after 500 rads to the mouse; (*c*) in the hamster, spermatids and mature sperm show an approximately similar response, the sensitivity pattern thus being different from that in the other three species; (*d*) for weeks 2-4, the yield of dominant lethals in hamsters after 200 rads is considerably lower than in mice after 500 rads; (*e*) in the mouse, hamster and guinea-pig, a large proportion of deaths occurs after irradiation whereas it occurs prior to implantation in the rabbit; and (*f*) after equal doses, the pre-sterile period in the rabbit and in the guinea-pig is about one week longer than in the mouse.

3. *Oöcytes*

24. Investigations on the sensitivity of the mouse oöcytes to the induction of dominant lethal damage at diplotene (dictyate) and at stages beyond diplotene were carried out earlier by Russell and Russell (434) and by Edwards and Searle (122). Similar studies had been performed with the golden hamster (172). To obtain more information on the sensitivity of mature diplotene oöcytes of guinea-pigs and golden hamsters, Lyon and Smith (289) irradiated young adults of these species. To ensure that the ova were at the diplotene stage at the time of irradiation, females were irradiated in middle dioestrus and immediately caged with fertile males. The females which mated at the first œstrus after irradiation were dissected during mid-pregnancy and the numbers of *corpora lutea* and of live and dead embryos were counted as in the experiments with irradiated males.

25. The results are given in table 3 which shows that (*a*) the mean number of ovulated eggs per female is slightly enhanced by the irradiation; (*b*) as after irradiation of males, most of the induced embryonic death occurs after implantation, although there is some pre-implantation loss after the highest dose to the guinea-pigs; (*c*) in both species it is in fact only the highest dose which gives a really marked yield of dominant lethals.

26. A comparison of the data of Lyon and Smith (289) with those published earlier (122, 434) shows that, at least at high doses, both the hamster and the guinea-pig are more sensitive to the x-ray induction of dominant lethals than the mouse. However, more data are needed to assess the significance of this finding.

4. *Summary and conclusions*

27. In meiotic and post-meiotic stages of the male mouse, the frequencies of dominant lethals increase linearly with increasing exposures; in contrast, in spermatogonia, the frequencies seem to level off at high exposures, as would be expected from the results of translocation studies.

28. Almost all the dominant lethality induced in mouse spermatogonia is due to the unbalanced products of translocations.

29. After an x-ray dose of 500 rads to males, both guinea-pigs and rabbits give a lower yield of dominant lethals than the mouse, but they show a similar pattern of relative sensitivity of germ-cell stages, spermatids being more sensitive than mature spermatozoa. Hamsters, after a dose of 200 rads, give a yield of dominant lethals from mature spermatozoa nearly as high as mice after 500 rads, but the pattern of sensitivity is different, mature sperm and spermatids being almost equally sensitive and giving a lower yield, close to that expected in mice after 200 rads.

30. Thus, in extrapolating from species to species, account must be taken of different patterns of relative sensitivity of germ-cell stages as well as of over-all differences in sensitivity.

31. At least at high doses, the mature diplotene oöcytes of the hamster and guinea-pig are more sensitive than those of the mouse to the induction of dominant lethals by x-irradiation.

B. Sensitivity of oöcytes to cell-killing effects

32. The most distinctive feature of oögenesis in mammals is the absence of oögenia from the postnatal adult ovary. Female mammals are born with a finite number of oöcytes formed already during embryonic development. These so-called primordial oöcytes are surrounded by a single layer of follicular cells. With maturation, the oöcytes grow and multilayered follicles are formed. In young adults of both the rat and rhesus monkey, the number of growing oöcytes amounts to 10 per cent of the total population, the remaining 90 per cent being primordial follicles (39).

33. In the oöcytes, the sequence of nuclear changes comprising meiosis is arrested at the diplotene stage which lasts until the time of ovulation. The nuclear morphology of the diplotene stage of the "arrested" oöcyte, however, varies widely between species. A "typical" diplotene is characteristic of man, the rhesus monkey, the goat and the dog. A synizesis-like diplotene (chromosomes clumped into a dense knot) is characteristic of the guinea-pig and a diffuse interphase-like diplotene (dictyate) is present in the mouse, the rat and a few related species of rodents such as the hamster, the deermouse and the gerbil (23, 24, 25, 359, 364).

34. The suggestion has often been made that differences in the radiation response of oöcytes to killing, both within and between species, may be correlated with variations in nuclear configuration (20, 23, 294, 359). The chromosomes in the nucleus of the primordial oöcyte in man and rhesus monkey are of the so-called lampbrush type, similar in form to those of amphibia and other lower vertebrates (58), consisting of a central axis from which lateral loops protrude on either side in association with clusters of ribonucleoprotein granules (25). The oöcytes in growing follicles in all the species examined possess lampbrush-type chromosomes.

35. Oöcytes with the lampbrush-type chromosomes have been found to be resistant to the cell-killing effects of irradiation. Baker (19, 21) observed that the primordial oöcytes in the rhesus monkey are eliminated

only after an x-ray exposure of 7,000-12,000 roentgens, and that the $LD_{50/30}$ is 5,000 roentgens. In contrast, exposure of mice to 15 roentgens, and of rats to 100 roentgens gives effects similar to those obtained with 5,000 roentgens in the monkey. In the mouse, Oakberg and Clark (364) have shown that almost all primary oöcytes are destroyed by 50 roentgens whereas in guinea-pigs they survive several hundred roentgens. Shapiro *et al.* (624) and Petrova (620) showed that in the guinea-pig and the golden hamster, œstrus cycles persist for several months after an exposure as high as 400 roentgens.

36. In contrast to the drastic differences in the response of the primordial oöcytes of the rhesus monkey on the one hand and of mice and rats on the other, the response of oöcytes in growing follicles is more comparable: exposures of mice to 2,000 roentgens and of rats to 4,400 roentgens result in approximately the same amount of killing as from 5,000 roentgens in the monkey (22, 39).

37. In an extension of their study, Baker and Neal (26) and Baker (20) found that the responses of the oöcytes of rats, mice, monkeys and humans maintained in organ cultures to the cell-killing effects of radiation are essentially similar to those reported from *in vivo* studies, i.e. monkey and human oöcytes are far more resistant than those of the rat and the mouse. Of particular importance is the observation that a majority of human oöcytes in organ culture survived for seven days after an x-ray exposure of 2,000 roentgens (almost all the cells were destroyed by 4,000 R) whereas in the rat an x-ray exposure of only 300 roentgens was sufficient to nearly deplete the population of primordial oöcytes.

38. Baker, Beaumont and Franchi (23) have proposed that the high radiosensitivity of the oöcytes at the dictyate phase may be related to the fact that, during this phase, the axial core and loops of the lampbrush chromosomes (of which DNA is a major constituent) become extended and the ribonucleoprotein (RNP) sheath more diffuse. Parts of the genome may thus become more sensitive to radiation damage because they lack the protection afforded in the monkey by the continuous RNP sheath. The latter may either shield the genetic material or, more probably, act as a "splint" allowing restitution and repair to take place. Miller, Carrier and von Borstel (305) reported that radiation-induced breaks in lampbrush chromosomes in the newt (examined *in vitro*) became apparent only when the sheath was dispersed by proteolytic enzymes.

39. Searle (480) has recently pointed out that it seems unlikely that the very drastic and rapid radiation killing of mouse and rat immature dictyate oöcytes (for example, loss of 93.5 per cent of all oöcytes in 10-day-old female mice within 3 days after a 25-R x-ray exposure (358)) can result just from the non-repair of breaks in the genetic material of cells at this non-dividing stage.

40. Whatever the underlying basis, judged from cell-survival experiments, monkey and human oöcytes are more resistant to radiation than mouse oöcytes. However, the differences in sensitivity to the induction of genetic damage (mutations, chomosome aberrations, etc.) may not be of the same magnitude as the one for cell survival and may also vary with the genetic criterion used to assess the difference. For example, the data of Lyon and Smith (289) (paragraphs 24-26) suggest that the hamster and the guinea-pig, at least at high doses, are more sensitive to x-ray induction of dominant lethals than the mouse. In contrast, the hamster and the guinea-pig are species in which the sensitivity of the oöcytes to cell-killing is much lower than in the mouse (paragraph 35). Results of this kind reinforce the need for caution in applying the quantitative rates obtained in the mouse to the problem of risk estimates in man.

C. Translocations

41. In its 1966 report, the Committee reviewed the evidence then available on the induction of translocations in pre-meiotic and post-meiotic germ-cell stages of the male mouse. It was pointed out that the presence of translocations is usually diagnosed through the incidence of semi-sterility in the offspring of those exposed, with cytological confirmation of translocation heterozygosity when possible. While this approach is still being pursued, attention is now focused on a direct cytological examination of the testes of the treated males (thus making possible the study of pre-meiotic germ cells) or of F_1 males sired by treated males, to investigate the induction of viable and transmissible chromosome rearrangement in both pre-meiotic and post-meiotic stages. The development of an air-drying technique for meiotic preparations of mammalian testes (131) has facilitated this line of inquiry and has greatly accelerated research.

42. Cytological examination of dividing primary spermatocytes of untreated mice at the diakinesis or first-metaphase stages of meiosis usually shows that 20 bivalents are formed. Because of the precise pairing of homologous chromosomes that exists at these stages, it is possible to correlate abnormal configuration with specific chromosomal changes induced by irradiation or by other treatments. The frequency of multivalent configurations gives a better indication of the frequency of induction of translocations than is obtainable from a genetic analysis, since the time available for the action of selective processes is shorter.

43. There have only been two studies on the induction of translocations in irradiated mouse oöcytes. In the late fifties, L. B. Russell and Wickham (435) reported a very small decrease in the fertility of male mice after acute x-ray exposures of 400 roentgens to their mothers, only 1 male in 320 being semi-sterile with semi-sterile offspring, and thus presumably heterozygous for a reciprocal translocation. However, a few others were sterile and so presumably may also have carried translocations, though cytological methods for determining this were then not available. Searle (479) and Searle and Beechey (482) carried out a large-scale study involving irradiation of late dictyate oöcytes at fast-neutron doses of 100 or 200 rads and at an x-ray exposure of 300 roentgens. There was no evidence of inherited semi-sterility in the neutron series; in the x-ray series, the results of tests completed thus far show that 1 out of 386 sons tested was sterile although no chomosome abnormality could be found. However, 8 out of 293 daughters were judged semi-sterile on the criterion of litter size and four of these showed definite evidence of being translocation-carriers. Thus the translocation frequency in daughters is probably between 1.4 and 2.7 per cent.

1. *Adult spermatogonia*

(a) *Acute exposures*

(i) *X rays*

44. The data obtained from experiments involving acute x-irradiation of spermatogonia are summarized in table 4 which indicates that heterogeneities often exist between investigators and within exposures studied at different times. In addition, differences between mice and between testes of a single mouse have sometimes been noted.

45. The frequencies of translocations seem to increase linearly with exposures, at least over the 25- to 600-roentgen range (15, 132, 139, 248, 250, 253, 254, 256, 258, 283, 284, 347, 463, 488, 491). This is unexpected since a dose exponent greater than one is normally found for the induction of two-track aberrations by low-LET radiations (326, 327). Using the data from four different sets of experiments each with different but occasionally overlapping exposure ranges, and excluding exposures higher than 600 roentgens, Léonard and Deknudt (256) arrived at the relationship

$$Y = 3.8\ 10^{-3} + (1.7 \pm 0.1)\ 10^{-4}X$$

where Y is the mean yield of translocations per spermatocyte and X the exposure in roentgens. Evans *et al.* (132) obtained a similar relationship, but with a significantly higher regression coefficient as is evident from the equation

$$Y = 3.6\ 10^{-3} + (2.9 \pm 0.4)\ 10^{-4}X$$

46. The relation between x-ray exposure and frequency of affected spermatocytes also appears to be linear. As in the case of translocations, the regression coefficient estimated by Evans *et al.* (132) — $(2.6 \pm 0.3)\ 10^{-4}$ — is significantly higher than the one ($1.6\ 10^{-4}$) calculated by Léonard and Deknudt (256).

47. Muramatsu *et al.* (347) expressed the linear dose-effect kinetics for translocation induction with the following equation (range : 50-700 R; 8 levels):

$$Y = 10.6\ 10^{-3} + (2.1 \pm 0.4)\ 10^{-4}X$$

This regression coefficient of $(2.1 \pm 0.4)\ 10^{-4}$ and that for affected spermatocytes $(2.2 \pm 0.4)\ 10^{-4}$ are nearly identical, but intermediate between those given in paragraphs 45, 46.

48. The reasons for the discrepancy in the slopes (for translocations as well as for affected spermatocytes) are not clear. Evans *et al.* (132) suggest strain differences in radio-sensitivity as one possibility. Whereas Léonard and Deknudt used the inbred BALB/C strain of mice, the studies of the Harwell workers had been carried out with hybrid mice and those of Muramatsu *et al.* (347) with a strain of mice maintained in a close colony of small size by random-mating after inbreeding for 14 generations. It may be pointed out that, in an earlier investigation, Léonard and Deknudt (250) had compared the radio-sensitivities of five inbred strains of mice using as end-point the induction of translocations in spermatogonia by an x-ray exposure of 400 roentgens. No significant differences, either in the nature or in the frequency of translocations, were found.

49. When the over-all dose response of translocation yield over the 25-1,250-roentgen range is considered, a humped dose-effect curve is obtained which is characterized by an apparent linear increase up to at least 600 roentgens followed by a marked falling off at higher exposures. Two major questions arise: (*a*) is the dose-response curve up to 600 roentgens really linear or is it likely that the initial curve has the square-law component expected of two-track aberrations but distorted by secondary factors intervening between irradiation and meiotic examination of the cells? and (*b*) what possible mechanisms could account for the reduction in yield at higher exposures?

50. Léonard and Deknudt (256) seem to favour the interpretation that translocation induction in spermatogonia is mainly, although perhaps not exclusively, the result of a one-track process. They are inclined to the view that the yield of translocations presumably consists of two components, a major one that increases linearly with dose and a minor one that increases as the square of the dose.

51. In a more recent paper, Gerber and Léonard (149) have examined mathematically the role of factors that may influence the dose-frequency relationship of these aberrations which, on theoretical grounds, will be expected to increase as the square of the dose. Their analysis reveals that selection by interphase death and/or by early elimination of severe, or delayed elimination of small, chromosome aberrations can convert a square-law curve into a linear one. The implication of this finding in general terms is that the observed linear dose-response of translocations in mouse spermatogonia may be a consequence of selective factors that operate between the induction of translocations in spermatogonia and their scoring in spermatocytes, a possibility which was put forth earlier by Lyon and Morris (283) and by Evans *et al.* (132) (paragraphs 52-55).

52. Lyon and Morris (283) and Evans *et al.* (132) have suggested that the observed linear response is probably secondary and that at least two plausible mechanisms might be postulated to explain the distortions of the dose-response curve at higher doses. Firstly, the chromosome aberrations reported in the preceding paragraphs are all stable, compatible with cell viability. However, it may be assumed that the aberrations actually induced in the spermatogonial stages include unstable ones, which after mitosis would give rise to inviable daughter cells lacking chromosomes or parts of chromosomes. If stable and unstable aberrations occurred independently, the death of cells carrying both aberration types would not lead to any decrease in the observed incidence of translocations. However, if the cell population was heterogeneous in radio-sensitivity so that the various types of damage tended to occur together in the same cells, the elimination of the unstable aberrations would lead to a fall in the observed incidence of the other types.

53. Another possibility envisaged by Lyon and Morris (283) and by Evans *et al.* (132) to explain the distortion of the dose-response curve is consistent with the interpretation proposed by Russell (437) for his specific-locus data at 1,000 roentgens, namely, that at higher exposures most of the spermatogonia are killed and that the mutation rate in the surviving cells is lower. A general theoretical model of the consequences of this type of heterogeneity in response has been put forth by Oftedal (367). According to this model, humped curves for mutant yield would be expected following acute irradiation of germ cell populations of heterogeneous sensitivity, provided the same

cells or stages are sensitive to both killing and mutation induction. The consistency of the translocation data with this model is clear enough and need not be detailed.

54. Elimination from one or both of these causes (paragraphs 52 and 53) would increase as induction increased and would tend to give a humped dose-response curve which might lead to an apparent linear relationship between translocation yield and exposure up to about 600 roentgens.

55. Lyon and Morris (283) mention one further, relatively less important, possible cause of elimination of translocations. This relates to those translocations (X-involved as well as autosomal) that may interfere with spermatogenesis so that cells carrying them seldom reach the stage of meiotic metaphase at which they are scored (paragraph 109).

56. The evidence for heterogeneity in radiosensitivity between cells of a spermatogonial population with respect to translocation induction is largely based on the statistical treatment of the relevant data of Searle *et al.* (491), Lyon and Morris (283), Morris and O'Grady (309) and Lyon, Phillips and Glenister (286). Briefly, the observed frequencies of spermatocytes with 0, 1, 2, etc., translocations were compared with those expected from a Poisson distribution. The analysis demonstrated the existence of significant deviations from expectations with a general tendency for a deficit of cells carrying one translocation and an excess of those carrying more.

57. Observations that depart from a Poisson distribution in the direction of over-dispersion can often be fitted satisfactorily by a negative binomial distribution which in this context could be interpreted as indicating heterogeneity of the irradiated gonia with respect to genetic sensitivity (381). If so, this is probably connected with differential radio-sensitivity during the gonial cycle for which there is good evidence from earlier fractionation experiments (442).

58. In view of the fact that a period of 12-14 weeks intervenes between x-irradiation and examination of the cells (during which interval the treated *A*-type spermatogonia must have undergone an unknown but large number of mitotic divisions) it is conceivable that deviations from an expected Poisson distribution may arise as a secondary effect. One factor that might lead to the observed divergence would be selection for or against particular translocation-carrying germ-cell lineages during the period of mitotic multiplication. There is some evidence in the work of Searle *et al.* (491) and of Lyon and Morris (283) for the existence of clones of spermatocytes with multiple translocations derived from x-irradiated spermatogonia; it is thus possible that the divergence from a Poisson distribution might originate from a tendency for spermatogonia carrying more than one translocation to show preferential clonal proliferation. An evaluation of the magnitude of the contribution of this factor to the observed divergence must, however, await further studies.

(ii) *Gamma rays*

59. The data obtained by Searle *et al.* (483) on the induction of translocation in mouse spermatogonia following acute, high-exposure-rate (95 R min^{-1}) gamma-irradiation (^{60}Co; 56 to 816 R) are presented in table 5. From an analysis of the data as a whole, taking into account the existence of significant heterogeneity between testes (both with reference to the frequencies of affected spermatocytes and of translocations per spermatocyte), the authors have concluded that the exposure-frequency relationship does not significantly depart from linearity. The regression coefficients[3] are $(1.67 \pm 0.18)\ 10^{-4}$ for affected spermatocytes and $(1.81 \pm 0.20)\ 10^{-4}$ for translocations per spermatocyte. In the 56-402-roentgen range, the exposure-frequency relationship looks concave[4] although, again, linearity cannot be excluded.

60. A comparison of tables 4 and 5 will show that for each comparable exposure, the yield of translocations is lower after gamma- than after x-irradiation. The ratio of the linear regression coefficients with respect to the frequencies of affected spermatocytes (paragraphs 46 and 59) is 0.62 which gives the best estimate of the RBE of acute gamma-irradiation relative to acute x rays.

(iii) *Neutrons*

61. Searle, Evans and West (492) investigated the effects of acute, high-dose-rate (49 to 55 rad min^{-1}) fast-neutron-irradiation (0.7 MeV) on the frequencies of translocations in spermatogonia. Their results are presented in table 6 which shows that the dose-response curve is markedly convex, the frequency of affected spermatocytes reaching a peak at 100 rads and then falling sharply so that 220 rads appear to be less effective than 25 rads.

62. The main explanation suggested for the humped dose-response curve is the same as the one discussed in connexion with a similar curve for acute x-irradiation (paragraphs 52-54). While the data from the acute neutron-irradiation are in general agreement with Oftedal's model, the position of the peak raises problems, since the dose giving the maximum yield (100 rad) is much higher than would be expected from Oftedal's curves and Oakberg's data (357) on spermatogonia survival following fast-neutron-irradiation. The peak frequency of translocations would be expected around a dose of 25 rads; it occurs instead at 100 rads which is expected to kill all the cells at a sensitive stage, as judged from cell-survival data. Further work is needed to resolve this contradiction.

63. It must be pointed out that, in these experiments, as in those with other types of irradiation, significant heterogeneities between testes (and to a smaller extent, between mice) were noted. Heterogeneity between testes might stem from preferential proliferation of particular clones of translocation-carrying germ cells (for which some evidence was presented in paragraph 58) but might also reflect chance differences in the proportion of sensitive cells (in a heterogeneous population) affected by ionizing tracks. Such an effect is more likely to arise from high-LET radiation (such as neutrons) in which the number of tracks is much less than with low-LET radiation (such as x rays) and the over-all heterogeneity correspondingly greater.

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[3] Since translocation frequencies in spermatocytes from unirradiated mice of the stock used in the present study are known to be extremely low, these regressions were computed so as to go through the origin.

[4] The quadratic equation $Y = 0{,}97\ 10^{-4}X + 3.04\ 10^{-7}X^2$ fits well the data on numbers of translocations per spermatocyte.

(b) *Dose rate*

(i) *X rays*

64. The effects of low- *versus* high-dose-rate x-irradiation on the frequencies of cytologically detectable translocations were examined by Searle and his co-workers in two series of experiments, the first at a dose level of 600 rads (range: 913 rad min^{-1} to 0.8 rad min^{-1} (490, 491)) and the second at 300 rads (range 93 rad min^{-1} to 0.09 rad min^{-1} (484)). The latter series was carried out in order to eliminate the possibility of any saturation effect. Data from both series are presented in table 7.

65. It can be seen that varying the dose rate over a thousand-fold range in the 600 rads series has no detectable effect on the frequencies of cells carrying translocations. At the lower dose of 300 rads, however, the frequency of affected spermatocytes at 93 rads per minute is more than twice that at 0.87 or 0.09 rad per minute. This difference is highly significant. These results suggest that, in spite of its linear dose-frequency relationship, the induction of translocations in mouse spermatogonia by acute x-irradiation is at least partly a two-track process.

(ii) *Gamma rays*

66. Searle, Evans, Ford *et al.* (491) published the results of a study in which translocation induction by gamma rays (^{60}Co) in mouse spermatogonia was investigated using 600 rads at five different rates. The data are presented in table 7 and show that the frequencies of translocations decrease steadily with decreasing dose rates. There is almost a nine-fold difference in the yield between the effects at the highest (83 rad min^{-1}) and at the lowest (0.02 rad min^{-1}) rate studied. Plotting the dose rates on a logarithmic scale and the frequency of affected spermatocytes on an arithmetic one, the authors find that there is no significant departure from log-linearity and obtain the relationship

$$F = 6.1 + 2.9 \log_{10} D$$

where F is the per cent frequency of affected spermatocytes and D the dose rate in rads per minute.

67. It may be noted that, over a comparable range of dose rates (80 rad min^{-1} to 0.09 rad min^{-1}), the reduction in translocation frequencies observed with gamma rays (600 rad) is much greater (12.1 to 2.9 per cent) than with x-irradiation (300 rad: 7.2 to 3.0 per cent). This differential response might be due to the different magnitudes of the one-track component, this being larger with x- than with gamma-irradiation.

(iii) *Neutrons*

68. In the study described in paragraph 61, Searle, Evans and West (492) also investigated the effects of low-dose-rate fast-neutron-irradiation (0.7 MeV) on the frequencies of translocations induced in spermatogonia. The data are presented in table 7. It is clear that, with 62 rads delivered at low dose rate, the frequency is 3.3 per cent and that there is a sharp increase with 214 rads, the frequency of cells carrying translocations being 21.7 per cent. Although only two points are available, the dose-response curve appears to depart significantly from linearity in the direction opposite to that recorded for acute neutron-irradiation. At high doses then, protracted neutron-irradiation is more effective than acute irradiation whereas the reverse seems to be true at low doses.

(c) *Fractionation*

(i) *Long intervals*

69. Lyon and Morris (283) compared the effects of a single x-ray dose of 1,000 rads with those of two equal fractions of 500 rads separated by a 24-hour interval, on the frequencies of translocations induced in spermatogonia. They recorded a much higher frequency (24.9 per cent) after fractionated than (5.3 per cent) after unfractionated irradiation (table 8). However, the observed frequency was merely twice that obtained with a single dose of 500 rads (463) differing in this respect from the high degree of enhancement observed with specific-locus mutations, under similar conditions of radiation exposure (paragraph 158).

70. In another study (309) where x-ray doses of 100, 300, 500, 600, 800, 1,200 and 1,400 rads were split into two equal fractions 24 hours apart, the incidence of translocations increased approximately linearly over the entire dose range studies (table 8). This finding is in marked contrast to the humped dose-response curve found with single doses of comparable size.

71. Table 8 also shows that up to 600 rads the results with fractionated doses (excluding experiments 2B, 2C and 2D which are discussed in paragraph 72) are remarkably close to those with single doses. Beyond 600 rads, the response to single doses declines and that of the split dose continues to increase linearly. When the effect is measured by the number of translocations per cell, the increase is somewhat faster than linear (last column of table 8) and at the higher doses (500 + 500 and 700 + 700 rad) fractionation results in frequencies somewhat higher than expected from single-dose experiments. It may be pointed out that the analysis of the data with respect to the numbers of translocations per spermatocyte is perhaps less reliable in view of the fact mentioned earlier (paragraph 56) that the distribution of 0, 1, 2, 3, etc., translocations per cell does not in fact fit a Poisson distribution.

72. In a third investigation (284) a total dose of 300 rads was delivered to spermatogonia in a single fraction or in daily fractions of 60, 10 or 5 rads (table 8). A comparison of these results with those at 150 + 150 rads indicates that (*a*) translocation frequencies remain approximately the same whether the dose is single or split into two fractions of 150 rads each; (*b*) when the dose is split into five fractions of 60 rads each, the effectiveness noticeably decreases; and (*c*) with 30 fractions of 10 rads each, the effectiveness decreases further and stays at approximately the same level even when the individual fraction is reduced to five rads.

73. To account for the drop in yield with repeated small doses of radiation, Lyon *et al.* (284) suggested two possible explanations. The first one is based on the repair hypothesis originally postulated by Russell and Kelly (451) to explain the reduction in specific-locus mutation frequencies after low doses and at low dose rates. These authors assumed that the observed reduction in mutation frequencies is a consequence of the operation of a repair process that is effective at low doses and dose rates, but is damaged or saturated

at high doses and dose rates. The interpretation of Lyon *et al.* (284) is essentially the same except that it is extended to the situation where repeated small daily doses are administered to spermatogonia and where the damage under consideration is that which leads to the production of translocations. The second interpretation assumes that a single small dose produces as much effect as one would expect, but that repeated irradiation changes the sensitivity of the spermatogonial cell population making it more resistant, with the result that later doses have less effect.

74. If the first explanation is correct, the translocation frequencies (after 10, 20, 30, etc., dose fractions, each dose fraction being small and equal in magnitude to the others) are expected to be linearly related to dose, with the dose-response curve passing through the control value. On the other hand, if the cell population sensitivity changed with repeated doses, then the dose-response curve would not be linear or would not pass through the control value.

75. The validity of these explanations was recently verified by Lyon, Phillips and Glenister (286). Male mice were given a total dose of 620 rads of gamma rays (^{60}Co; 17-18 rad min^{-1}) either singly or in successive daily fractions of about 10.4 rads (5 fractions a week for 12 weeks). After treatment, the mice were kept for appropriate periods, then killed and cytological preparations made using standard procedures. The results are given in table 9.

76. It can be seen that (*a*) the yield of translocations in spermatocytes after 620 rads delivered in 60 fractions is only about one fifth of that with the same dose delivered singly, a result which is in agreement with that discussed in paragraph 72; (*b*) after 30 fractions of 10.4 rads each (total dose about 300 rad), 1.6 per cent of the spermatocytes showed translocations, again in agreement with the x-ray data (paragraph 72). A weighted regression analysis of translocation yield *versus* number of weeks of exposure (for repeated doses) gave the following equation:

$$Y = (6.16 \pm 2.96)\ 10^{-3} + (1.39 \pm 0.41)\ 10^{-3}X$$

where Y = the proportion of affected spermatocytes and X = number of weeks. For the number of translocations per spermatocyte, analysed in a similar way, the relationship was expressed as:

$$Y = (5.69 \pm 2.96)\ 10^{-3} + (1.52 \pm 0.41)\ 10^{-3}X$$

where Y = the proportion of translocations per spermatocyte and X is defined as before.

77. There was no significant departure from linearity, whichever measure of translocation yield was used. The intercepts on the ordinate, however, were much higher than the observed frequency of translocations in unirradiated mice which in previously reported experiments (258, 283, 488, 492) was only two in 27,200 cells or 0.07 10^{-3}. The difference between the Y intercepts (paragraph 76) and the control value of 0.07 10^{-3} is significant at the 5 per cent level or on the border-line of significance (P = 0.04, and 0.058, respectively, for the first and second).

78. These data are interpreted by Lyon *et al.* (286) as providing evidence for the possibility that under conditions of repeated irradiation, changes in sensitivity of the spermatogonial cell population arising from the selection of radio-resistant cell-lines might be quite important.

79. In a more recent study of Lyon, Phillips and Glenister (287) male mice received 600 rads at high dose rate or in 12 fractions of 50 rads each, at daily or weekly intervals. The frequencies of translocations observed in spermatocytes (irradiated as spermatogonia) were compared in the three groups.

80. It was found (table 10) that the yields after either type of repeated irradiation were similar (6.1 ± 0.7 per cent with daily intervals and 7.1 ± 0.9 per cent with weekly intervals) but significantly lower than that after unfractionated irradiation. These results are in agreement with those from an earlier experiment (paragraph 72) and appear to suggest that the size of each dose fraction rather than the interval between them is important in determining the effect of repeated radiation doses.

81. In work similar to that outlined in paragraphs 79 and 80 but in which specific-locus mutations were scored (paragraphs 159-160), the mutation rates after single (600 rad) or fractionated doses (12 × 50 rad; weekly intervals) were not significantly different (15.4 10^{-5} locus^{-1} *versus* 12.6 10^{-5} locus^{-1}), thus differing from the situation discussed above.

82. Searle, Evans and Beechey (485) studied the induction of translocations in mouse spermatogonia by fractionated, high-dose-rate (49 to 55 rad min^{-1}) fast-neutron irradiation (0.7 MeV). A total dose of 276 rads was delivered to male mice in two fractions of 184 and 92 rads, the interval between the fractions being eight weeks. In one parallel experiment, the order of the radiation doses was reversed (92 rad, first and 184 rad, second) and in another, the mice received a single dose of 92 rads.

83. The above experiment was designed to examine whether there was selection (of the kind envisaged by Lyon *et al.* (286); paragraph 78) for radio-resistant spermatogonial stem cells after a large initial radiation dose which would kill most of the cells sensitive to both killing and translocation induction. If this occurred, the final yield of translocations after dose fractionation (184 + 92 rad) would be low and close to that obtained with 184 rads alone. If, on the other hand, there was no such selection for radio-resistant cells, the final yield would be closer to the sum of the yields of the two dose fractions.

84. The results show that the frequency of affected spermatocytes after a single dose of 92 rads is 6.5 ± 1.5 per cent and that expected (on the basis of earlier data (492)) from 184 rads (single) is 3.5 per cent. The observed frequency after fractionated irradiation is 9.4 ± 1.0 per cent (184 + 92 rad) and 8.4 ± 2.0 per cent (92 + 184 rad), frequencies consistent with the expectation of additivity of response to the dose fractions (when there was a long interval between them) and not in line with that based on the presence of any radio-resistant population of spermatogonial stem cells as the result of a large first dose.

85. The above data have led the authors to suggest that either (*a*) there are no radio-resistant cell lines of spermatogonia or (*b*) such lines are present and initially predominant after a large radiation dose, but tend to disappear after further cell generations, unless selected for by repeated irradiation. The latter interpretation does not conflict with the possibility envisaged by Lyon *et al.* (286) to explain their fractionation results, namely, continuing selection for radio-resistant cells by repeated irradiation.

(ii) *Short intervals*

86. Léonard and Deknudt (259) and Searle *et al.* (489) carried out a study to examine the effects of short-interval fractionation (exposures separated by 1, 2, 3 hours etc.) on the induction of translocations in mouse spermatogonia. Earlier work along similar lines had been carried out on human and plant cells. In one of the human leucocyte experiments (130), for example, it was found that, with fractionation intervals of between one half and five hours, the yield of dicentrics and of rings *declined* to a minimum that was slightly below the expected base-line. With a six-hour interval, however, the yield significantly *increased* and was equal to the yield obtained with the single dose. With about eight hours, the yield *declined* again and was back to its base-level at 12 hours.

87. This "fall-rise-fall" pattern has been called the "Lane effect" or the "Evans effect". In the study of Léonard and Deknudt (259), an essentially similar pattern is observed. After a single x-ray exposure of 500 roentgens to mouse spermatogonia the frequency of affected spermatocytes was 8.1 ± 0.8 per cent, and 4.2 ± 0.2 per cent after an exposure of 250 roentgens. With the exposure (500 R) split into two equal fractions, the frequency fell to 5.7 ± 0.8 per cent at two hours and rose to 8.8 ± 0.9 per cent at four hours. With a four-hour interval, the yield dropped to 4.4 ± 0.8 per cent and rose again to 8.4 ± 1.3 per cent with a 16-hour interval. With a 24-hour interval, the yield was slightly reduced to 6.8 ± 0.9 per cent. The authors interpret these variations in the frequencies with different fractionation intervals as a possible consequence of differential radio-sensitivity of the cell-cycle stages.

88. In the study of Searle *et al.* (489) a marked fall in translocation frequency was also observed when a dose of 300 rads was split into two equal fractions separated by an interval of half or one hour between them; with longer intervals (up to eight hours) however, fluctuations in frequency were less pronounced than in the experiments of Léonard and Deknudt (259).

(d) *Intervals between irradiation and examination*

89. Evans *et al.* (132) investigated the dependence of the frequency of translocations induced in spermatogonia on the interval between acute x-irradiation and examination. As the data in table 11 clearly show, no significant differences are seen between the three groups at any of the exposures, except for a possible decline in frequency 210 days after 800 roentgens.

90. A similar study was carried out by Léonard and Deknudt (258) over a still longer period of time, up to 600 days, following an acute x-ray exposure of 600 roentgens. The frequency of spermatocytes with translocations increased from 8.4 per cent after 60 days (1,000 cells scored) to 12.6 per cent after 100 days (1,800 cells scored), remained at approximately the same level up to 200 days, decreased 250 days later, and remained reasonably steady for the following 200 days. At 500 and 600 days, there was a slight non-significant tendency towards an increase. No chromosome rearrangements were recorded in controls after 60, 100, 200 and 300 days (3,400 metaphases examined). However, after 400 days one abnormal metaphase was found in 800 cells examined (0.13 per cent) whereas after 500 and 600 days, 2 out of 1,000 cells (0.20 per cent) and 9 out of 1,200 cells (0.75 per cent), respectively, were found to be abnormal.

91. The authors suggest that the presence of chromosomal rearrangements after 400, 500 or 600 days might be related to the ageing effect described in mice by Curtis *et al.* (99) and in man by Jacobs *et al.* (187). The small increase observed after 500 and 600 days in the radiation experiment might be related to the same phenomenon.

92. It must be pointed out that none of the changes in frequencies outlined in paragraph 90 for the irradiated groups appears to be significant when the frequency obtained at 60 days is used as a base-line although the response as a whole can hardly be characterized as uniform. With a higher exposure (1,200 roentgens in two equal fractions separated by eight weeks) Ford *et al.* (139) observed 41.6 per cent (623 cells examined) and 32.5 per cent (4,000 cells examined) of the spermatocytes with one or more multivalent configuration when the mice were killed 91-126 days and 413 days, respectively, after the second dose (table 8, experiments 7B, 7C).

(e) *Cytological* versus *genetic observation*

93. All the experiments reported thus far employed the cytological technique to screen for the presence of translocations in the irradiated males themselves. With the genetic experiments, on the other hand, the irradiated males have to be bred to raise the F_1 generation and the male or the female progeny further test-crossed to ascertain the incidence of heritable semi-sterility. A comparison of the data from the cytological experiments with those from the genetic experiments therefore necessitates that the primary cytological data be manipulated to derive the expected frequencies.

94. From a comparison of the genetic and cytological results on translocations, Ford *et al.* (139) concluded that the frequency of translocation heterozygotes in the progeny of irradiated male mice (spermatogonial irradiation) was only about one half of what would have been expected from the frequencies of multivalent configurations observed in the spermatocytes of their fathers (table 1).

95. It is therefore easy to understand that in the cytological studies of Léonard and Deknudt (255) on 121 F_1 males (300 R paternal irradiation; spermatogonia) no translocation heterozygosity could be found since the expected translocation frequency in F_1 generation with this exposure is quite low.

96. Griffen and Bunker (161) have published data showing that the incidence of semi-sterility in the offspring derived from gonial stages of x-irradiated males given 350 and 700 roentgens was 4.6 and 3.9 per cent, respectively. Since the presumed semi-sterility was not shown to be inherited and since only some sterile and semi-sterile animals were studied cytologically (from squash preparations of the seminiferous tubules and not with the air-drying method of Evans *et al.* (131)) a quantitative comparison of these data with those of Léonard and Deknudt (255) and of Ford *et al.* (139) is difficult.

(f) *Radio-sensitivity of wild mice*

97. Searle *et al.* (488) investigated the sensitivity of house mice living under natural conditions on the Pembrokeshire (Wales, United Kingdom) island of Skokholm to the induction of reciprocal translocations

following spermatogonial x-irradiation (300 rad, whole body; 75 rad min^{-1}). Eleven out of 528 metaphases examined were abnormal, giving a frequency of 2.1 per cent compared to only 0.2 per cent (1/500) in controls.

98. The frequency in the irradiated series appears to be about 3-4 times lower than the frequencies found in laboratory strains of mice after the same whole-body exposures to x rays (table 4, experiments 14, 15 and 16). In further experiments involving simultaneous x-irradiation of Skokholm wild, mainland wild and laboratory male mice, the authors were unable to confirm the apparent difference in radio-sensitivity discussed above (489).

(g) *Differences between species*

99. Work on the genetic radio-sensitivity of post-meiotic stages of male mammals has shown that at present there are no sure grounds for extrapolating from one stage or type of genetic damage to another (paragraphs 105, 106). To throw further light on this problem, Lyon and Smith (289) conducted an experiment in which translocation induction in spermatogonia was studied in the guinea-pig, the rabbit, the hamster and the mouse. The notable difference in the cytological procedure used in this study and in other mouse studies is that preparations of the spermatocytes were made using Meredith's method (302).

100. The results are given in table 12 which shows that (*a*) the mouse data obtained using Meredith's method are in good agreement with those obtained previously with the method of Evans *et al.* (131); (*b*) translocations are induced in the spermatogonia in all the experimental species although the dose-response relationship differs from that in mice; (*c*) in both rabbits and guinea-pigs, the over-all dose-response curve appears humped (as in mice) but the peak incidence occurs at doses around 200-300 rads, compared with 600-800 rads in mice (table 4); and (*d*) in hamsters at the one dose level tested (200 rad), translocations are indeed induced.

101. The interpretation of the humped dose-response curve in mice is that the spermatogonial cell population is heterogeneous in sensitivity to both mutagenesis and cell-killing. The sensitive cells are killed at high radiation doses and the mutation rate represents that of the resistant population (paragraph 53). On this basis, in rabbits and guinea-pigs, either the range of sensitivities or the proportions of sensitive and resistant cells might differ from those in the mouse. The point of greatest interest would be the form of the curves at doses below the peak, but on this the available data are insufficient.

2. Differences between pre- and post-meiotic germ cells

102. The existence of pronounced differences in radio-sensitivity between pre-meiotic and post-meiotic stages of spermatogenesis with reference to the induction of translocations and other kinds of genetic damage is now well-documented in mice, is in line with similar findings in *Drosophila* and in other species, and has now been confirmed and extended at the cytological level. Léonard and Deknudt (255) examined the F_1 male progeny (sires exposed to 300 rad at a dose rate of 100 rad min^{-1}) obtained by mating each treated male to one virgin female per week for a total period of nine weeks. With this mating scheme which is essentially similar to the brood-pattern technique employed by *Drosophila* workers, progressively younger stages at the time of irradiation would be sampled in successive weeks.

103. The incidence of males with aberrations was 5.1 (6/117), 10.4 (11/106), 21.7 (20/92), 2.2 (1/45) and 6.3 per cent (3/48), respectively, during the weeks 1-5 whereas in weeks 6-9 no males with aberrations were found. The germ-cell stages samples would, at irradiation, approximately correspond to sperm from vas deferens and epididymis (first week), testicular sperm (second week), spermatids (third week), spermatocytes (fourth and fifth weeks) and spermatogonia (sixth, seventh, eighth and ninth weeks), respectively (365). The peak sensitivity to translocation induction is clearly found in the third week corresponding to spermatids at the time of irradiation, in good agreement with the data of L. B. Russell (428) on induced X-chromosome anomalies.

104. The data of Griffen and Bunker (161) show that the frequencies of semi-sterile offspring of x-irradiated males (350 and 700 rad) are 7.2 and 11.8 per cent among the progeny sired during the pre-sterile period (spermatozoa, spermatids and spermatocytes) whereas in the post-sterile period (spermatogonia) these are 4.6 and 3.9 per cent. Cytological anomalies were more frequent in the F_1 males sired during the pre-sterile period.

105. In order to study whether the spectrum of translocation induction in post-meiotic male germ-cell stages of the hamster follows a pattern similar to that for the induction of dominant lethals (paragraphs 22, 23) Lyon and Smith (289) irradiated male hamsters with x rays (200 rad) and measured the incidence of translocations in the various post-meiotic stages. The testes of F_1 males were examined cytologically using Meredith's method for translocation configurations. It was found that the frequencies of males carrying translocations were 0/50, 0/11, 1/7 and 1/9, respectively, in male progeny sired during weeks 1 to 4.

106. Except for week 1, the number of F_1 sons tested is obviously too small for an accurate estimation of translocation frequency. However, it is clear that week 1, with the highest incidence of dominant lethals (paragraph 23) does not have a correspondingly high incidence of translocations. Rather, the pattern in the hamster is generally similar to that recorded for the mouse (paragraphs 102, 103). This and other observations recorded earlier (paragraph 40) are quite important in extrapolating from one criterion of radiation damage to another and from species to species.

3. Embryonic irradiation

107. Léonard and Deknudt (252) studied the possibility of inducing viable and transmissible chromosome rearrangements by irradiating mouse embryos *in utero* during the pre-implantation period. The timing of the irradiation of the pregnant females was such (day 0.5 of gestation) that the eggs received the irradiation at the pronuclear stages (100 R; whole body; 100 R min^{-1}). A total of 38 males and 24 females irradiated at the pronuclear stage survived and were available for testing. The testes of 141 sons of the 38 males and of 100 sons of the 24 females were examined for the presence of chromosome re-

arrangements by analysing, for each son, 50 spermatocytes at diakinesis-first metaphase. Whereas no chromosome rearrangements were found in the spermatocytes of the sons of the irradiated females, some sons of three irradiated males showed spermatocytes having translocation configurations. Using the method of Falconer (134) the authors estimate that the over-all rate of induction of translocations when irradiation is delivered to the embryos *in utero* is 2.5 10^{-4} per genome per roentgen, in good agreement with the rate observed in adult spermatogonia as discussed in paragraphs 45-47.

108. Searle and Phillips (494) used fast neutrons (0.7 MeV; 108.5 rad plus 20.5 rad gamma contaminations; 0.011 rad min^{-1}) to irradiate mouse embryos between the blastocyst stage and the beginning of somite formation. Twenty of the males irradiated *in utero* were examined cytologically for the presence of translocations. It was found that two of the males had high and two had low frequencies of translocations. The over-all translocation frequency was 1.2 per cent which is lower than that found after fast-neutron irradiation of adult spermatogonia which, at a dose of 62 rads spread over 12 weeks, gave a mean frequency of 3.3 per cent (paragraph 68). This reduction is of the same order as that for specific-locus mutations. Since, however, a protracted exposure (600 R) of adult males to gamma rays gave a yield of only 1.4 per cent translocations (table 7) it can be seen that irradiation of male embryos with fast neutrons at low dose rate is much more effective for translocation induction than gamma-irradiation of adult males. The same is true for the induction of specific-locus mutations (table 14).

4. Types of translocations and their effects on fertility and viability

(a) *Autosomal translocations*

109. Lyon and Meredith (282) exposed males to x rays (600 rad) and carried out a genetic analysis of the female progeny obtained in the pre-sterile period (spermatids or sperm sampled). Forty-six of the 168 daughters (27.4 per cent) studied were semi-sterile and of these 26 carried translocations causing semi-sterility in both sexes. Five carried translocations causing semi-sterility in females and full sterility in males, and five had translocations giving some semi-sterile and some sterile males. All the translocations were autosomal. The five translocations causing male sterility were studied more fully. All gave chain quadrivalents and some univalents at male meiosis. Examination of the male progeny in the first and later generations showed that spermatocytes were present (though in reduced numbers) in four cases in stages up to first metaphase but that there were very few, if any, spermatids or mature sperm.

110. This investigation provides important evidence of two kinds: first, certain autosomal translocations in the heterozygous state can be fully viable but yet lead to male sterility through failure in spermatogenesis; second, the failure may not be specific to a particular stage or cell type but occur with variable incidence throughout the meiotic process and possibly at earlier steps in the germ-cell sequence. The fact that autosomal translocations associated with male sterility can be induced in sperm or spermatids has been further substantiated by the work of Cattanach *et al.* (68) with ethylmethane sulphonate treatment and of Léonard and Deknudt (255) with x-irradiation.

111. If translocations with genetic properties similar to those described in paragraph 110 are induced in spermatogonia, and if these behave autonomously, they will *not* be represented in the effective sperm population. It follows therefore that male sterility attributable to translocation heterozygosity will *not* be expected in the progeny of fathers whose spermatogonia have been exposed to irradiation or other mutagenic treatments. The failure to detect translocations in the sterile sons from the irradiation experiments of Ford *et al.* (139) is in line with this expectation.

(b) *X-autosome and Y-autosome translocations*

112. In contrast to the ease with which autosomal translocations can be induced and recovered, those involving the X chromosome have been recovered only rarely. This rarity of induced X-autosome translocations seems to be the rule in experiments involving spermatogonial irradiation. The X-autosome translocations that have actually been discovered were found as a result of experiments designed for other purposes (431).

113. Analysis of the data from all experiments (involving irradiated spermatogonia and cytological scoring in descendent spermatocytes) published by Searle and his collaborators (15, 132, 139, 483, 491, 492) shows that 24 out of 7,898 presumptive translocations were diagnosed as being between the X chromosome and an autosome. Their over-all frequency is thus 0.30 per cent. Since there are 38 autosomes in the mouse, there are 38 possible paired combinations of X chromosome and autosome which could be involved in a translocation, while there are $38 \times 36/2$ possible paired combinations of non-homologous autosomes which could be involved. Therefore, if an X-autosomal translocation was as likely to occur as a completely autosomal one (the X chromosome is about as long as the average autosome), its expected frequency would be about 1/18 of all translocations, namely, 5.56 per cent. It thus seems likely that there is selective elimination of this type of translocation (483). Probable reasons for this have been discussed by Lyon and Morris (283).

114. Similar calculations made by L. B. Russell and Montgomery (431) from genetic data obtained from irradiation experiments involving post-spermatogonial stages also showed that there was a discrepancy between the estimated (estimated because some were not adequately tested) and the expected incidence of X-autosome translocations, the former being about one quarter to one half of the latter.

115. All the known X-autosome translocations seriously interfere with spermatogenesis when a male mouse is hemizygous for them (431, 483). For example, L. B. Russell (427) found that spermatogenesis was interrupted before meiotic metaphase in six of her translocations. Translocations with these types of effects, if induced in spermatogonia and if they act autonomously, will normally be eliminated before meiosis and thus will not contribute to the zygotic population of the next generation.

116. Léonard and Deknudt (257) have reported the first case of a cytologically-diagnosed radiation-induced Y-autosome translocation observed in the F_1

son of a male mouse given an x-ray exposure of 300 roentgens. Genetic testing, however, has not been made.

5. *Summary and conclusions*

117. Translocations can be induced by ionizing radiations at all stages of spermatogenesis and in late dictyate oöcytes of the mouse.

118. The pattern of radio-sensitivity as it emerges from the cytological studies closely parallels that from genetic studies in demonstrating that post-meiotic germ cells are more radio-sensitive with regard to translocation induction than pre-meiotic stages; among the post-meiotic stages, spermatids are by far the most sensitive.

119. Some translocations induced in spermatogonia can successfully pass through the remaining stages of spermatogenesis and can contribute to zygotic populations.

120. Certain autosomal translocations can be fully viable in the heterozygous state and yet cause male sterility through failure in spermatogenesis. If such translocations are induced in spermatogonia, they will not be represented in the effective sperm population and consequently will not be expected in the progeny of fathers whose spermatogonia have been exposed to irradiation. A similar argument is true for translocations involving the X chromosome.

121. A marked discrepancy exists between the frequencies of translocations diagnosed cytologically and genetically in that the expected frequency in the F_1 was about twice that actually observed. It is considered that selection operating on diploid and haploid genomes between the spermatocyte stage and maturation of the sperm is sufficient to cause the observed discrepancy.

122. The data obtained from experiments involving high-dose-rate x- or fast-neutron-irradiation of spermatogonia are consistent with a linear kinetics (up to 600 R with x rays and up to 100 rad with neutrons) after which the yield falls off drastically, giving an over-all humped dose-response curve. With high-dose-rate gamma-irradiation, however, there may possibly be a small square-law component, although a linear relationship cannot be excluded when the data are analysed as a whole. All these responses are very probably the result of secondary distortions of the primary dose-response curves which may well have a more marked square-law component in the case of x and gamma rays.

123. A dose-rate effect has been observed with x-, gamma- and neutron-irradiation, the effect being most pronounced with gamma rays.

124. Acute x-irradiation is mutagenically more effective than acute gamma-irradiation; acute gamma-irradiation is more effective than chronic gamma-irradiation; and the efficiency of chronic neutrons at high doses is about 20-25 times that of chronic gamma-irradiation.

125. The effects of fractionation are dependent on total doses and on fractionation procedures. Especially important from the standpoint of human genetic risks is the observation that the fractionation of a total dose of 300 rads of x rays into several small fractions of 10 or 5 rads leads to a significant reduction in translocation yields as compared with the effects of a single dose.

D. INVERSIONS

126. Roderick and Hawes (418) and Roderick (417) reported the first radiation-induced chromosomal inversions recovered in mice. Male inbred mice received x-ray exposures of 700 to 900 roentgens and the F_1 male progeny from matings during the presterile period were used for the cytological screening of the inversions. The procedure included removal of one testis from each F_1 male, appropriate fixation and sectioning, and examination of the sections for meiotic anaphase bridges. The males suspected to have induced inversions were later used to build up stocks.

127. Anaphase bridges were used as indicators of inversion heterozygosity since it is well-known that a single crossing-over within the inverted segment in a paracentric[5] inversion heterozygote will generate a dicentric and an acentric chromatid, in addition to two normal chromatids. At anaphase the dicentric chromatid will form a bridge and the acentric a fragment, both of which can be scored.

128. Approximately 30 first meiotic anaphases were examined in each F_1 male from the control and irradiated groups. Out of 915 anaphases (from 30 animals) in the control, 31 (3.4 per cent) showed bridges. Among the irradiated males, those which gave 10 per cent (or more) anaphase bridge frequencies were more intensively investigated. In cases suspected of being inversions heterozygotes, additional anaphase up to a maximum of about 130 were examined.

129. Until now 18 males with presumptive inversions have been isolated. Of these, two inversions (anaphase-bridge frequencies of 34 and 21 per cent, respectively) were followed for more than two generations and checked cytologically and genetically. One inversion on the XIII linkage group (In (13)1 Rk) is approximately 17 map units long and spans the distance between loci *Id*-1 (isocitrate dehydrogenase) and the *Dh* (dominant hemimelia). The other is on linkage group XVII (In (17)2 Rk), is approximately 10 map units long, and is closely linked with *bf* (buff) which is at one end of the known group of markers for linkage group XVII; preliminary data also show that this inversion is linked with *rd* (retinal degeneration) and *Pgm*-1 (phosphoglucomutase) loci that also belong to linkage group XVII.

130. Using the data pertaining to the 15 presumptive inversions recovered among the first 541 F_1 males screened (exposures between 700 to 900 R with an average of 814 R), Roderick (417) has estimated that the rate of induction for post-meiotic male germ-cell stages is about 3.4 10^{-5} inversions per gamete per roentgen. This is an underestimate, since small inversions cannot be efficiently recognized by this method. Since it is doubtful that a linear relationship exists between irradiation dose and number of inversions per gamete, other exposures may give different results.

131. The major advantage of having these as well as more and longer inversions will be their usefulness in uncovering and then retaining recessive lethals. The inversion on linkage group XIII is particularly suited for this purpose since the inverted segment is opposite to loci that can be used to construct a balanced

[5] Because the chromosomes of the mouse are all acrocentric, the great majority of inversions should be paracentric.

lethal system of the kind that had so many practical advantages in *Drosophila* genetics.

132. In trying to use the anaphase-bridge method to screen for the induction of inversions, it should be remembered that differences with regard to the incidence of natural inversion polymorphism are likely to exist between species as well as between sub-species. For example, in F_1 males obtained in crosses of laboratory strains of mice (*Mus m musculus*) and a Japanese sub-species (*Mus m molossinus*; originally trapped in Kyushu), Roderick (417) found that the average anaphase-bridge frequency was 20.3 per cent, much higher than the 3.4 per cent observed in the laboratory strains of *Mus m musculus* used in his study.

E. Loss or addition of chromosomes

133. Loss of any autosome is probably lethal in the mouse while loss of a sex chromosome causes few adverse effects provided one X remains (the OY condition results in lethality) and is phenotypically detectable by the use of appropriate markers. Induction of sex-chromosome losses has been used by L. B. Russell (428) to compare a large number of germ-cell stages for radiation sensitivity to chromosomal damage. Most of the earlier work on this subject was reviewed in the 1966 report from which the following conclusions, which are still valid, can be drawn: (*a*) losses of sex chromosomes can be easily induced in the mouse; (*b*) by far the highest yields of these losses are obtained by irradiating zygotes from the time of sperm entry (second meiotic division) through early pronuclear stage; the maternal X chromosome may be relatively more sensitive than the paternal X chromosome or than the Y chromosome during the first part of this period; (*c*) there is a sharp drop in sensitivity between early and late pronuclear stages; (*d*) among the germ cells tested, the ones yielding the highest XO frequencies are the dictyate oöcytes in mature follicles of the female and the spermatids in the male; (*e*) taken as a group, leptotene-through-diplotene oöcytes and spermatocytes give a lower, and roughly equal, yield; and (*f*) among spermatocytes, post-pachytene stages give the lowest frequency of XOs. These comparisons must, however, take account of the fact that YO yield from irradiation of spermatocytes and pre-dictyate oöcytes is presumably being measured in selected populations.

134. Although the XXY and XYY (but not XXX) type of sex-chromosomal aneuploidy are known in the mouse, there is as yet no evidence of their being induced by irradiation.

1. *Male germ cells*

135. The induction of X-chromosome loss after an x-ray exposure of 600 roentgens to mouse spermatogonia was studied by Léonard and Schröder (260). The paternal X chromosome was marked by the dominant sex-linked gene, Tabby (*Ta*). In all, three XO exceptions were recovered, one among 1,347 F_1 females in the irradiated group (0.07 per cent) and two among 1,508 females in the control (0.13 per cent). Since all the three XO exceptions were of the genotype *Ta*/O, their X chromosomes were of paternal origin. Consequently, this study provides no evidence for the induction of paternal-X losses. It is likely that the observed XOs were either due to the mothers being XOs (the mothers of the exceptions were not cytologically tested) or to the spontaneous loss of the maternal X chromosome, although the incidence of the latter is known to be extremely low (426).

136. L. B. Russell and Montgomery (432, 433) irradiated male mice with x rays (600 R, 66 R min^{-1}) either in a single exposure or in two exposures of 100 and 500 roentgens separated by 24 hours. The latter régime was chosen in order to examine whether sex-chromosome losses would also show an enhanced response to fractionation similar to what was already known regarding the response of the specific-locus mutations induced in spermatogonia (439).

137. Immediately after completion of irradiation, these two groups and a sham-irradiated control group were mated to females (homozygous for the sex-linked dominant gene *Greasy*) for 10 days in order to obtain data on spermatozoal sensitivity; males were then removed and re-mated shortly prior to the estimated end of the sterile period and for the remainder of their lives (spermatogonial data). Paternal sex-chromosome losses are detectable by the occurrence of *Gs*/O daughters. The exceptional progeny were tested genetically and cytologically.

138. The results analysed thus far indicate (*a*) no significant differences between the effects of single and fractionated exposures (the frequencies are so small that differences cannot be picked up at present); (*b*) with spermatozoal irradiation, the induced rate of loss of the X (or the Y) chromosome is 0.8 10^{-5} per roentgen (results of single and fractionated irradiation considered together, 2 XOs among 538 as against none among 538 female progeny in the controls); and (*c*) with spermatogonial irradiation, the frequency of induction is much lower, being 0.02 10^{-5} per roentgen (16/7789 in the irradiated; 10/5190 in controls).

2. *Female germ cells*

139. Russell *et al.* (452) investigated the effect of dose rate on the induction of X-chromosome loss in female mice. Mature hybrid female mice (X chromosomes unmarked) were exposed either to x rays at a rate of approximately eight roentgens per minute or to gamma rays (^{137}Cs) at about 0.6 roentgen per minute, the total exposure being in both cases 400 roentgens. On the day following the irradiation, the females were mated to males carrying the dominant sex-linked gene *Greasy* (*Gs*) and the progeny from the litters conceived within the first seven weeks after irradiation were screened for exceptional females of the genotype *Gs*/O. The presumed exceptions were checked by breeding tests and chromosome counts. Chromosome counts of the mothers of these females were also made to exclude cases in which the parent was also XO.

140. The results show that the frequency of exceptional females (*Gs*/O) at the low dose rate is significantly below that at higher rate (21 out of 6,674 female progeny *versus* 50 out of 7,576 female progeny). Tests are not yet completed on a few additional exceptions (6 in the low-dose-rate series and 14 in the high-dose-rate series). The frequency of exceptions in the control series currently stands at 0.05 per cent (3/5,547) and the test on one more presumed exception is incomplete.

141. In a translocation study involving irradiation of mouse dictyate oöcytes with 200 rads of fast neutrons Searle (479) obtained one definite and one presumptive case of XO out of 37 females tested.

F. Point mutations

1. *Spontaneous mutations*

142. Schlager and Dickie (467-469) have published the results of their very extensive study on spontaneous mutations and mutation rates in the mouse incorporating also the earlier data of the Bar Harbor group (158, 466). Taylor (541) investigated this problem in the rat populations that were used as controls in experiments designed to study the genetic effects of cumulative spermatogonial irradiation (paragraph 216). The data are given in table 13.

143. According to the latest results of Schlager and Dickie (469) (*a*) the average forward mutation rate per locus per gamete for the five coat-colour loci studied (estimate based on mutations that occurred in both males and females) is about four times that for back mutation at these loci; (*b*) the confidence interval of their estimate ($7.3\ 10^{-6}$; $16.6\ 10^{-6}$) encompasses the rates ($7.5\ 10^{-6}$ and $10\ 10^{-6}$) for the seven loci reported by Russell (440) and by Lyon *et al.* (285) from data collected, respectively, at Oak Ridge and Harwell; and (*c*) the over-all rates of forward mutations to recessive alleles at 26 unselected loci and to dominant visibles at 12 other unselected loci are not significantly different from one another but significantly lower than that for the specific loci.

144. Batchelor *et al.* (36) and Russell (448) recovered a total of seven specific-locus mutations[6] among 202,812 offspring of control females (0/37,813 and 7/164,999, respectively). In Russell's experiments, six of the seven mutants were recovered among the progeny of the same female, representing a cluster of mutant germ cells occurring early in development. This complicates the computation of the spontaneous mutation rate in females.

145. If it is assumed that the chance of a mutation occurring in the limited number of germ cells in early development is much less than the chance of occurrence among the numerous germ cells available later, then this leads to the conclusion that, in spite of the finding of a cluster, clusters will usually be much rarer than single mutants. On this basis, one can assume that there will be little error in assuming the mutation frequency to be 2 in 202,812 which gives a rate of $1.4\ 10^{-6}$ per locus per gamete.

146. On the other hand, if it is assumed that the only estimate of the frequency of clusters is that observed in Russell's experiments, namely, one out of two mutational events, then the sample size should be corrected to get an estimate of the number of independent observations. This gives 2/7 of 202,812, i.e., 57,946. The frequency of independent mutational events will then be 2 in 57,946 which gives a rate of $4.9\ 10^{-6}$ per locus per gamete.

147. The estimate of Taylor (541) on spontaneous mutation rates in rats cannot be directly compared with the other data presented in table 13 since the former is on a per gamete and not on a per locus basis.

148. Since all estimates of specific-locus mutation rates in *Drosophila* and the mouse as well as in man are based on loci at which mutations were known to have occurred before, they must be considered as possibly biased. This point has been particularly stressed by Cavalli-Sforza and Bodmer (69).

[6] The seven-locus tester stock was used; see foot-note 7.

149. Of the five coat-colour loci used in the study of Schlager and Dickie (469), the highest rate of spontaneous mutation from wild type was recorded for the *a* (non-agouti) locus (table 13). This is in contrast to the low rate of mutation recorded for this locus under acute spermatogonial x-irradiation. Russell and Russell (453) found only two mutations at the *a* locus out of 174 mutations recovered from x-irradiated spermatogonia (300 to 1,000 R; 90 R min^{-1}). Lyon and Morris (283) found no mutations at the *a* locus in their irradiation experiment (600 R) involving over 24,000 progeny. Further comparisons of the spontaneous and induced mutation rates of the four loci common to the study of Schlager and Dickie (469) and of Russell and Russell ((453) show an inverse relationship between the two rates in rank order: $b > d > c > a$ under irradiation *versus* $a > c > d > b$ for spontaneous mutations.

150. With reference to the discrepancy between induced and spontaneous rates at least at the *a* locus, it must be pointed out that most of the mutations observed in radiation studies at this locus were of a type which could not have been picked up in the usual kind of specific-locus experiment; the hybrid stock normally used in radiation experiments has the genotype AA^w at the *a* locus which means that $A^w A$ or $A A^w$ mutations cannot be detected (430, 480). It should also be borne in mind that the spontaneous mutations recorded by Schlager and Dickie (469) could have occurred in any of the male or female germ-cell stages whereas in the radiation experiments (paragraph 149) they were specifically recovered from irradiated spermatogonia. Because of these reasons, the apparent discrepancies between the spectra of spontaneous and induced rates at the loci compared are presumably not as big as they appear to be.

2. *Specific-locus mutations*

151. In its 1962 and 1966 reports, the Committee discussed data on the induction of recessive mutations at 12 specific loci[7] in the mouse. Tables 14-17 summarize the major results and include new data from experiments that have since been completed. In the following paragraphs, attention will be focused on the new data.

(a) *Adult spermatogonia*

(i) *Acute irradiation*

152. The complete results of the specific-locus experiment (six loci) carried out by Lyon and Morris (283) show that seven mutations were obtained out of a total of 24,834 offspring giving a rate of $0.78\ 10^{-7}$ mutation per locus per rad with 95 per cent confidence limits, $0.16\ 10^{-7}$ and $2.5\ 10^{-7}$ (600 rad: x rays). This estimate is not far from the approximate one derived on the basis of limited data in the 1966 report ($0.50\ 10^{-7}$). The confidence ranges of the present estimate overlap those for Russell's estimate of $2.2\ 10^{-7}$ for the seven loci ($0.89\ 10^{-7}$; $4.75\ 10^{-7}$).

153. Tests of viability effects of five mutations (out of the seven recovered) revealed that only one (at the

[7] The seven loci: *a* (non-agouti), *b* (brown), c^{ch} (chinchilla), *d* (dilute), *p* (pink-eyed dilution), *s* (piebald spotting), *se* (short ear).

The six loci: *a* (non-agouti), bp^R (brachypody-Harwell), *fz* (fuzzy), *ln* (leaden), *pa* (pallid), *pe* (pearl).

bp^R locus) mutation was lethal in the homozygous condition in contrast with the observations of Russell and Russell (453) that 77 per cent of the specific-locus mutations recovered in their study were lethal when homozygous.

154. The data of Lyon and Morris (283) permit the conclusion that the over-all rate of mutation induction at the six loci is about one third of that at the seven loci. It should, however, be mentioned that the point estimates for the individual loci (in either group) vary a great deal and have wide confidence limits. Consequently, it is not unreasonable to assume that the mutation rate of the average mouse locus (based on all 12 loci and with equal weight to each locus) in the spermatogonial stage is of the order of 1.7 10^{-7} mutations per roentgen per gamete.

(ii) *Dose rate*

155. Russell's earlier data from exposure-rate studies in spermatogonia revealed that the maximal effect of reducing the exposure rate is already obtained at 0.8 R min^{-1}, namely, a reduction of the yield to 30 per cent of that obtained at high dose-rate. This has been confirmed by a repetition of the 0.001 R min^{-1} gamma-ray experiment. In addition, the effects of an exposure rate much higher than the highest one (90 R min^{-1}) used previously were also studied by Russell (446). With an x-ray exposure of 300 roentgens delivered at a rate of 1,000 R min^{-1} to spermatogonia, 24 specific-locus mutations were recovered among 38,207 F_1 offspring, giving a rate of 3.0 10^{-7} mutations per locus per roentgen per gamete which is almost identical to the figure (2.9 10^{-7}) obtained from earlier experiments with the same x-ray exposure of 300 roentgens, but delivered at 90 R min^{-1} (table 14).

156. Batchelor, Phillips and Searle (35) have published the final results of their dose-rate study with 0.7-MeV neutrons. Mouse spermatogonia were given either a dose of 188 rads (+ 18 rad gamma contamination) delivered in 3-4 minutes or a total dose of 62 rads (+ 42 rad gamma contamination) delivered over a period of twelve weeks. The induced rates of mutation at the *PT* loci were 0.15 10^{-6} per locus per rad per gamete (188 rad, acute) and 1.33 10^{-6} per locus per rad per gamete (62 rad, chronic). This reverse dose-rate effect is in line with earlier findings reported by Russell (440).

157. The amount of germ-cell killing with chronic neutron irradiation at a dose of 62 rads was much less than that found in an earlier experiment in which 214 rads were delivered over a 12-week period (34). With 62 rads, the mean testis weight decreased to only about 50 per cent of normal whereas with 214 rads the decrease was greater (20 per cent of normal).

(iii) *Fractionation*

158. The fractionation effect leading to a striking increase in mutation frequency observed by Russell when 1,000 roentgens were administered to spermatogonia in two equal fractions separated by 24 hours has now been confirmed and extended by Lyon and Morris (283) using both sets of specific loci. With the seven loci, 16 specific-locus mutations were recovered among 5,462 offspring, giving a mutation rate per locus per rad of 4.2 10^{-7}. This figure is not far from that (4.9 10^{-7}) obtained by Russell (438). With the six loci, 14 mutations among 17,301 offspring were found. The mutation rate per locus per rad is 1.4 10^{-7} with 95 per cent confidence limits 0.74 10^{-7} and 2.27 10^{-7}. When the differential mutability of the two sets of loci is taken into account, the agreement between the new data of Lyon and Morris (283) and those of Russell's group is quite good. Viability tests showed that three out of the nine mutations in the six-locus fractionated series, and two out of seven mutations in the seven-locus fractionated series, were lethal when homozygous.

159. In subsequent experiments, Lyon, Phillips and Bailey (285) examined the mutagenic effects of repeated small radiation doses delivered to spermatogonia at different dose rates; with a total dose of 600 rads of ^{60}Co gamma rays (at 17 rad min^{-1}) delivered in daily doses of 10 rads each, the yield of specific-locus mutations (at seven loci) was one third of that after the single exposure, under otherwise similar radiation conditions (compare treatments 1 and 2, table 15) and was close to that found after the low-dose-rate irradiation at 0.008 rad min^{-1} (treatments 2 and 3, table 15). Thus repeated small doses produce less effect than a single dose of the same size and the reduction in yield is of the same general order as in the case of translocations (table 9).

160. However, when a similar total dose was split into 50-rad fractions and administered at weekly intervals, the yields depended on the dose rate, being about twice at 60-70 rad min^{-1} than at 0.05-0.07 rad min^{-1} (treatments 4 and 5, table 15); the yield with the higher dose rate is close to that after the single exposure, thus differing in this respect from the response observed for translocations (paragraph 80).

(b) *Oöcytes*

(i) *Low-dose-rate neutron- and gamma-irradiation*

161. Since it is known from earlier work that chronic fast-neutron-irradiation is nearly 20 times as effective as chronic gamma-irradiation in inducing specific-locus mutations in mouse spermatogonia, and since it is also well established that gamma-irradiation at low dose rate induces even fewer mutations in female mice than in males, a series of experiments were carried out to investigate the relative radio-sensitivity of the dictyate oöcytes to chronic neutron and gamma irradiation (36, 493). The seven locus stock was used. In this large-scale study (79.7 rad 0.7 MeV neutrons + 57.8 rad gamma contamination; 412 rad ^{60}Co gamma-irradiation; both irradiations were over a 12-week period) involving a total of over one hundred thousand F_1 mice, only one mutation was recovered in the first litter of the neutron series (among 32,221 progeny) and none in the gamma or in the control series.

162. From the results of neutron-irradiation, the mutation rate can be estimated to be 0.3 10^{-7} per locus per rad per gamete, or less than 5 per cent of that found when spermatogonia are exposed to a similar dose of fast neutrons over the same 12-week period (table 16).

163. The absence of specific-locus mutations after 412 rads received chronically from a gamma source is in line with previous findings of Russell (440). All the oöcyte studies so far carried out with different exposures of chronic gamma-irradiation (258, 400 and 412 R, table 16) have yielded only three specific-locus

mutations in about 100,000 progeny. This frequency is of the same magnitude as the maximal estimate of the spontaneous frequency (paragraph 146) and roughly three times that of the minimal one (paragraph 145). In view of the uncertainty as to which of the spontaneous estimates is to be used for comparison, any firm statement on the mutagenic efficiency of chronic gamma-irradiation is difficult except that it is very low.

164. While considering the low mutagenic effectiveness of chronic gamma irradiation, the possible effects of the interval between irradiation and conception should also be taken into account. This aspect is discussed in paragraph 172.

(ii) *Small single doses*

165. The low mutational yield obtained with small single doses of high-dose-rate irradiation and with medium-sized doses split into several fractions, which is predicted on the hypothesis of repair of one-hit mutational events and for which preliminary evidence was presented in the 1966 report, has now been fully confirmed (443-446, table 15). The mutation rate after 50 roentgens is only one third of that after a single exposure of 400 roentgens; with eight fractions of 50 roentgens each, the mutation rate is less than one half of that after a single exposure of 400 roentgens.

(iii) *Interval between irradiation and conception*

166. In adult male mice, no effect of the interval between irradiation and fertilization has ever been observed on the induced specific-locus mutation frequency in spermatogonia. This holds true even to the end of the animal's reproductive life (440).

167. In contrast, the results from experiments involving irradiation of female mice clearly show that the interval between irradiation and conception has a dramatic effect on the mutation frequency observed in the offspring. This effect was first discovered with high-dose-rate fast-neutron-irradiation (441); at a dose of 63 rads, the mutation frequency was high in those litters conceived within seven weeks after irradiation but zero or nearly so in later litters (table 16). This finding was subsequently extended to low dose-rate neutrons and high-dose-rate x rays (445, 448; table 14).

168. The failure to recover mutations from earlier dictyate stages could be due to their low intrinsic mutational sensitivity, to the high efficiency of their repair or to selection, since in these experiments large numbers of oöcytes in early follicle stages are killed by radiation. Of these possibilities, selection perhaps is the least likely one (448).

169. The autoradiographic study of Oakberg (360) on the relationship between stage of follicular development and RNA synthesis in the mouse oöcyte shows that the oöcyte stages with high mutation frequency may correspond to those in which uridine incorporation has stopped, whereas the earlier stages with low mutation frequency probably correspond to those that show heavy labelling. Oakberg concludes that, since it is likely that capacity for repair is closely correlated with metabolic activity, the change in mutation frequency with time after irradiation may be explained by a changing capacity for repair of genetic damage. He cautions, however, that "a better understanding of normal oögenesis and the ability to relate specific follicular stages to specific post-irradiation litters is mandatory for a critical evaluation of the possible relationships between metabolic activity and sensitivity to mutation induction of the mouse oöcyte".

170. While it is quite possible that ability to repair genetic damage is correlated with metabolic activity, it should be borne in mind that there are other systems where such a correlation does not seem to exist. The rate of incorporation of ^{3}H-uridine is low during the first three cleavage divisions of the fertilized egg, but then increases sharply and rapidly to a high level (306, 308). High metabolic activity presumably continues during the period of differentiation and active multiplication of the primordial germ cells, which nevertheless show a high level of mutational sensitivity (paragraphs 176-177). These findings argue against metabolic activity being the sole determinant of mutational insensitivity of the early dictyate oöcytes (480).

171. These findings have led to the suggestion that the mutational insensitivity of the immature dictyate oöcyte depends on some other factor or factors besides the level of metabolic activity (494). However, a positive correlation between mutational sensitivity and a sudden and dramatic change in ^{3}H-uridine incorporation within the dictyate oöcyte may still be indicative of repair processes associated with a specific kind of metabolic activity occurring within this cell stage (449).

172. After chronic gamma irradiation of oöcytes, the mutational yield is so low that the effect of interval between irradiation and conception is not very obvious; as a matter of fact, the mutation frequencies recorded for oöcytes sampled during the first seven weeks and those for oöcytes sampled subsequently are not significantly different from one another (table 16). Nonetheless, the interval effect presumably operates here too; the observation that the mutation frequencies for later matings are lower than those for earlier matings is in keeping with this line of reasoning (1/21,854 *versus* 1/15,195; 0/18,684 *versus* 1/8,373).

173. The exposure rate of 0.009 R min^{-1} in the 258 and 400 roentgen experiments involved exposure durations of approximately three and five weeks, respectively. The progeny from matings made within seven weeks after the termination of these exposures obviously included some derived from oöcytes that received a sizeable proportion of their radiation while in a resistant stage (earlier dictyate stages: paragraph 167); most of the oöcytes responsible for later litters would have been in a resistant stage during the entire duration of irradiation. Thus the low total mutation frequency over the first seven-week mating period and the still lower one over the subsequent period could be explained as due to the operation of both the dose-rate effect and the interval effect although the latter, as discussed above, is not as dramatic as after acute irradiation.

(c) *Neonatal and embryonic germ cells*

174. Selby (498) has obtained data on the x-ray induction of specific-locus mutations (300 R; 80 R min^{-1}) in male mice at various ages from new-born to young adult. For day one, the results obtained thus far show 16 mutations among 55,126 offspring or a rate of about 1.4 10^{-7} per locus per roentgen, less than one half of that obtained in adults with the same exposure, and the difference between the two is sta-

tistically highly significant. The combined data from nine groups of males irradiated at ages ranging from 2 to 35 days show 43 mutations among 77,429 offspring yielding a rate of 2.6 10^{-7} per locus per roentgen. This rate is quite close to that (2.9 10^{-7} per locus per roentgen) calculated from the results of adult irradiation (table 14).

175. In another study Selby (499) exposed within nine hours after birth new-born female mice to 300 roentgens at high rate and obtained three specific-locus mutations in a total of 14,259 offspring. This gives a rate of about 1.0 10^{-7} per locus per roentgen, one which is only about one sixth of that expected from similar irradiation of adult females.

176. Searle and Phillips (494) compared the mutagenic response of mitotically dividing primordial spermatogonia and oögonia with their precursors, following protracted *in utero* irradiation of mouse embryos. A neutron dose of 108.5 rads (plus 20.5 rad gamma contamination) at 0.011 rad per minute was given to pregnant females over a period of one week before the twelfth day of embryonic life. Weaned males and females were appropriately mated at eight weeks of age to mice of the *PT* tester stock and the offspring were scored for mutations at the specific loci.

177. The large clusters of specific-locus mutations found in both the male and female series show conclusively that mutations can be readily induced in embryonic germ cells. Using cluster size to estimate the mean number of germ cells at risk, Searle and Phillips (494) calculated the mutation rates to be 5.3 10^{-5} per locus and 6.4 10^{-5} per locus, respectively, in male and female primordial germ cells with induced rates per locus per rad 4.2 10^{-7} and 5.8 10^{-7} in male and female germ cells, respectively. The difference between the two rates is not significant. If dose attenuation is allowed for (because of the depth of the embryonic germ cells within the pregnant females) the rates are one third higher (5.6 10^{-7} and 7.7 10^{-7}).

178. A comparison of these rates with those obtained after irradiation of spermatogonia and oöcytes in adults (tables 14 and 16) shows that (*a*) the rate of induction of specific-locus mutations in primordial spermatogonia is somewhat lower than that obtained after neutron-irradiation of adult spermatogonia and (*b*) the rate in primordial oögonia is less than that in mature oöcytes irradiated at 0.17 rad per minute although very much higher than that after chronic irradiation of oöcytes (79.7 rad; 0.0007 rad per minute).

179. Further comparisons of the data of Searle and Phillips (494) can be made with those of Carter (62, 63) and Carter, Lyon and Phillips (66). Carter (62) reported a very low mutation rate of 4.7 10^{-8} per locus per rad after x-irradiation at 300 rads (70 rad min^{-1}) of male fœtuses 13½ days after conception, but this may have been mainly the result of strong germinal selection, since spermatogonial killing was so high that 30 per cent of males proved infertile. The mutation rate after a dose of 200 rads at a high dose rate given to 17½-day-old male fœtuses was 2.1 10^{-7} per locus per rad (66), not significantly different from the rate in adults and in fœtuses of 13½ days of age; 7.6 per cent of males were sterilized by the radiation exposure and so, again, germinal selection may have tended to reduce the yield of mutations. The general conclusion that can be made then is that the genetic sensitivity of the primordial germ cells in the male may not in fact be much less than that of spermatogonia in the adult.

180. In other experiments, Carter (63) gave female fœtuses between 12½ and 18½ days of age 300 rads (gamma rays) at 0.05 rad per minute and obtained a mutation rate of 1.02 10^{-7} per locus per rad which is much higher than 0.23 10^{-7} per locus per roentgen obtained after low-dose-rate gamma-irradiation of oöcytes in adult females (table 16). In Carter's experiment, the irradiated germ cells would have been oögonia and pre-dictyate oöcytes in early meiotic stages. In another study (66), high-dose-rate x-irradiation of 17½-day-old fœtuses at 200 rads yielded a mutation rate of 0.7 10^{-7} per locus per rad which is significantly lower than the rate of 4.02 10^{-7} in mature dictyate oöcytes.

181. It thus seems clear that the mature dictyate oöcyte is genetically rather more radio-sensitive than pre-dictyate and pre-meiotic germ-cell stages. It is also becoming increasingly likely that the immature dictyate oöcyte is the only germ-cell stage (among both male and female germ-cell stages) which is insensitive from the point of view of mutation induction.

(d) *Nature of specific-locus mutations*

182. A careful examination of tables 14-17 will reveal that the pattern of response of the specific-locus mutations to changes in the radiation variables is in certain respects qualitatively similar to that of translocations. This feature has been noted by several workers (280, 478) and suggests that there is something in common between the primary lesions leading to gene mutations and translocations. In particular, the response of specific-locus mutations to changes in dose rate, to some fractionation procedures and to high-LET radiation is so similar to what is usually observed with translocations and to what is known about the response of chromosome-breakage events in general, that it has been argued that specific-locus mutations are really two-track chromosome deletions, rather than one-track events (524, 604). However, the evidence presented below does not support this view.

183. Especially pertinent in this context is the recent work of L. B. Russell (430) who has been able, by means of complementation tests, to make a detailed genetic analysis of the *d se* region of linkage group II of the mouse (recombination frequency of 0.16 per cent). While the original screening for mutants employed only two markers (*d, se*), subsequent analysis (using nearby markers *sv, tk* and *sg* in addition) has so far revealed 16 complementation groups spanning eight or nine functional units. Mutations used for this purpose were derived from specific-locus experiments of W. L. Russell and co-workers at Oak Ridge, and were detected by their visible phenotype in combination with tester-stock's markers, *d* and *se*.

184. The results given in tables 18 and 19 (involving well over 800 combinations and a total of about 40,000 progeny) show that there is a strong effect of the irradiated germ-cell stage, as well as of the type of radiation, on the locus spectrum (i.e., on the relative frequencies of events involving *d*, *se* or both) and on the involvement of single functional unit as against that of two or more functional units. In the case of x- or gamma-irradiated spermatogonia, the spectrum is very similar to that of controls, with a majority of mutations being at the *d* locus (table 18).

185. With 24-hour x-ray fractionation and with neutron-irradiation again in the same germ-cell stage, the spectrum of events is different (and, with neutrons, significantly so) with relatively fewer *d* and relatively more *se* and Df[8] (*d, se*) events. In addition, in the neutron series, a somewhat higher percentage of events is pre-natally lethal.

186. The spectra obtained after irradiation of post-spermatogonial stages and oöcytes are very clearly different from those obtained after spermatogonial irradiation. In each case, the proportions of the three types of events are much more nearly equalized (table 19). The post-spermatogonial stages and oöcytes do not differ significantly in total distribution, but there is evidence of a higher proportion of pre-natal lethals among the latter group.

187. The frequency of mutations interpreted as aberrations ranges from 13.5 per cent in most x- or gamma-irradiated spermatogonia to 42.3 per cent in post-spermatogonial stages and 65.6 per cent in oöcytes (table 19). The recombinational length of most of the aberrations is very small, 75 to 80 per cent of them spanning less than two cross-over units. Even in those groups that have a high total frequency of aberrations (post-gonial stages and oöcytes) no more than 23 per cent of all mutations exceed this length and the figure is zero per cent for x- or gamma-irradiated spermatogonia (excluding the 24-hour fractionation group).

188. The findings presented in paragraph 187 lend strong support to W. L. Russell's conclusion that the specific-locus mutations recovered in his studies are predominantly single-track events. In what follows, the validity and/or usefulness of other criteria that have been used to characterize the specific-locus mutations as point mutations or as resulting from chromosome breakage events will be discussed.

189. The mutational spectrum of specific-locus mutations at high exposure rates is expected to be different from that at low rates if these mutations are predominantly two-track in origin (442). Information bearing on this point is given in table 20 for specific-locus mutations induced in spermatogonia. It is clear that the spectrum is hardly affected by the exposure rate, even though the spectrum itself is characterized by marked differences between loci. Although the data for oöcytes are less extensive, Russell points out that the results of the analysis of spermatogonial mutations apply to them also. This is so even with regard to the relative frequency of *d* and *se* presumed deficiencies which is greater in oöcytes than in spermatogonia and large enough for a more meaningful dose-rate comparison. These observations, then, seem to be more compatible with the one-track nature of the origin of these mutations.

190. In oöcytes, about half the mutations induced at high exposure and high exposure rate that involve either the *d* or the *se* locus also affect the other locus, i.e., they are genetically-detected deficiencies. Tests with marker genes close to the *d-se* region (430) show that these deficiencies are also small, most of them probably involving less than two cross-over units. The assumption that these are predominantly two-track events implies that most of those that involve only one of the two loci may also be two-track in origin but must, on average, be smaller than those which affect both loci. If these small deficiencies, are the result of two independent hits occurring close together, the probability of hits occurring farther apart and causing larger deficiencies must be greater.

191. Russell (447) argues that even if the probability were only three times as great, a single acute exposure of 400 roentgens would, on the above assumptions, bring about more than one large deficiency per genome, which would be lethal either in the germ cells or during development. Since only enough oöcytes mature in each oestrus to produce the number of eggs ovulated, an average frequency of at least one lethal deficiency per genome, regardless of whether death occurred in the germ cell or during development, would usually eliminate most of the offspring in the first litter after irradiation. However, there is only a small reduction of litter size in litters conceived shortly after an exposure of 400 roentgens, strongly suggesting that most of this reduction may not result from two-break aberrations causing dominant lethality. Thus, one can conclude (although by somewhat indirect reasoning) that most of the specific-locus mutations observed are not due to two-break aberrations, a conclusion which is in line with the findings of L. B. Russell presented earlier (paragraphs 183-187).

192. When the effect of a single exposure of 1,000 roentgens to spermatogonia is compared with that of an exposure split into two equal fractions separated by a 24-hour interval, it is seen that the specific-locus mutation frequency increases nearly five-fold with fractionation (table 14). On the other hand, with similar exposure and similar fractionation procedure, the frequency of translocations is no greater than expected on the basis of the additivity of yields of two well-separated 500-rad fractions (table 8). Furthermore, at doses of 600 rads and below, the translocation yield of a single dose and of fractionated doses (two fractions, 24 hours apart) are the same. These observations raise the question as to whether the presence or absence of a fractionation effect is sufficient *per se* to decide on the nature of the events involved in specific-locus mutations.

193. The results of the fractionation experiment in females where a total exposure of 400 roentgens was split into two fractions separated by a 24-hour interval (table 16) show that the observed specific-locus mutation frequencies are the same irrespective of whether the exposure is single or fractionated. This finding would be unexpected if the specific-locus mutations were predominantly two-track events. The difficulties encountered in upholding the two-track interpretations to explain the lack of fractionation effect in the above experiment have been summarized by Russell (445).

194. One additional argument against the specific-locus mutations being predominantly two-track events comes from work on chemical mutagenesis carried out at Oak Ridge (447). Four different methane sulphonates were tested both for dominant lethal and specific-locus mutation induction. All gave a dominant lethal frequency and some a translocation frequency (449) equivalent to that yielded by a large dose of radiation, but only one gave any significant increase over control values for specific-locus mutations, and even there the effect was small. Since there is strong evidence that dominant lethals are due to chromosome breakage, Russell considers that the evidence from the chemical work suggests that chromosome aberrations, including

[8] Df = deficiency.

two-break deficiencies, are unlikely to be the source of most specific-locus mutations.

195. From the foregoing discussions it will be clear that the results of the various dose-rate and dose-fractionation experiments with specific loci should be compared in the wider context of the recent data on translocation induction; the results of complementation tests at the *d-se* region, however, have led to an improvement of our understanding of the nature of radiation-induced mutations in the mouse and strongly support the idea that the specific-locus mutations studied in the mouse may predominantly be one-track events. Work on other closely linked loci in the mouse would seem desirable, in order to find out whether the *d-se* pair presents a typical picture.

3. *Dominant and recessive visibles and recessive lethals*

196. Recent data on these mutations have been obtained from straightforward mutation experiments as well as from long-term population experiments designed to assess the magnitude of the genetic load under different conditions of irradiation and its effects on several measurable components of fitness. Specific-locus mutations which turn out to be homozygous lethals, thus fulfilling the criterion of recessive lethality, will not be discussed here since this aspect has already been considered in the section on specific-locus mutations.

(a) *Dominant visibles*

197. The data on dominant visibles summarized in table 21 lead to the following conclusions for mouse spermatogonia: (*a*) the frequency of dominant visibles increases with exposure fractionation; (*b*) high doses of fission neutrons lead to higher yields at low than at high dose rates; (*c*) at low dose rates, neutron-irradiation is mutagenically more effective than gamma-irradiation; (*d*) the general pattern of response of the dominant visibles to irradiation is similar to that of specific-locus mutations; (*e*) the frequency of dominant skeletal mutations induced by x-irradiation of post-spermatogonial stages after 600 roentgens is 2.6 times that induced in spermatogonia. The magnitude of the difference in response between spermatogonial and post-spermatogonial stages observed in Ehling's study (124, 125) is strikingly similar to that recorded by Russell, Bangham and Gower (450) for specific-locus mutations; and (*f*) the dose response for 14.1 MeV neutrons in post-spermatogonial stages is approximately linear (572).

198. The mutational nature of the events involved in the induction of dominant skeletal mutations was examined by Ehling (125) and by Tutikawa (572) in experiments designed to permit breeding tests on a sample of presumed skeletal mutations, the first generation offspring being sacrificed only after they had produced one litter. In Ehling's study three out of five mutations were found to be transmitted to the second and later generations. One of these mutants was found in an earlier experiment (124) in which spermatogonia had been irradiated and two others were from a study involving irradiation of post-spermatogonial stages. The test of two additional presumed mutations is incomplete. In Tutikawa's work, 2 out of 11 presumptive mutations were found to be autosomal dominants.

(b) *Recessive lethals and visibles*

199. In recent years, there have only been a few investigations aimed at studying the induction of sex-linked lethal mutations in mice or in rats. In the absence of efficient screening methods, the techniques thus far employed have relied on changes in sex proportion and reduction in litter size as possible indicators of lethals induced in the X chromosome. In some experiments, use was made of X chromosomes marked with suitable dominant genes to identify at least those lethals that happen to be induced in the vicinity of the marker(s). The closer the lethal to the marker(s), the greater the chance of detecting it. The results obtained using any of these approaches have so far yielded equivocal evidence for the induction of sex-linked lethals, and the estimates, where given, seem open to question on grounds outlined in paragraphs 208-210.

200. Auerbach *et al.* (16) exposed male mice to x rays (500 R) and carried out a test for sex-linked lethals in post-meiotic germ cells using bent-tail (*Bn*), tabby (*Ta*) and brindled (Mo^{br}) as sex-linked markers. Among 176 tested gametes, there was no indication of a lethal in the segments adjoining the markers.

201. In one of the two experiments of Schröder (475), male mice of *Ta*/Y constitution were x-irradiated at exposures of 600 or 1,200 roentgens and mated to unirradiated females (X chromosomes unmarked) after the period of sterility. The F_1 females heterozygous for *tabby* (*Ta*/+) were outcrossed to normal inbred males (+/Y) to produce an F_2. If an F_1 *Ta*/+ female carried a recessive lethal on the X chromosome marked by *Ta*, no viable *Ta* sons would be expected among her progeny. If no *Ta* son was produced in 20 offspring, the F_1 female in question was suspected to be a carrier of a recessive sex-linked lethal mutation and would be expected to have transmitted the lethal to all her *Ta*/+ daughters (the situation is not so simple because of crossing-over). All the *Ta*/+ daughters of "suspect" females were retested to confirm the absence of *Ta*/Y sons.

202. In the second experiment, Schröder irradiated females homozygous for *Ta*(*Ta*/*Ta*) (x rays, 300 R) and mated them to normal males (+/Y). The F_1 *Ta*/+ females were handled in the same manner as outlined above. Appropriate controls were maintained.

203. Out of a total of 3,504 X chromosomes (in both groups together with their respective controls) screened, no true recessive *Ta*-linked lethal mutation could be found that satisfied the criterion of non-occurrence of *Ta* males in both the F_2 and F_3 generations.

204. In the study of Grahn *et al.* (153), irradiated (500 R; spermatogonia) and control males were mated to females heterozygous for the dominant sex-linked gene *Tortoise* (*To*). F_1 *To*/+ females carrying the irradiated X chromosome from the father were outcrossed to +/Y males to raise an F_2 generation and the suspected lethal-carriers were appropriately retested.

205. In the F_2 generation, the female progeny will be of two types, i.e., *To*/+ and +/+, the latter carrying the irradiated X chromosome, but there will be only one class of males (+/Y) since *To*/Y males are inviable. If an F_1 *To*/+ female carries no lethal on the X chromosome, her progeny will occur in the ratio of

two females to one male (sex proportion: 0.33). However, if that female carries a lethal on her X chromosome, such a lethal can be "transferred" through crossing-over to the other X chromosome carrying the *To* gene with a probability that depends on the distance of the lethal from the *To* gene. Under the extreme assumption that the chance of crossing-over is 0.5, female and male progeny of a carrier F_1 female will occur in a 4:1 ratio. As will be obvious, the probability of detecting a lethal will increase as the distance between the lethal and the *To* gene decreases.

206. In analysing the F_2 data using Haldane's swept-radius method of detecting lethals linked to a visible marker (*To* was the point marker and the presence and location of the lethal was determined by the degree of deficiency in number of males), Grahn *et al.* (153) found that no estimate of sex-linked lethal damage could be arrived at. However, when the data were analysed taking into account the distributive properties of sex proportion and litter size and their variances, the authors noted that (i) there was good evidence for induced sex-proportion changes at birth and litter-size reduction in F_1 and F_2 generations; and (ii) the sex-proportion changes at birth were consistent with sex-linked lethals and detrimentals being induced in mouse spermatogonia at a rate of 0.85 10^{-4} per roentgen per X chromosome with 95 per cent confidence limits of 0.2 10^{-4} and 1.5 10^{-4}. The assumption used here in making this estimate was that the difference between the control and the irradiated groups (with regard to sex-proportion changes) was a measure of the induced lethal and detrimental genetic burden specific to the X chromosome. This assumption, as will be shown below (paragraphs 208-210), seems questionable.

207. In attempts to perpetuate the suspected sex-linked lethals to generations beyond F_2, Grahn *et al.* (153) found that only two lethals (one in the control and the other in the irradiated group) continued to give positive evidence for segregating lethals; these two were discarded as "indeterminate" after the sixth generation. In all of these generations, the suspect carriers had been identified by the occurrence of a significant sex-proportion deviation.

208. Lüning and Sheridan (279) tested the hypothesis whether sex-proportion shifts and litter-size reduction could be used as reliable criteria for the detection of sex-linked lethals. No X-linked marker genes were employed, and the material for this study was derived from their irradiated (276 R to spermatogonia in each generation) and control mouse populations. Production records from single-pair matings of offspring of the ninth and fourteenth generation were examined. The irradiated series gave, in both generations, a lower proportion of males than the control although only the results of the fourteenth generation test showed a significant difference.

209. If these observed changes were due to the circumstance that some of the females tested were heterozygous for sex-linked lethals then (*a*) the causal basis should be more easily demonstrable and the presumed lethals identifiable in families with a significant as well as in those with a considerable but non-significantly decreased sex proportion and (*b*) such selected families should provide more clear evidence of reduced litter size. These expectations were not fulfilled; there seemed to be no correlation between the sex-proportion shift observed in the "index cases" and that in their mothers and/or sisters. Furthermore, there was no indication of a reduced mean litter size in the selected group relative to its appropriate control, nor was there any evidence for a decreased sex proportion in families with fewer litters (one to three) and small mean litter size (of up to six) relative to those with more litters (ten or more) and large mean litter size (more than six). On the basis of these results, the authors have concluded that the sex-proportion shift is an unreliable indicator for the presence of sex-linked lethals.

210. In a study on the genetic effects of spermatogonial irradiation (1,200 R of x rays in two equal fractions separated by eight weeks) on productivity of F_1 female mice, Searle (477) observed a significant deficit of males. However, a familial analysis of cases with such a deficit and a comparison of families with small and large sibships showed that sex-linked lethals were responsible for very little, if any, of the reduction in litter size and productivity, from which it was concluded that "the sex-ratio change was probably mainly a chance effect or due to some other unknown factors".

211. In view of the uncertainties involved and of the divergence of views on the use of sex-proportion shifts in identifying sex-linked lethals (paragraphs 206-210) it does not seem feasible at present to use the data on sex-proportion shifts to compute the rate of induction of sex-linked lethals.

212. Lüning and Searle (275) have recently summarized the results of studies on the induction of autosomal recessive pre-natal lethals in the mouse. These data from experiments involving single or fractionated x-ray exposures of spermatogonia in one generation only, and those from experiments involving irradiation of spermatogonia over several generations can be used to compute the rate of induction of recessive lethals in mouse spermatogonia. The derived estimates are presented in table 22.

213. It can be seen that (i) with reference to spontaneous recessive lethals, the three separate experiments give widely divergent results, presumably because of the low number of spontaneous lethals expected per experimental group under test and the resultant large random variation. Combining the three, the best estimate of the incidence of spontaneous recessive lethals can be arrived at, and this is of the order of 29 10^{-4} per gamete with an upper 95 per cent confidence limit of 65 10^{-4} per gamete; and (ii) there is variation in the estimated induced rates (experiments 5-7) although this seems to be of a lesser magnitude than in the control groups. Averaging results from the three separate sets of data, the induced rate can be estimated as 0.9 10^{-4} per gamete per roentgen, with 95 per cent confidence limits of 0.4 10^{-4} and 1.5 10^{-4} per gamete per roentgen.

214. The estimates derived from population studies (table 22, experiments 8-9) are not directly comparable with those presented in the preceding paragraph since (i) there were no precautions to exclude semi-sterile animals, with the consequence that the results may and do show considerable variation; and (ii) consecutive generations are not independent of each other. Nevertheless, it is worth pointing out that the estimates derived from the study of these irradiated populations are of the same magnitude as the upper limit of those presented earlier (paragraph 213).

215. In the investigation discussed in paragraph 7, Chambers (71) also studied the induction of autosomal recessive lethals in rat spermatogonia. It was found that the estimated rate (based on litter size at one day of age) ranged from $(8.4 \pm 7.6)\ 10^{-4}$ to $(9.1 \pm 3.3)\ 10^{-4}$ per gamete per roentgen, being about five times higher than those obtained in other studies with rats (paragraphs 216-217). The latter might be due to the experimental scheme employed, in which the lethality caused by induced reciprocal translocations could have had a significant contribution (the experimental design was based on a combination, with appropriate modifications, of Haldane's method in which marker genes were used to scan the genome for recessive lethal mutations and of Russell's specific-locus method).

216. Havenstein *et al.* (174), Havenstein and Chapman (173) and Taylor and Chapman (543) have presented some data on the x-ray induction of sex-linked lethals and of autosomal lethals and visibles. The basic data are derived from their two albino rat populations (and two contemporaneous controls) started around 1960 with highly inbred strains. Males of one and females of the other received whole-body irradiation (450 R) every generation, the total exposure being administered in three fractions of 100, 150 and 200 roentgens at 10, 12 and 14 weeks of age in each generation. This schedule of irradiation was designed to minimize somatic effects. The germ cells sampled were spermatogonia in one population and oöcytes in the other.

217. A total of nine generations were irradiated in each group and data were collected for five subsequent generations after irradiation was discontinued. Full sib-matings were made at appropriate generations, and estimates of both sex-linked and autosomal recessive lethals obtained using sex-ratio shifts and litter sizes at various ages after birth as measured end-points. The following main conclusions were drawn: (i) the pattern of response of the genomes of the rat and the mouse is essentially similar; (ii) the rate of induction of sex-linked lethals in rat spermatogonia is $(1.6 \pm 0.6)\ 10^{-4}$ per gamete per roentgen. Despite its closeness to the available estimates for mouse spermatogonia, the reliability of this estimate is open to question in view of the fact that sex-ratio shifts were used as indicators of sex-linked lethal damage (see paragraph 211); and (iii) the rate of induction of autosomal recessive lethals (table 22) in rat spermatogonia (based on litter size at one day of age) is $(1.0 \pm 0.8)\ 10^{-4}$ per gamete per roentgen, in general agreement with the rate based on embryonic survival in mice (table 22; 1966 report, annex C, paragraphs 142-144); for oöcytes, the rate is similar to that for spermatogonia and also has wide confidence limits.

4. *Effects of induced mutations on components of fitness*

218. Fully recessive mutations have relatively little importance in determining the fitness of individuals in large random-breeding populations, except that at the human level they can be regarded as being roughly equivalent to recessive genetic diseases. There is, however, the possibility that mutations considered to be recessive because of their visible or lethal phenotypic effects may have deleterious effects in heterozygotes either singly or in combination with other heterozygous recessives. This problem has been debated for well over a decade, discussed in the earlier reports of the Committee and reviewed recently by Searle (478), Green (155) and Lüning (273) for mammalian experimental populations and by Spiess (531) for insect populations. Table 23 summarizes the more recent results of studies with mammals.

219. Many but not all (see for example Russell (436), Russell and Russell (453)) of the results that bear on the problem of detrimental effects of induced mutations in the heterozygous condition are either negative or just on the border line of significance. The weight of evidence thus far accumulated tends to suggest that such effects are of a lesser magnitude in mammalian populations than those that have been observed in *Drosophila studies* (155, 273). As Green (155) summarized, the generally negative results of the mammalian studies may be due to the "non-existence of induced mutations having only moderate individual effects on heterozygotes, to the failure to find the right indicator trait or to the relatively small sizes of the experiments so far conducted and their relative lack of power for discriminating small genetic differences in the presence of large amounts of non-genetic variability".

5. *Summary and conclusions*

220. The average spontaneous forward mutation rate at the five coat-colour loci studied in the mouse (*a, b, c, d* and *ln*) in the course of routine breeding is $11.3\ 10^{-6}$ per locus per gamete, based on mutations that occurred in both males and females. This rate is about five times that for spontaneous back-mutations at these loci.

221. At other loci studied in conjunction with radiation experiments the average forward mutation rate is $7.9\ 10^{-6}$ per locus per gamete in males and $1.4\ 10^{-6}$ or $4.9\ 10^{-6}$ per locus per gamete (depending on the method of estimation) in females.

222. The over-all rates of spontaneous forward mutations to recessive alleles at 26 unselected loci and to dominant visibles at 12 other unselected loci are not significantly different from one another, but significantly lower than the specific-locus rate in males mentioned above.

223. The recent data on the induction of specific-locus mutations, dominant and recessive visibles and recessive lethals in spermatogonia and oöcytes of the mouse and of the rat are in essential conformity with the earlier mouse data and strengthen the conclusions reached by the Committee in 1966.

224. The available data from experiments involving acute x-ray exposures of up to 600 roentgens (adult spermatogonia) permit an estimate of $1.7\ 10^{-7}$ per locus per gamete per roentgen as the average rate of induction of specific-locus mutations, this figure being based on all the 12 loci studied with equal weighting given to each locus.

225. The rate of induction of specific-locus mutations in the spermatogonia of new-born mice (on day of birth) is less than one half of that obtained after irradiation of adults with the same x-ray exposure of 300 roentgens; the combined data from nine groups of males irradiated at ages ranging from 2 to 35 days give a rate of induction not significantly different from that recorded for adult spermatogonia. For new-born female mice irradiated (300 R of x rays) within nine hours after birth, the rate of induction is only about

one sixth of that expected from similar irradiation of adult females.

226. Specific-locus mutations can be readily induced by low-dose-rate neutrons (0.011 rad min^{-1}) in primordial spermatogonia and oögonia by irradiating mouse embryos *in utero*. The rate of induction per locus in primordial spermatogonia (4.2 10^{-7} rad^{-1}) is somewhat lower than that obtained after irradiation of adult spermatogonia; the rate per locus in primordial oögonia (5.8 10^{-7} rad^{-1}) is less than that in mature oöcytes irradiated at 0.17 rad min^{-1} and very much higher than that after low-dose-rate irradiation (0.0007 rad min^{-1}) of oöcytes.

227. The results of genetic analysis and complementation tests at the *d se* region (linkage group II of the mouse) have led to an improvement of our understanding of the nature of radiation-induced mutations in the mouse and strongly support the idea that the specific-locus mutations studied in the mouse may be predominantly one-track events.

228. There is controversy on the use of sex-proportion and litter-size changes as measures of sex-linked lethal damage in mice; there is evidence showing that these changes can be due to factors other than sex-linked lethals and until the exact role of sex-linked lethals in causing these changes is more clearly defined, the meaning of the estimates derived using these changes as criteria must, for the time being, be regarded as open to question.

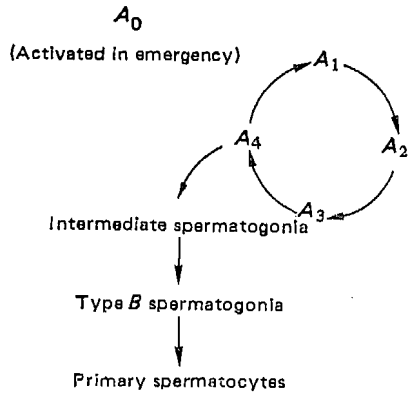
229. Attempts at measuring the over-all effects of induced mutations using several measurable end-points believed to be components of fitness have, in general, yielded negative results and suggest that the deleterious effects in heterozygotes are presumably much less severe than would be expected from the results of *Drosophila* experiments.

G. Spermatogonial stem-cell renewal and its relationship to genetic effects

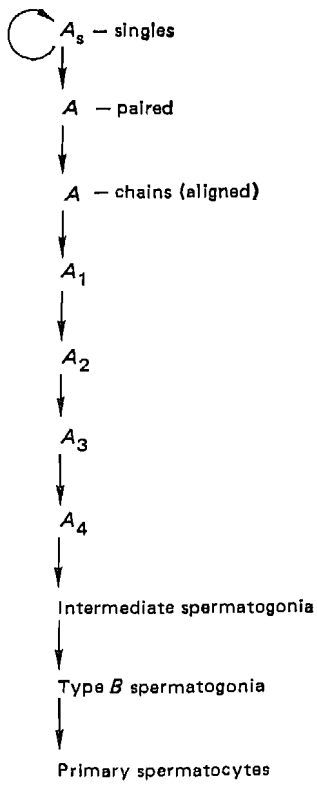
230. Description of the stages of the cycles of seminiferous epithelium has made possible the accurate identification of cells, the determination of cell lineages, the quantitation of cells and the elucidation of cell development times in spermatogenesis (233, 356). It became clear that the stem cell of the seminiferous epithelium is a type *A* spermatogonium which, by a series of divisions plus differentiation, gives rise to an unlimited number of intermediate spermatogonia irreversibly committed to the production of more mature cell types (87, 233, 356). Some type-*A* cells fail to differentiate and become the stem cells for the next multiplication cycle. This process has been termed stem-cell renewal.

231. Currently, the most widely accepted model of spermatogonial stem-cell renewal is that proposed by Clermont and Bustos-Obregon (88) as a result of a study of tubule whole mounts in the rat. According to these authors, the spermatogonial population of the rat is made up of two groups of cells. The first constitutes the actively renewing population and is comprised of spermatogonial types A_1-A_4. Spermatogonia of each type divide sequentially, each type giving rise to the one next to it in the series, and are involved in the four mitotic peaks of spermatogonial multiplication. Most A_4 cells divide to form intermediate spermatogonia; a few, however, give rise to A_1 cells which later initiate a new cycle.

232. The second group of type-*A* spermatogonia occurs as single for paired cells that do not normally contribute to the replenishment of *A* spermatogonia and are considered to function as "reserve stem cells". These cells designated as A_0 constitute about 20 per cent of the *A* population and become active only if the more mature classes of cells become depleted by some agent such as radiation. The whole sequence can be diagrammed as follows:



233. The recent studies of Oakberg (361, 362, 363) in the mouse and of Huckins (182) in the rat, however, have led to a different model of spermatogonial stem-cell renewal (as diagrammed below) which casts the A_0 spermatogonium of Clermont and Bustos-Obregon (88) in the role of the active stem cell. Accordingly, the authors have proposed the designation A_s for this type of cell.



234. According to the Oakberg-Huckins model, renewal of stem cells occurs by the division of some A_s spermatogonia to form more isolated A_s cells; other divisions of A_s spermatogonia result in the formation of "paired" cells and constitute the initial step in differentiation. Further divisions of the pairs result in

mates of mutation rate, however, can be made from their data.

257. In a further extension of their study, Albertini and de Mars (7) have obtained evidence showing that (*a*) the exposure-frequency relationship for the x-ray induction of HG-PRT mutations may be non-linear (exposure levels: 75, 125, 150 and 250 R); (*b*) the HG-PRT activity varied among the mutants tested. Approximately one half of the derived strains had very low activity comparable to that found in Lesch-Nyhan cells, while the remainder showed intermediate activity and one strain had activity in the normal range; and (*c*) surprisingly, all but one of the mutants were able to utilize hypoxanthine for growth in the presence of an aminopterin block; they did this as well as normal cells, regardless of the apparent HG-PRT activity. Current attempts of Albertini and de Mars are directed towards an understanding of whether the various phenotypic classes of AG-resistant mutants represent a multiple allelic series of one gene or mutations at different loci.

258. In table 25, the mutation rates in mammalian somatic cells *in vitro* are compared with the rates known in germ cells of the mouse and in some other organisms on the one hand, and with the rates in micro-organisms on the other. It can readily be seen that the mutation rates per locus per cell (or gamete) per roentgen are considerably higher in animal cells and cell systems than those in micro-organisms. Bridges and Huckle (51) suggest that the high mutability of animal cells may be a general property of both somatic and germinal cells, not specifically associated with meiotic stages.

259. From what has been presented in this section, it seems clear that biochemical mutations can be induced in mammalian cells in culture after exposure to UV light and to ionizing radiation suggesting that such studies have a great deal of potential to permit insights into the mutagenic sensitivity of mammalian cells, information which will be of great value in facilitating comparisons with what is already known for germ cells. It is hoped that somatic cell genetics studies will eventually complement studies with germ cells and will provide a surer basis to evaluate the genetic sensitivity of the human species to ionizing radiations.

II. Effects in fish

260. Schröder (473) studied the genetic effects of x-irradiation in male and female germ-cell stages of the guppy, *Lebistes reticulatus*, a viviparous species of fish. A hybrid (obtained by crossing two inbred lines) and one inbred line were employed as experimental material. To sample presumed primordial gametogonial stages, new-born male or female guppies of the hybrid line were given x-ray exposures of 1,000 roentgens. To sample later stages, adults of the inbred line were exposed to radiation (500 and 1,000 R to males or females). Irradiated fish were appropriately mated to raise the F_1 and subsequent generations, without any further irradiation.

261. The results showed that: (*a*) irradiation of primordial germ cells led to no significant changes in litter size (live-born per litter; first four litters) though in the inbred lines a trend towards increasing litter size was seen in the F_2 to F_4 generations; (*b*) the frequency of still-born fish (expressed as per cent live-born) was higher only in the F_1 and F_2 offspring after spermatogonial irradiation; (*c*) in experiments in which the gonial stages were irradiated, post-natal mortality (per cent dead between birth and 90 days) was enhanced only in the F_2; and (*d*) the incidence of skeletal abnormalities (curvatures of the vertebral column) and of pigmentation defects of the body was higher in the generations of the hybrid and inbred lines that were born after irradiation.

262. Newcombe and McGregor (351) investigated the incidence of major malformations (eyes, head, tail, etc.) and of several minor ones in the embryos and fry of the rainbow trout, *Salmo gairdnerii*, derived from *in vitro* irradiated sperm or eggs. Fertilization was accomplished by mixing and stirring the gametes in petri dishes. The sperm or the eggs were given x-ray doses of 200, 2,000 and 20,000 rads in 2.1, 2.4 and 13.4 minutes, respectively. Screening for malformations in embryos was done by stereo-microscopic examination.

263. Major malformations, equivalent to the skeletal mutations described by Ehling in the mouse, were substantially more frequent following irradiation. The response per unit dose fell off at high doses but at 200 rads, the lowest dose used, the yield was approximately 300 10^{-6} per embryo per rad for irradiations of either gamete.

264. In an extension of this work to low doses, McGregor and Newcombe (299) showed that, following gamma irradiation (^{60}Co) of sperm, the frequency of major eye malformations in the immediate offspring followed a linear relationship in the 25-400 rad range (5 levels). Analysis of the survival data revealed that, at doses of 25 and 50 rads, there was a significant increase (by about 35 and 40 per cent, respectively) in the proportion of eggs with embryos as compared with unirradiated controls. After 400 rads, however, the yield of embryos was greatly reduced (352). The "beneficial" effect of the lower doses is more apparent during the early and intermediate stages of embryonic development while the "harmful" effect of the higher dose is expressed mainly during the intermediate stages (300).

265. In another study (298) the embryos received x-ray doses of 10, 100 and 1,000 rads at early cleavage, late cleavage, blastula and germ-ring stages. Ten and 100 rads had little or no effect on egg mortality. The loss of ability to produce visible embryos was greatest following 1,000 rads, and resistance to the lethal effects of this dose increased progressively with the age of the embryo. More interesting, however, is the finding that the embryos developed a high incidence of major malformations when irradiated prior to active organogenesis, there being a peak effect of 40 per cent eye malformations and 35 per cent body malformations at late cleavage. This observation is in contrast with the evidence from studies in the mouse (429). The authors suggest that the apparent lack of quantitatively similar responses in mammals must be due to loss of potentially malformed individuals resulting from selective failures to implant or from post-implantation deaths.

III. Effects in insects

A. Loss or addition of chromosomes

266. It is known from earlier studies in *Drosophila* (462, 533, 556, 615) that (*a*) for the induction of

X-chromosome losses in males, spermatocytes are the most sensitive stage, followed by spermatids, spermatozoa and spermatogonia in that order (the situation being thus different from that in the mouse where spermatids have been found to be the most sensitive stage, paragraph 133); (*b*) below 1,000 roentgens, the results in spermatocytes are consistent with a linear dose-effect relationship, the rate of induction of XOs being approximately 2.3 10^{-5} per roentgen, a figure which is close to that obtained for spermatocytes in the mouse; (*c*) for the induction of X-chromosome non-disjunction, spermatocytes again are the most sensitive stage; (*d*) in females, the sensitivity of the germ cells to radiation-induced X-chromosome losses varies strikingly during oögenesis; (*e*) in stage-7 oöcytes (Prophase I of meiosis), the frequency of X-losses increases faster than linearly with exposures in the range from 500-5,000 roentgens; and (*f*) in the same exposure range, the dose-effect relationship for X-chromosomes non-disjunction in stage-7 oöcytes follows some kind of step-wise pattern and is not amenable to any simple interpretation. The data that have been collected in recent years confirm and extend these findings.

1. *Chromosome loss in* Drosophila

(a) *Male germ cells*

267. Traut, Scheid and Wind (566) observed that the frequency of X-chromosome losses induced in mature sperm increases with exposure (1,000-4,000 R) with a dose exponent greater than one. In a parallel cytological study, the authors obtained evidence indicating that more than 90 per cent of the losses at 4,000 roentgens were partial (detected as ring and rod fragments). Since partial losses are expected to be two-hit events, the results of the cytological and genetic study complement each other well.

268. In an investigation designed to study the induction of ring-X-chromosome losses in various stages of spermatogenesis, Leigh (241) observed that in post-meiotic stages the frequencies of XO males increase linearly with x-ray exposures (500-3,000 R). In spermatocytes, however, the yield increases faster than linearly over the same range of exposures, indicating that a two-hit mechanism might be involved. The author suggests that induced crossing-over may be the mechanism largely responsible for the production of high frequencies of XO males in spermatocytes.

269. In another study (240) the frequencies of ring-X-chromosome losses induced in mature spermatozoa were found to be almost identical whether the males were x-irradiated in nitrogen or in oxygen atmosphere. This observation is in line with that reported by Baker and von Halle (27) for the loss of inverted rod-X chromosomes, but is in contrast to that recorded for the loss of structurally normal rod-X chromosomes (269, 462, 605), for sex-linked lethals and for autosomal translocations, where a marked oxygen enhancement effect has been found. The induced rate of ring-X-chromosome loss, however, is greater than the rate of both normal and inverted rod-X chromosomes and this may be in some way related to the configuration of the ring-X chromosome. No satisfactory explanation is yet available to account for the refractoriness of ring-X-chromosome losses to changes in oxygen tension.

270. Würgler and Maier (604) have recently reported that the x-ray induced loss of ring-X chromosome in *Drosophila* sperm is profoundly influenced by the genotype of the females with which the irradiated males are mated. Furthermore, the rate of loss observed in brood 1 (first day of egg-laying) was twice that in brood 2 (second to fourth day of egg-laying) this being true for all types of females used. The authors suggest that a plausible interpretation for the observed "brood-pattern" is that there may be a difference in the maternal effect depending on whether aged stage-14 oöcytes (first day sampling) or newly produced stage-14 oöcytes (not aged, second to fourth day sampling) are fertilized by irradiated spermatozoa.

(b) *Female germ cells*

(i) *Exposure-frequency relationships*

271. Traut (557) compared the frequencies of X-chromosome losses induced in mature (stage-14) and immature (stage-7) oöcytes of *Drosophila melanogaster* at x-ray exposures of 100, 200 and 400 roentgens. In stage-7 oöcytes, the frequencies increased linearly with increasing exposures. In stage-14 oöcytes, however, the relationship was non-linear. In the exposure range studied, stage-14 oöcytes seem to be 23 to 31 times as sensitive as stage-7 oöcytes depending on the definition[9] used to calculate the frequencies of X-chromosome losses.

272. In view of the fact that in Traut's experiments a 24-hour period was employed to sample stage-14 cells and in view of the known heterogeneities in sensitivity within such samples (616) the sensitivity ratios given in the preceding paragraph are to be regarded as only approximate.

273. In a subsequent study Traut and Scheid (564) studied the problem in relatively more homogeneous samples by restricting the period of egg-laying to eight hours so as to sample stage-14 cells. The x-ray exposures employed were 100, 200, 300 and 400 roentgens. The earlier general conclusion of a non-linear dose-response for induced X-chromosome losses was confirmed, but the absolute frequencies at comparable exposure levels in the present study were much higher than in the previous one, obviously a result of improved sampling technique.

274. Kiriazis (219) investigated the induction of X and chromosome IV losses in stage-14 oöcytes at x-ray exposures of 100, 200, 300, 400 and 500 roentgens. Egg-laying was restricted to the first 12 hours following irradiation. At comparable exposures, the frequencies of XO males recorded in this study were of the same magnitude as those found by Traut and Scheid (564).

275. The data of Kiriazis on the loss of chromosome IV in the same germ-cell stage are not in agreement with the X-chromosome results. There is no effect observed for the loss of chromosome IV. Although the numbers are small at some exposures, the probable explanation is that the majority of the haplo-IV individuals are not viable and have died before eclosion.

276. In summary, in spite of differences in absolute frequencies at comparable exposures observed between

[9] Frequency of X-chromosome losses:

Definition 1. $\dfrac{\Sigma \text{ XO males}}{\Sigma \text{ XY males} + \Sigma \text{ XO males}}$

Definition 2. $\dfrac{\Sigma \text{ XO males}}{\Sigma \text{ XX females} + \Sigma \text{ XO males}}$

experiments of different investigators, it is safe to conclude that in stage-14 oöcytes, the yield of X-chromosome losses in the range 100-500 roentgens increases with exposure with a dose exponent greater than one. In stage-7 oöcytes, however, the dose-response curve is linear between 100 to 400 roentgens after which level it becomes non-linear, suggesting that, at higher exposures, there might be a two-track contribution in the induction of this type of genetic damage.

(ii) *Exposure fractionation and exposure rate*

277. Traut (560) investigated the effects of x-ray dose fractionation and of dose rate on the yield of XO males obtained from stage-14 and stage-7 oöcytes. Egg-laying was restricted to 18 hours in sampling stage-14 oöcytes and to 48 hours in sampling stage-7 oöcytes. In stage-14 oöcytes, when a total exposure of 400 roentgens was split into two equal fractions separated by a 20-minute interval, no fractionation effect was observed.

278. The lack of fractionation effect might be due to the fact that chromosome-breaks induced in stage-14 oöcytes do not rejoin before fertilization. In stage-7 oöcytes, however, when total exposures of 2,000, 4,000 and 5,000 roentgens were split into two equal fractions separated by either 20 or 60 minutes, there was a decrease in the yield of XO males relative to single exposures but this decrease was significant only with 2,000 + 2,000 roentgens separated by 60 minutes.

279. With an exposure of 2,000 roentgens delivered at a rate of 50 R min^{-1} (as compared with 850 R min^{-1}) to stage-7 oöcytes, no dose-rate effect could be detected. But with 3,000 roentgens at 100 R min^{-1}, there was a significant decline relative to the single acute exposure.

280. It is known that repair of radiation damage in stage-7 oöcytes is completed within approximately 15 to 20 minutes following irradiation (389). On this basis, and because of the multi-hit dose response for XO induction in this germ-cell stage (219, 556), one would expect that the fractionation procedure and the dose rate employed by Traut should lead to a reduction in the frequencies of XO males. However, such a reduction was observed in only two out of seven experiments. The causes for the discrepancy are not known.

281. In more recent work with stage-7 oöcytes, Traut (563) found that the frequency of X-chromosome losses decreased highly significantly with fractionated exposure (1,800 R in two equal fractions separated by one, three or five hours) and at lower exposure rates (10 R min^{-1}) relative to those obtained with single exposures delivered at 850 R min^{-1}. The reduction, however, was more pronounced with the lowering of the exposure rate than with fractionation. In the latter series of experiments, a one-hour interval between the exposure fractions was found to be already sufficient to cause a reduction in the X-loss frequencies such that there was no further decrement in frequency with increasing intervals.

(iii) *Cytological analysis*

282. Traut and Scheid (565) carried out a cytological study of X-chromosome losses induced in oöcyte stages 7 and 14, similar to the one reported earlier for mature sperm (paragraph 267). About 39 per cent (13/33) of the losses induced in stage-14 oöcytes after an x-ray exposure of 400 roentgens were partial. Since partial losses are in general expected to be two-track events, this result corroborates that obtained in genetic studies (paragraphs 266, 273). It is considered that the frequency of partial losses observed in this study is sufficient to account for the rise above linearity of the dose-effect curve observed in experiments with mature oöcytes.

283. Similar results were obtained for X-chromosome losses induced in stage-7 oöcytes after an x-ray exposure of 3,500 roentgens. Nevertheless, the proportion of partial losses (amounting to between 7(3/43) and 23(10/43) per cent depending on whether the dot-like small fragments observed were chromosome IV or of X-chromosomal origin) is not large enough to account for the whole two-track component observed at this exposure.

284. In order to determine the nature of the unclassified dot-like fragments Traut and Scheid (567) resorted to staining with quinacine dichloride and fluorescence microscopical analysis of the cerebral ganglia of F_1 larvæ originating from complete or partial X-loss induced by x rays (3,500 R) in stage-7 oöcytes. As has been demonstrated recently (584) the fourth chromosome is more strongly fluorescent than any other chromosome of *Drosophila melanogaster* except for parts of the Y and a short region at the centromere of the X chromosome.

285. Fluorescence analysis permitted an unambiguous identification of the seven cases recovered in this study which were characterized by a third (instead of the normal two, corresponding to the two fourth chromosomes) dot-like fragment as being fourth chromosomes. The results demonstrate a positive correlation between the x-ray induction of complete X chromosome loss and chromosome IV non-disjunction in stage-7 oöcytes.

286. A possible mechanism underlying the correlation observed might be radiation-induced interchange between chromosome X and chromosome IV followed by the separation of the heterologues at the first meiotic division. Consequently, the homologues of the interchange-involved X and IV might segregate more or less at random, thus producing (among other non-disjunctional types) nullo-X, diplo-IV gametes. This attractive hypothesis has been developed by Parker (388) from his work on x-ray induced detachment of the attached-Xs. After irradiation of attached-X females, Parker also recovered relatively frequently mono-X, triplo-IV individuals. However, it remains to be seen whether in immature oöcytes with free X chromosomes (as used in the study of Traut and Scheid) interchanges between the X chromosomes and chromosome IV are induced at frequencies high enough to correspond to the mechanism postulated above.

287. Grell *et al.* (160) investigated the role of chromosome size in radiation-induced loss of chromosomes by irradiating newly eclosed females (most advanced stage: stage-7 oöcytes) carrying two extra small chromosomes of equivalent length, one a free IV and the other a free X duplication. These two extra chromosomes constitute approximately one tenth of the length of the normal X chromosome. With x-ray exposures of 4,000 roentgens the normal X chromosomes are lost about three times as often as the X

of sex-linked lethals and autosomal translocations induced in the successive stages of spermatogenesis. Of particular importance was the finding that for any given radiation exposure in nitrogen, the frequencies of lethals and of translocations are almost identical in mature spermatozoa and in late spermatids. However, when irradiation is carried out in air, the frequencies are higher in mature spermatozoa than in late spermatids, and significantly so at higher exposures.

314. In an independent study designed to explore the basis for the differences in radio-sensitivity between mature spermatozoa and later spermatids, Sobels (523) found that these differences disappeared when irradiations were performed in either nitrogen or oxygen but were quite pronounced with irradiations in air. The observations of Shiomi and Sobels are best interpreted as indicating that, under normal conditions in air, mature spermatozoa are relatively more oxygenated than late spermatids. These findings have since been extended to dominant lethals in these two germ-cell stages (460). This interpretation finds further support in studies with fast-neutron irradiation where it has been found that neutrons are more efficient than x rays in inducing genetic damage in late spermatids than in mature spermatozoa (526; table 26).

315. Inagaki and Nakao (184) observed that in *Drosophila* spermatozoa the frequencies of complete mutations at four X-linked recessive visible loci increased non-linearly with increasing exposures (1,000-4,000 R). However, the frequency of 0.05 per cent does not differ significantly from the control frequency and consequently, it seems doubtful whether mosaic mutations were induced at all.

(ii) *Silkworm*

316. Tazima and Onimaru (550) irradiated wild-type silkworm males with gamma rays (2,500-15,000 R; 4 levels; 331 R min^{-1}) and found that in mature sperm the exposure-frequency relationship was linear for complete mutations and exponential for mosaic mutations at the *pe* and *re* loci. With x-ray exposures of 2,500 to 10,000 roentgens (4 levels; 100 R min^{-1}) Inagaki and Nakao (185) also obtained similar results. The kinetics of the induction of mosaics in silkworm spermatozoa thus differs from that observed for *Drosophila* spermatozoa (paragraph 315).

317. The results of Tazima and Onimaru (550) also indicate that the rate of induction of mosaic and complete mutations varies with the progress of spermatogenesis. In spermatogonia, mosaics are induced at extremely low rates (1-2 10^{-6} R^{-1}) relative to complete mutations (39-354 10^{-6} R^{-1}, depending on the exposure and locus). The frequencies of both mosaics and complete mutations increase sharply through meiotic prophase up to V-4.5 (fifth instar larvæ, day 4.5) although most mutations are still complete. Around V-4.5 the ratio of complete to mosaic mutations reaches unity and, from then on, relatively more mosaic than complete mutations are produced.

318. Tazima and Murakami (549) have recently summarized the data on the mutational response of male germ cells to x-irradiation of several sensitive and resistant strains of silkworm studied by them. The original criterion of selection was based on the LD_{50} values for embryonic killing by x-irradiation, which varied over a threefold range from about 670 roentgens for the most sensitive strain to about 2,000 roentgens for the most resistant strain. Sensitivity to mutation induction was compared at three different stages of spermatogenesis: spermatogonia, spermatids and spermatozoa.

319. Marked (up to tenfold) differences were observed among the strains when spermatogonia were irradiated. The differences, however, diminished as more advanced stages were sampled, being about two- to threefold in spermatids and only about 1.5-fold in spermatozoa.

320. Murakami *et al.* (344) showed that fractionating a 1,000-rad dose of 14 MeV neutrons (two equal fractions separated by 10 to 45 hours) can more than double the frequency of specific-locus mutations when spermatogonia are irradiated soon after hatching of the silkworm egg. This is similar to the previous finding with low-LET radiation (548) but the peak yield was obtained with a 36-hour interval for neutrons, in contrast to 18 hours for x or gamma rays. The enhancing effect is probably the result of differential radio-sensitivity within the gonial cycle.

(b) *Internally-deposited radio-active isotopes*

321. Earlier work with radio-active nucleosides such as ^{3}H-thymidine, ^{3}H-uridine etc., showed that these substances are capable of producing mutations in *Drosophila* germ cells (204, 205, 374). Recently, Kieft (214) studied the induction of recessive lethals by ^{3}H-uridine and ^{3}H-thymidine following injection of these nucleosides into adult *Drosophila* males. Six successive two-day broods were taken and the maximum sensitivity was observed in the broods corresponding to spermatocytes and late spermatogonia. This finding is similar to the one reported by Olivieri and Olivieri (374).

322. In Kieft's work, uridine with tritium in the 5 position of the pyrimidine ring produced approximately twice as many lethals as an equivalent dose of 6-^{3}H-thymidine. This finding might indicate a possible transmutation effect at the site of tritium decay.

323. Kaplan and Oftedal (206) made a similar study using ^{3}H-thymidine. Each brood was of one-day duration. Elevated mutation rates were observed earlier than the tenth day post-injection, when the first labelled sperm cells are expected to be available for fertilizations. Radio-autographs prepared from testes of males taken from successive broods disclosed a cytoplasmic label which was removable by DNAse. The authors suggest that beta rays from the labelled cytoplasmic DNA was responsible for the mutations produced in the early broods. The nature of the cytoplasmic body which had incorporated the ^{3}H-thymidine is not known.

324. In early work on ^{32}P mutagenesis in *Drosophila* (reviewed in reference 366) there were difficulties in critically separating the mutagenic effects of beta irradiation from those from transmutation of ^{32}P to ^{32}S. Lee *et al.* (236) showed that these two effects could be separated by storing labelled spermatozoa in unlabelled females and found no mutagenic effects of transmutation that could be detected by genetic tests in the F_1 and F_2 generations. However, when the experiments were extended for an additional generation, a significant increase of sex-linked recessive lethals (detected in the F_3) was observed (235). The authors have attributed this increase to transmutation effects.

325. In another study concerned with the mutagenic effects of transmutation of ^{14}C to ^{14}N, Lee (234) obtained results similar to those outlined in paragraph 324, in the F_1 and F_2 tests; tests are not yet completed for the F_3 and later generations.

2. *Female germ cells*

(a) *Drosophila*

(i) *Introduction*

326. Much of the radiation genetics work in female *Drosophila* has been concerned with two stages, designated in the terminology of King, Rubinson and Smith (218) as 7 and 14, which are, respectively, the oldest stages in newly emerged females and the fully mature chorionated oöcyte found in females ready to begin egg-laying (usually during the second day of adult life). Stage-7 and stage-14 oöcytes are in prophase I and in metaphase I of meiosis, respectively. In recent years, other meiotic stages in the eggs and early mitotic stages in embryonic development have also received attention. The sensitivity varies widely over these stages. Stage 14 and division stages are much more sensitive than stage 7 and the stages preceding it, the extent of the difference depending very much on the kind of genetic damage under observation (383, 386).

(ii) *Recessive lethals*

327. Parker (382) published a brief paper on recessive lethals induced in stages 7 and 14 showing that in both stages, a quadratic equation fits the data better than a linear one. Here the apparent differences in sensitivity are at their smallest. The dose required to give equivalent damage in stage 7 is only about two to three times that required in stage 14, and the major increase in stage 14 seems to be the component that is increasing approximately as the square of the dose.

328. Markowitz (295) investigated the effects of exposure rate in stage-7 oöcytes by irradiating *Drosophila* females with gamma rays (^{137}Cs) at about 4.8 and 300 roentgens per minute (total exposures 2,000 and 4,000 R). Sex-linked lethals were the measured end-point of genetic damage. In the experiments of Meyer quoted in Markowitz's paper, an essentially similar scheme was used except that in her study, chromosome II recessive lethals were scored. In neither series of experiments was there any evidence of a dose-rate effect.

329. With an x-ray exposure of 3,000 roentgens delivered to stage-7 oöcytes at rates of approximately 3,000, 150 and 50 R min^{-1}, Sankaranarayanan (460) also found that sex-linked and autosomal (chromosome II) recessive lethal frequencies were nearly the same irrespective of the exposure rate. However, the results of dominant lethal tests in the same germ-cell stage likewise irradiated showed that the damage was less at lower exposure rates.

330. Meyer and Abrahamson (303) have recently obtained data on exposure-frequency relationship for sex-linked lethals in oögonia after x-irradiation with 20, 100, 500 and 6,000 roentgens. Over 166,000 X chromosomes have so far been tested. The mutation frequencies (in per cent) recorded in this study are as follows: Control: 0.17 ± 0.02; 20 R: 0.17 ± 0.02; 100 R: 0.14 ± 0.02; 500 R: 0.27 ± 0.03 and 6,000 R: 2.81 ± 0.27.

331. It can be seen that the frequency after 6,000 roentgens (corrected for controls) is in line with the expectation based on 0.5 per cent lethals per 1,000 roentgens found by many investigators (329, 414); however, those at the lower exposure levels are low suggesting the lack of a linear exposure-frequency relationship in this range. As a working hypothesis the authors suggest that low doses of radiation may induce repair of mutational damage (compare with the results of Newcombe and McGregor (352) in fish; paragraph 264).

332. Rinehart and Lee (414) have presented the results of a large-scale study (over 70,000 X chromosomes tested) with oögonia where sex-linked lethal frequencies were determined following gamma (^{137}Cs) or x-ray exposures (2,000 or 4,000 R) delivered at intensities in the range from 0.13 to 4,000 roentgens per minute. The results indicate a small reduction in mutation frequencies (2.6 *versus* 2.0 per cent: 4,000 R) when the exposure rate is lowered from 4,000 roentgens per minute to 50 roentgens per minute. Below this exposure rate, there was no further reduction.

333. These results are thus qualitatively similar to those obtained in mouse spermatogonia (440, paragraph 155) although, in the latter experiments, the range of intensities was different and the magnitude of the effect was greater (table 14). As in the case of mouse spermatogonia, the *Drosophila* results with oögonia show that there is no threshold exposure rate below which no mutations are induced. It may perhaps be mentioned that the results of earlier investigators (329, 401, 403, 404) have not provided unequivocal evidence for a dose-rate effect (of the type found in the mouse) for the induction of recessive lethal mutations in any of the *Drosophila* germ-cell stages tested thus far. Even in the one experiment with spermatogonia where an exposure rate of 0.01 roentgen per minute (200 R) produced a significantly lower mutation frequency than that of 2.6 roentgens per minute, this single observation was not regarded as conclusive (401).

(iii) *Autosomal translocations*

334. Traut (558, 559) has published the results of a genetic investigation in which the induction of reciprocal autosomal translocations (between chromosomes II and III) by x-irradiation of mature (stage 14) and immature (stage 7) oöcytes of *Drosophila* was studied. In stage-14 cells, frequencies of 0.25 per cent (13 out of 5,158 tested gametes), 0.36 per cent (14/3935), 0.36 per cent (12/3334) and 0.22 per cent (6/2699) were recorded after exposures of 250, 500, 750 and 1,000 roentgens respectively. The dose-effect relationship thus bears a general resemblance to that obtained with x-irradiated mouse spermatogonia (paragraph 49). In stage-7 oöcytes, Traut obtained a frequency of 0.32 per cent translocations (7/8877) after 4,000 roentgens.

(iv) *Chromatid interchanges (half-translocations)*

335. While attempts to recover reciprocal translocations from irradiated *Drosophila* females have not been very successful, it has been possible, when the effects of aneuploidy are not so great, to recover one of the two products of exchange between two chromosomes. These "half-translocations" as some workers call them (3) are chromatid interchanges and have

been found as detachments of attached-X chromosomes involving exchanges between X and Y, X and IV, X and tips of major autosomes or as gross deletions of an X from the attached-X chromosome (386).

336. Parker's data on the frequency of detachments at various exposure levels in stages 7 and 14 show that the exposure-frequency relationship is of the form $(1-\exp(-kD))^2$ where k is the mean number of breaks in a chromosome in a site per roentgen and D the exposure in roentgens. That is, the yield increases as the square of the exposure at low exposures, while at high exposures the curve begins to saturate. Consequently, when the exposure is doubled, there is less than a fourfold increase in effect. Therefore, a $D^{3/2}$ kinetics is simulated over the biological range (603).

337. In the ranges where meaningful comparisons can be made, the difference in sensitivity between stages 7 and 14 can be expressed by a dose-reduction factor of about five, i.e., an exposure of 200 roentgens produces damage in stage-14 oöcytes approximately equivalent to that produced by 1,000 roentgens in stage-7 (383).

338. Abrahamson *et al.* (2) have recently shown that in stage-14 oöcytes, the exposure-frequency relationship for induced detachments of attached-X chromosomes appears to fit a linear response between 10 and 50 roentgens; from 50 to 500 roentgens, the frequency follows the conventional $D^{3/2}$ relationship. Their results also show that an x-ray exposure of 10 roentgens to stage-14 oöcytes causes a significant increase over control frequencies and more than doubles them.

339. The exposure fractionation experiments of Parker and Hammond (389) showed that in stage-7 oöcytes when the fractions were separated by 15 minutes or more, there was a significant decrease in detachment frequency, indicating that chromatid breaks rejoin in about 15 minutes or so. In contrast, in stage-14 oöcytes there was no fractionation effect even with a 24-hour interval between the exposure fractions. It was therefore concluded that the broken ends did not rejoin before fertilization. Abrahamson (1) reported similar findings.

340. More recent studies of Parker (384, 385, 387, 388), Parker and Williamson (390) and Williamson (594) have been concerned with devising sensitive methods for the detection of various kinds of aberrations induced in female germ cells, detailed genetic analysis of the aberrations so recovered, examination of their disjunctional properties and so on (see also paragraph 286).

341. Rinehart and Ratty (415) used a brood-pattern technique to study the x-ray induction of aberrations in stage 7 and earlier stages. When the number of aberrations recovered from individual females was compared, it was found that there was a significant departure from expectation based on a Poisson distribution in which the aberrations were assumed to have been recovered as independent events, i.e., there was a deficit of females with fewer aberrations and an excess of females with more aberrations. Furthermore, most aberrations occurred among the earliest broods and when individual females had multiple aberrations among their offspring, such aberrations occurred as early as the first brood, which is far too early to assume an oögonial origin of these events. No satisfactory explanation is yet available to account for these results.

(v) *Meiotic stages beyond metaphase I*

342. Würgler and his colleagues have made extensive studies on the variations in radio-sensitivity during meiotic and early cleavage stages of *Drosophila* eggs (152, 393, 471, 607, 611). In newly-inseminated eggs laid within the first three minutes or so, the oöcyte nucleus progresses from metaphase I to anaphase I and the paternal genome is still contained within the condensed sperm head. It has been shown by Schneider-Minder (471) that meiosis of the maternal genome is completed and maternal pronucleus formed within the first 15 minutes after the egg is laid. Simultaneous with the meiotic divisions, the initially condensed sperm head changes into the paternal pronucleus.

343. Petermann (393) and Graf *et al.* (152) found that in newly-inseminated eggs the paternal X chromosome is more sensitive to the x-ray induction of recessive lethals than the maternal X which shows no change in sensitivity at any stage from anaphase I until the completion of meiosis. In contrast, the paternal X in the sperm head which changes into the paternal pronucleus goes through a transient phase of very high sensitivity (nearly twice that of the maternal X and of the paternal X in eggs 10-16 minutes after egg deposition). The authors speculate that this transient high sensitivity may be connected with the replacement of the arginine-rich histone by a lysine-rich histone in the paternal chromosomes.

344. Würgler (608) observed essentially a similar pattern with regard to the x-ray (500 R) induction of autosomal translocations in newly-inseminated eggs. A constant rate of 0.3 per cent translocations within the maternal chromosome set was found throughout the period during which the maternal genome passes from meiotic metaphase I to the pronuclear stage. However, during the period when the sperm head transforms into the paternal pronucleus, a rate of 2.3 per cent translocations within the paternal chromosome set was found. This rate fell to 0.2 per cent with the progression towards the pronuclear stage. From the recovery of three maternal-paternal translocations, Würgler estimates that the maximum rejoining time for chromosome breaks induced in newly-inseminated eggs is of the order of 10 minutes.

(b) *Silkworm*

345. Inagaki and Nakao (185) observed that x-irradiation (1,000-4,000 R) of mature silkworm oöcytes produced predominantly complete mutations. The exposure-frequency relationship was non-linear, with a dose exponent greater than one. In contrast, the frequency of mosaics increased only slightly with increasing exposures.

346. Murakami (336) treated silkworm oöcytes at different stages during meiosis I with x-ray exposures ranging from 1,000 to 6,000 roentgens. Using embryonic mortality as the criterion of genetic damage, Murakami found that the silkworm oöcytes were more radio-sensitive in metaphase I–anaphase I than in other phases, a finding which is in line with the results obtained in other insect species (383, 583). The LD_{60} values for embryonic mortality increased from 2,100 roentgens for oöcytes in metaphase I and early anaphase I to 4,150 roentgens for prophase-I oöcytes sampled from late pupae.

3. *Summary and conclusions*

347. The results presented in the preceding paragraphs entirely confirm and extend the conclusions reached in the 1966 report of the Committee on the existence of radio-sensitivity differences among germ-cell stages in *Drosophila* and in silkworm. The magnitude of the difference varies between the stages and depends very much on the kind of genetic damage under observation.

348. In silkworm, the rate of induction of complete and mosaic mutations at the *pe* and *re* loci varies in different male germ-cell stages. In spermatozoa the exposure-frequency relationship is linear for complete mutations and exponential for mosaics.

349. Fractionated neutron-irradiation of silkworm spermatogonia leads to an enhancement of the mutation frequencies similar to what has been known for low-LET radiations.

350. In *Drosophila*, it has been shown that the sensitivity differences between mature spermatozoa and late spermatids is due to a higher degree of oxygenation of the former germ-cell stage under normal conditions in air.

351. There is no dose-rate effect for the induction of recessive lethals in stage-7 oöcytes of *Drosophila*, the situation being thus different from that obtaining in mouse oöcytes. However, such an effect has been observed in oögonia, the frequencies of sex-linked lethals at 50 R min^{-1} being lower than those at 4,000 R min^{-1}.

352. The exposure-frequency relationship for sex-linked lethals induced in *Drosophila* oögonia deviates from linearity in the range from 20 to 500 roentgens, namely, a reduction from that expected from higher exposures (e.g. 6,000 R).

353. Autosomal translocations are induced at very low frequencies in *Drosophila* oöcytes. Sensitive methods, however, are available to study the induction of another type of chromosome aberration, namely, detachment of the attached-X chromosomes. The frequencies of detachment induced in mature (stage-14) oöcytes appear to increase linearly with exposures in the range between 10 and 50 roentgens; beyond this exposure and up to 500 roentgens, the kinetics follows the $D^{3/2}$ relationship.

354. In newly-inseminated *Drosophila* eggs, the paternal X chromosome passes through a period of high sensitivity during the transformation of the sperm head into the paternal pronucleus. The maternal X chromosome, however, shows no change in sensitivity at any stage from anaphase I until the completion of meiosis.

D. Relative biological effectiveness

355. Earlier studies in insects designed to estimate the RBE of high-LET radiations, especially of neutrons, in inducing different kinds of genetic damage were comprehensively reviewed in the 1966 report of the Committee. In general terms, the conclusions were that (*a*) compared to x or gamma rays, neutrons have RBE values almost always in the range from one to six and (*b*) these values vary with the dose, the dose rate and the energy spectrum of the neutrons and may be different for different germ-cell stages and for different types of genetic damage. Since 1966 some new data have become available and these will be reviewed in the following paragraphs. Whenever necessary for purposes of comparison, earlier results will also be considered.

1. *Drosophila*

356. There have been several recent studies on neutron RBEs for the induction of recessive lethals, translocations and dominant lethals in *Drosophila* and these are summarized in table 26, which shows that the RBE values are dependent on the germ-cell stage, being lower in mature sperms than in spermatids. In addition, they are higher for translocations than for recessive lethals. The stage-dependent differences in RBE reflect differences in the degree of oxygenation of the treated cells (paragraph 314). The disparity in the RBE values for comparable stages might be related to the differences in neutron energies and to the lack of standardized mating procedures in sampling given germ-cell stages.

357. It may be noted that, for mature spermatozoa, the RBE values recorded by Sobels and Broerse (526) for the induction of sex-linked lethals (0.8) and translocations (1.0) are lower than those found by others (table 26) and those reported earlier by Edington (120) and Edington and Randolph (121). Sobels and Broerse have argued that the discrepancy between their estimates and those of Edington (120) and of Edington and Randolph (121) might stem from the possibly mixed population of germ-cell stages sampled (mixture of late spermatids, with a lower x-ray sensitivity, and mature spermatozoa) and the use of gamma rays as a standard to compute the RBE values (which are known to be slightly less efficient in the production of mutations than x rays) both of which in these earlier studies would lead to higher RBE values than those estimated from the data of Sobels and Broerse.

358. Beside the data given in table 26, Traut's conclusions (555) may be also mentioned. He compared his results for translocation induction after x-irradiation of *Drosophila* spermatozoa at low doses with those obtained by Muller (325) for fast-neutron-irradiation of males and concluded that the RBE at low doses was in the range of 4.5 to 5.9 depending on the dosimetric criteria used.

359. Nakao and Machida (348, 349) found that the RBE of 2.5 MeV neutrons for dominant lethal induction in spermatozoa increased markedly with decreasing dose, reaching a higher value than that given in table 26 at low doses (experiments 11 and 12). Sobels and Broerse (526) also found that the neutron versus x-ray RBEs for the induction of translocations in late spermatids increased at low doses because of the linearity of the neutron response while the x-ray yield increased more than linearly, as expected.

360. Panikovskaia and Troitzky (619) found that intermediate neutrons (200 keV) were more effective than gamma rays in inducing X-chromosome deletions in spermatids and spermatocytes but showed about the same effectiveness as gamma rays when spermatogonia or spermatozoa were irradiated.

361. Here it may be pointed out that the RBEs of neutrons for the induction of recessive lethals in *Drosophila* spermatozoa are somewhat lower than those for the induction of specific-locus mutations found by earlier investigators (186, 304, 375). The latter values range from 4.0 to 5.3 in the different studies.

362. Of the experiments reported in table 26, only in one (experiment 1) were spermatogonia sampled. The RBE of 2.1 can be compared with the mean value of 3.9 obtained by Murakami and coworkers (table 27) for the induction of specific-locus mutations in silkworm after fission-neutron irradiation of late spermatogonia.

363. Lamb *et al.* (231) studied the mutagenic effectiveness of 600-MeV protons (LET of about 0.26 keV μm^{-1} in water) using second-chromosome recessive lethals as the measured end-point of genetic damage. A wide range of male germ-cell stages were sampled (six successive three-day broods). Over-all, the data suggest that 600-MeV protons do not differ from 250-kVp x rays in their mutagenic effectiveness This result is similar to the one reported by Rappoport *et al.* (623) for the induction of sex-linked lethals in spermatozoa with 660-MeV protons.

364. In order to investigate mutation induction by the heavy primaries of cosmic radiation, Malich *et al.* (292) exposed *Drosophila* males to carbon ions (max. LET 630 keV μm^{-1}) and studied the rates of induction of various types of mutation in spermatozoa. No actual RBE values were calculated, but the mutation rates observed were "several times smaller" than those induced by uniform irradiation with protons, alphas and boron ions (LET 1.5, 20 and 120 keV μm^{-1}). The authors concluded that the affected cells are usually killed, while those surviving carry few mutations.

2. *Silkworm*

365. Most of the silkworm studies, like those in the mouse, have been concerned with the induction of specific-locus mutations (*pe* and *re* loci). Both old and new data are given in table 27 which shows that, as in *Drosophila*, the RBE depends on developmental stage. However, this is largely due to variations in the gamma rather than in the neutron response. It is suggested by Murakami and Kondo (342) and Murakami *et al.* (343) that the capacity for repair of gamma-ray-induced mutational damage may differ in different stages but that neutron damage is probably not reparable (see also paragraph 415). It can be seen that with the exception of primordial spermatogonia in embryos, RBEs are higher in later stages than in earlier ones and reach a maximum with irradiation of spermatozoa. At low doses, oögonia and spermatogonia gave similar mutation frequencies.

366. The RBEs of 1.5-MeV fission neutrons follow the same pattern as those of 14 MeV, but are markedly higher. On average, fission neutrons are 1.7 times as effective as 14-MeV neutrons for the four comparable stages studied by Murakami and colleagues.

367. Murakami (339) compared the mutagenic response in five silkworm strains known to differ markedly in their sensitivities to embryonic killing by x rays. Primordial gonial cells in newly-hatched larvae were given either 970 rads of x rays or 910 rads of 14-MeV neutrons. It was found that the average mutation rates (at the two loci studied) in the male germ cells of the most sensitive strain were 31.7 10^{-7} per rad with x rays and 14.4 10^{-7} per rad with neutrons while the comparable figures for the resistant strain were 3.0 10^{-7} and 3.8 10^{-7}. A similar trend was observed in the female germ cells. The estimated RBE values consequently are dependent on the strain, being 0.44 and 1.11 for primordial spermatogonia and oögonia of the sensitive strain and 1.29 and 6.06 for those of the resistant strain. Thus the strain with the highest sensitivity to embryonic killing by x rays is the one giving the highest mutation rates and the lowest RBE values.

368. The induction of dominant lethals in silkworm germ cells by 14-MeV and fission neutrons has also been studied by Murakami (337, 340); with 14-Mev neutrons and ^{137}Cs gamma rays, RBEs of 1.6, 4.4. and 8.2 were found with primordial germ cells, spermatogonia in larvae and mature spermatozoa, respectively. A linear dose-reponse relation was established only for spermatozoal irradiation. Effects on other germ cells were compared at the 50 per cent survival level. At the same level the RBE of 1.5-MeV fission neutrons relative to gamma rays was 11.2 with spermatogonial irradiation, 2.5 times the figure for 14-MeV neutrons. Thus the general pattern is very similar to that for the induction of specific-locus mutations: higher RBEs with fission neutrons than with 14-MeV neutrons and with more mature than with less mature cells.

3. Dahlbominus *and* Mormoniella *(Hymenoptera)*

369. One great advantage of *Dahlbominus* for mutational studies is that unmated females produce only haploid male progeny in which all mutations are expressed. Baldwin (32) and Baldwin and Cross (33) studied especially four classes of eye-colour mutants (carmine, claret, chestnut and russet), which arise at a minimum of eight loci. In earlier studies reported in the 1966 report, Baldwin and Cross compared the frequencies of such eye-colour mutations in female *Dahlbominus* of different ages after exposure to 14.6-MeV neutrons (80 rad min^{-1}) or to ^{60}Co gamma rays (100 rad min^{-1}) at a dose of 750 rads. Mutation frequencies rose with the age of the insects at the time of exposure, i.e, with increasing numbers of mature oöcytes. The RBEs calculated as ratios of mutation frequencies were 1.2-1.4.

370. In separate experiments, Baldwin (32) showed that when oöcytes are irradiated at a stage of constant radio-sensitivity, the yield of mutations is higher with low-dose-rate than with high-dose-rate gamma-irradiation. Germinal selection did not appear to be responsible for the lower yield with low-dose-rate irradiation.

371. Work similar to that of Baldwin was carried out by Kayhart (212) on *Mormoniella vitripennis*, another hymenoptera. Like Baldwin, Kayhart irradiated virgin females and looked for eye-colour mutations in their haploid sons. However, the effects of thermal neutrons, fast neutrons from detonation of nuclear devices and acute x-irradiation were studied rather than those of 14-MeV neutrons and gamma rays. Dose-response curves were linear at low doses but became exponential at higher ones. Kayhart reported that the RBE for fast neutrons relative to x rays was 17-21 at lower doses and 2-4 at higher ones, but no figures for thermal neutrons were given. It was considered that the decreased effectiveness of fast neutrons at high doses was to be expected if many of the mutations were due to minute rearrangements and deletions.

4. *Summary and conclusions*

372. In general, recent work on insects suggests that the RBE of neutrons for recessive visibles are

higher than for recessive lethals. For chromosome aberrations, the shape of the dose-response curves indicates that RBEs will tend to increase with decreasing doses, except with spermatozoa where they also tend to increase with decreasing neutron energy, from 15 MeV to the fission energy spectrum.

E. Radiation-resistant populations

373. In a laboratory population of *Drosophila melanogaster* in which males and females were x-irradiated (2,100 R) in every generation for a period of over 10 years (220 generations) with an accumulated exposure of over half a million roentgens, Nöthel (354) obtained evidence for resistance to irradiation. In spite of the fact that spermatozoa, spermatids and oöcytes from stage 14 to possibly stage 6 were irradiated in every generation, only stage-7 oöcytes showed radiation resistance (354). At comparable exposures, the frequencies of induced dominant lethals, X-chromosome losses and recessive sex-linked lethals in the irradiated populations (tested in stage-7 samples drawn from the population) were approximately one half of those in the control population.

374. This pronounced difference in radio-sensitivity was not associated with oxygen-dependent sensitivity differences and/or oxygen-mediated repair. The results of experiments designed to localize the gene loci responsible for radiation resistance show that at least two different factors, one on the X chromosome and the other on chromosome II, might be involved (355).

375. Sensitivity differences, as measured by relative mortalities of adults at specific times (days) after irradiation, among natural populations of *Drosophila* (370, 371, 391), among different strains of *Drosophila* (535, 536), and among silkworm strains (338, 549) have also been reported.

F. Mutation rates to recessive lethals and polygenic mutations

376. Data on spontaneous rates of mutation to recessive lethals at loci on the X chromosome are quite extensive in *Drosophila melanogaster,* for these constitute the controls in a large number of experiments on induced mutation rates. Information regarding other chromosomes and other species, although less extensive, is sufficient to make meaningful comparisons. During the past 15 years or so, increasing attention has been paid to the study of polygenes, especially those affecting viability, to obtain estimates of their mutation rates and gain an insight into their effects in heterozygotes and their role in the maintenance of genetic variability. The literature on this subject has recently been reviewed by Crumpacker (98), by Mukai (311), and by Spiess (531). The following paragraphs will be devoted to an examination of some of the representative data that bear on the problem of mutation-rate estimates.

1. *Sex-linked recessive lethals*

377. Using the published data of several earlier investigations, Crow and Temin (97) estimate that the weighted average mutation rate for the X chromosome of *Drosophila melanogaster* is 2.6 10^{-3} lethals per X chromosome per generation. The authors found no significant rate differences between laboratory stocks and wild flies nor between sexes.

378. Wallace (587) found that the over-all mutation rate for sex-linked lethals in the same species was 2.8 10^{-3} per X chromosome per generation (75 lethals in 27,094 tests), a direct estimate not significantly different from the one given in the preceding paragraph and close to the indirect estimate (2.0 10^{-3}) based on the Poisson distribution. No significant differences in rates between the sexes were found although there were some differences between the strains tested.

379. Rinehart (413) studied the effects of ageing of spermatozoa on the spontaneous rate of mutations to sex-linked recessive lethals in a laboratory stock of *Drosophila melanogaster.* Females inseminated by 2-3 day old males were either allowed to lay eggs in a single four-day brood ("non-aged" sperm sampled) or held for three weeks on sugar-agar food ("aged" sperm sampled) and later allowed to lay eggs. In the "non-aged" group, the frequencies of sex-linked lethals were 0.142 ± 0.027 (22/15,449) and in the "aged" group 0.283 ± 0.049 (29/10,216), suggesting a rate of increase of 0.047 per cent of lethals per week. The rate of increase found in the present study is approximately of the same magnitude as the one reported by Muller (324) (8.6 10^{-5} lethals per day) from earlier studies.

2. *Autosomal lethals*

380. In the same paper discussed in paragraph 377, Crow and Temin (97) arrived at a weighted average of 5.0 10^{-3} as the mutation rate per generation for chromosome-II lethals, in agreement with the expectation based on the physical length of chromosome II relative to the X chromosome.

381. After comparing the rates of recessive lethal mutations for chromosomes II and III, Wallace (586) concluded that there was no significant difference between the chromosomes (or between the sexes), the over-all average mutation rate being 6.9 10^{-3} per chromosome per generation (direct estimate: 80/11,655) or 5.9 10^{-3} (indirect estimate) in both cases in good agreement with the estimate made by Crow and Temin (97).

382. Purdom *et al.* (402) made a study similar to that of Rinehart (413) (paragraph 379) on the effects of ageing of the spermatozoa on the spontaneous mutation rate, but using the induction of chromosome-II recessive lethals as the criterion. The age of the gametes was varied by ageing male flies and by storage of spermatozoa in inseminated females held at 10° C under conditions which precluded egg-laying for four, six or eight weeks. It was thus shown that mutation frequencies increased with time in each case, but the rates were low compared with the normal spontaneous mutation rate observed in spermatozoa of young male flies, the latter ranging from 4.4 10^{-3} to 7.2 10^{-3} in the different experiments.

3. *Viability polygenes*

383. The past 15 years have witnessed an increasing interest in the study of polygenic mutations controlling viability in natural and laboratory populations of *Drosophila,* with and without irradiation (95, 96, 310-320, 522, 585). The results of these studies, while contributing to our knowledge of the genetic architecture of the populations, have raised certain interesting problems concerning the dynamics of detrimental genes in populations and thus are of relevance for human risk estimates as well.

384. Mukai (310) conducted an experiment in which spontaneous polygenes controlling viability were allowed to accumulate under minimum selection pressure generation after generation in 104 second chromosomes, kept heterozygous by means of appropriate markers and balancers. All these second chromosomes were derived from a single second chromosome (from a natural population of *Drosophila melanogaster*) which was chosen because of its high viability when homozygous.

385. In generations 10, 15, 20 and 25, the chromosomes under test were made homozygous and the viability and genotypic variance among the lines were examined. From these tests, Mukai has estimated that polygenic mutations controlling viability arise at a rate of 0.1411 mutation per second chromosome per generation, in contrast to the rate of 0.0063 per second chromosome per generation for recessive lethals. Mukai and Crow (315) later repeated this experiment with concordant results.

386. A high mutation-rate ratio (~28) was obtained by Mukai *et al.* (320) for x-ray-induced polygenic mutations relative to recessive lethals (0.79 *versus* 0.028 per second chromosome after 500 R to spermatozoa).

387. In a series of papers (312-320) Mukai and his colleagues analysed several aspects of these polygenic mutations in relation to their effects on fitness and their role in the maintenance of genetic variability in populations. One of the most important findings relates to the fact that polygenic mutations (spontaneous or induced) manifest overdominance in heterozygous condition when they arise in an otherwise homozygous background. When, however, they arise in a heterozygous background, they are detrimental or, at best, neutral (317-320). This result helps to reconcile much of the controversy that exists in the literature on the effects (in heterozygous condition) of newly-arising mutations (reviewed in references 98, 311, 531). The implication of this finding is that, since natural populations of sexually reproducing organisms are normally heterozygous at most of their loci, newly-induced mutations are expected to manifest a certain degree of deleteriousness (semidominance) rather than overdominance.

4. *Relevance for man*

388. The foregoing evidence from *Drosophila* suggests that polygenic mutations with very minor deleterious effects occur at a much higher rate than conventional recessive lethals. As pointed out by Crow (96) their very mildness usually means that these mutants will have a correspondingly mild effect on fertility and therefore be transmitted from generation to generation. Although at present we have no knowledge about the incidence of this type of mutations in human populations, they probably exist and their effects would roughly correspond to a whole array of possible physical, physiological and mental impairments, each causing a small deleterious effect with the effects spread over some dozens of generations. As Crow points out, the over-all effect of these in terms of morbidity and mortality as well as of economic costs is probably great, but it may be so diluted in space and time as not to be recognizable as being of mutational origin.

G. Nature of radiation-induced lethals

389. Lifschytz and Falk (263, 264) studied a small, proximally-located, region (about 1.5 cross-over units long) of the X chromosome of *Drosophila* along lines similar to those followed by Benzer (40) in phage and de Serres (111) in *Neurospora crassa*. By using a Y chromosome carrying a duplication for the region, they were able to construct a complementation map based upon radiation- and chemically-induced lethals. The map contained 34 functional units.

390. With an x-ray exposure of 3,200 roentgens to mature spermatozoa and possibly late spermatids, a total of 413 chromosomes carrying recessive lethals were recovered, out of which 42 (10 per cent) were covered by the duplication. Appropriate complementation tests of the 35 lethals analysed showed that nearly 85 per cent were deletions of various lengths with breakage points distributed non-randomly in the segment and with some "hot spots".

391. In contrast, nearly 80 per cent of the lethals induced by ethyl-methane sulphonate in the same germ-cell stages involved single functional units, operationally indistinguishable from point mutations.

392. In a subsequent study involving irradiated spermatogonia, Falk (135) found that the proportions of aberrations were smaller than among those induced in spermatozoa.

393. It should be borne in mind that the conclusion of Lifschytz and Falk that, at least in post-meiotic germ-cell stages, most radiation-induced recessive lethals may be deletions, is based on an analysis of only the proximal part of the section of the X-chromosome covered by the Y-chromosome duplication. Furthermore, that region is atypical and not representative of the *Drosophila* genome, if only because it is immediately adjacent to the "proximal heterochromatin" (division 20 of the salivary chromosome map) in which 30 per cent of all x-ray-induced breaks have been found to be located (210). Thus, the region studied by Falk and Lifschytz would be expected to yield an unusually high frequency of x-ray-induced deletions, as has long been known to be the case for other regions when they are placed adjacent to the proximal heterochromatin by means of inversions (238, 327).

394. Recently it has been shown by Schalet, Lefevre and Singer (465) that at least the distal part of division 20 of the salivary chromosome contains loci capable of producing ordinary sex-linked lethals. Consequently, about one half of the 34 functional units mapped by Lifschytz and Falk are actually located *within* the segment of the X chromosome found to contain 30 per cent of all x-ray-induced breaks (210), and about two thirds of the lethals obtained in their experiment lie entirely within division 20.

H. Repair of radiation damage

1. *Drosophila*

395. In earlier investigations, Sobels (521) demonstrated that in *Drosophila* spermatozoa, repair of radiation damage is favoured by post-irradiation treatment with nitrogen but adversely affected by that with oxygen. Sex-linked recessive lethals and autosomal translocations were the scored end-points of genetic damage. In experiments in which dominant lethals

were used as criteria, Sankaranarayanan (456) found that the frequencies were precisely the same, irrespective of the gas used for post-treating the flies.

396. Mukherjee and Sobels (321) studied the effects of pre-treatment with sodium fluoride (NaF: a known inhibitor of glycolysis) on x-ray induced sex-linked lethals in *Drosophila* spermatozoa and found that the pre-treatment led to a consistent and highly significant increase in mutation frequency (relative to saline-injected controls). When the action of NaF was studied in combination with pre- and post-treatment with nitrogen or oxygen, it was observed that (i) irrespective of pre-treatment with nitrogen or oxygen, NaF enhanced the mutation frequency over that in the saline controls and (ii) following irradiation under anoxia, post-treatment with nitrogen reduced the mutation frequency below that observed with oxygen post-treatment, even when the flies had been pre-treated with NaF.

397. These additive effects of NaF pre-treatment and oxygen post-treatment have been taken to indicate that, even when glycolysis is inhibited by NaF, some energy is left, which is still available for repair by post-radiation anoxia. This interpretation that the amount of repair in spermatozoa depends on different levels of available energy is supported by the observation that NaF pre-treatment is still effective in increasing the mutation frequency over that in the controls when nitrogen has been given before, during and after irradiation. Thus, repair is maximal in the saline-nitrogen-radiation-nitrogen group, intermediate in the NaF-nitrogen-radiation-nitrogen group and minimal in the NaF-nitrogen-radiation-oxygen group.

398. In contrast to the situation obtained in mature sperm (paragraph 395), the repair process in spermatids is oxygen-dependent (522, 588). However, repair occurs only when x rays are delivered at a high exposure rate of about 2,700 R min^{-1}. With exposures of 1,250 or 2,500 roentgens delivered at 1,000, 500, 250 or 120 R min^{-1}, the yields obtained with nitrogen as well as with oxygen post-treatment are nearly the same (457). Such a peculiar dose-rate effect was also observed by Sobels (520) with hydrocyanic-acid post-treatment under similar conditions of radiation exposure.

399. As early as 1940, Muller proposed, on the basis of the lack of dose-fractionation effects in x-irradiated mature sperm, that chromosome breaks remain open until fertilization (323). The work of Leigh and Sobels dealing with the recovery of homoisochromosomes from irradiated post-meiotic germ cells (paragraphs 307-309), among other things, confirmed this possibility and extended it to spermatids as well. Additional evidence from exposure-fractionation experiments has substantiated the above thesis (525).

400. The experimental procedure consisted of irradiating adult males with the first fraction of a dose, sampling the various germ-cell stages by means of a brooding technique and giving the second fraction of the dose to the mature sperm in the inseminated females in the various broods. Appropriate controls where only males were irradiated (RM) and brooded as in the fractionation series or only sperm in inseminated females were irradiated (RF) were run concurrently. The progeny were scored for translocations between the second and third chromosomes. The frequencies in the fractionation series were compared with those expected on the basis of additivity or of interaction of breaks in the RM and RF groups.

401. The results showed that the translocation frequencies in spermatids of the fractionated group were significantly higher than the sum of the yields of the separate fractions. This indicates that a considerable proportion of the breaks produced in spermatids of the adult testis remains open until fertilization.

402. An important point that emerges from the work of Würgler and Maier (609; paragraph 270) and other related work referred to in their paper, is that the repair machinery in the females plays a role in determining the magnitude of the genetic damage in the paternal genome. This raises the possibility of manipulating the genetic constitution or the physiological environment of the oöcytes by appropriate methods to gain an insight into, and define the role of, maternal repair processes on various kinds of genetic damage in the male genome.

403. Proust (398) and Proust *et al.* (399) studied the effects of treating (injection) females with actinomycin-D on the frequencies of dominant lethals, autosomal translocations and sex-linked lethals induced in mature sperm by x-irradiation. When compared to the appropriate controls, it is found that such treatment of the females with actinomycin leads to an increase of the frequency of dominant lethals and to a decrease of those of translocations and recessive lethals; the modifying effect on the translocation and recessive lethal frequencies is most pronounced in oöcyte stages utilized four to six days after injection.

404. The likely interpretation of these findings is that actinomycin acts by partially inhibiting the restitution of chromosome breaks thereby increasing the frequency of dominant lethals and decreasing those of translocations and recessive lethals. This implies that maternal repair processes acting at the stage of pronucleus formation are required for the repair (restitution) or misrepair (reunion giving rise to translocations) of chromosome breaks induced in mature sperm.

405. Traut and Schmidt (568) studied the x-ray induction of dominant lethals in stage-7 oöcytes (850 R min^{-1}). Exposures ranging from 1,000 to 6,000 roentgens were used and these were delivered either singly or in two equal fractions separated by one-hour intervals. The dose-response survival curves with single and fractionated exposures were sigmoidal and the survival with fractionated exposures always higher than with single exposures. In addition, survival significantly increased relative to that at single exposure (*a*) when an exposure of 3,000 roentgens was split into six equal fractions separated by two-hour intervals and (*b*) at lower dose rates (100 R min^{-1}, 5 R min^{-1}).

406. The effects of oxygen and nitrogen post-treatments on x-ray-induced dominant lethality in stage-7 oöcytes were studied by Sankaranarayanan (458). Irradiations (2,700 R min^{-1}) were carried out in anoxia, in air or in an oxygen atmosphere. A wide range of exposures from 1,000 to 14,000 roentgens was used, the actual range depending on the gaseous atmosphere in which the flies were irradiated. The results indicate that the dose-response survival curves are predominantly two-hit and that with oxygen post-treatment the egg survival is consistently higher relative to that observed with nitrogen post-treatment. The latter observation is interpreted as indicating oxygen-mediated repair of dominant lethal damage. The oxygen-enhancement ratio is estimated to be about 2.6.

407. In a similar study on stage-14 oöcytes it was found that the dose-effect relationship (with either oxygen or nitrogen post-treatment) was consistent with a one-hit survival kinetics and that with post-irradiation oxygen the survival was significantly higher than with post-irradiation nitrogen much as has been observed in stage-7 oöcytes (459). In stage-14 oöcytes, however, the effect of oxygen post-treatment can be easily reversed by subsequent post-treatment with nitrogen, suggesting that the mechanisms responsible for the post-radiation modifications observed with these gases are probably not the same in stage-7 as in stage-14 oöcytes.

408. The data also suggest that under normal conditions stage-14 oöcytes have more oxygen available than stage-7 oöcytes. It is likely that this differential oxygenation in air may constitute one of the factors contributing to the higher radio-sensitivity of stage-14 oöcytes relative to those in stage 7. Sobels (523) found a somewhat parallel situation in comparing the radio-sensitivities of late spermatids and mature sperm (paragraph 314). The oxygen-enhancement ratio for stage-14 oöcytes is about 3.6 (459).

409. Würgler and Matter (610) observed a small but measurable reduction compared with the effect of the single dose in the mortality of stage-14 oöcytes when an exposure of 600 roentgens was split into two equal fractions separated by a time interval ranging from 10 minutes to 8 hours. The fractionation effect, however, was pronounced only with longer intervals between exposures. These authors interpret this finding as indicating that stage-14 oöcytes are capable of repairing some of the radiation-induced damage although, as they themselves point out, only about 10 per cent of the damage can be repaired in eight hours.

410. In a study on the x-ray induction of dominant lethals in stage-7 and stage-14 oöcytes of a recombination-deficient mutant of *Drosophila*, Watson (589) obtained evidence that this strain was also more radio-sensitive than wild-type flies.

411. Seeley and Abrahamson (496) found that in stage-14 oöcytes the frequency of chromatid aberrations can be slightly but significantly enhanced by post-irradiation anoxia.

2. *Silkworm*

412. Tazima (547) compared the responses of normal, weakly radio-sensitive, intermediate and highly radio-sensitive strains to irradiation. Spermatids in full-grown larvæ were exposed to 1,000 roentgens delivered either singly or in two fractions separated by intervals of 3, 6 or 12 hours and the incidence of complete or mosaic mutations at the *pe* and *re* loci was studied. The results show that fractionation reduces the mutational yield only in the normal and weakly radio-sensitive strains, suggesting that the other strains presumably lack the ability to repair radiation damage.

413. In parallel experiments with the normal strain, larvæ were irradiated (1,000 2,000 or 3,000 R) in nitrogen or in air and then post-treated with either nitrogen or oxygen. Oxygen post-treatment in the nitrogen-pre-treated group resulted in a slight non-significant decrease in mutation frequencies whereas the opposite effect was found in the air-irradiated group. These effects are thus different from those reported for *Drosophila* spermatids.

414. Mutation frequencies obtained after irradiations in nitrogen are roughly one half of those obtained at the same dose after irradiations in air. In particular, the dose-modifying effect of nitrogen was more pronounced for mosaic mutations than for complete mutations.

415. Evidence that neutron-induced mutational lesions are poorly reparable was obtained by Murakami and colleagues in experiments involving post-irradiation treatment of silkworm spermatogonia with the base analogue 5-bromodeoxyuridine (BUDR). With x-irradiation, BUDR enhanced the yield of specific-locus mutations at most 2-3 times (345), but there was very little effect with 14-MeV neutrons (335). Murakami and Ito (341) interpreted these results as being due to the replacement of thymine by BUDR during the course of the repair resynthesis that follows the degradation of DNA segments once the lesions have been induced by radiation. Such replacement would lead to mutations of base-substitution type and they have proposed the term "co-mutagenesis" to describe this synergistic effect. They considered that the smallness of the enhancement with neutrons was because more double-strand breaks, not susceptible to repair, were induced by densely ionizing radiations.

416. In line with this view are the findings of Tazima *et al.* (551) that a much higher proportion of mosaics are recovered after treatment with chemical mutagens than with 14-MeV neutrons. The predominantly whole-body (complete) mutations obtained with the latter treatment are believed to result from lesions affecting both strands of the DNA double helix.

3. *Summary and conclusions*

417. The results of studies on repair of radiation damage in *Drosophila* and in silkworm germ cells carried out during the last few years are in essential conformity with those reported in the 1966 report of the Committee. The new data have come from experiments in which (*a*) the effects of nitrogen and oxygen post-treatments following irradiation in nitrogen, air or oxygen were compared and (*b*) dose-rate and dose-fractionation procedures were employed. The most frequently used criteria of genetic damage were: sex-linked recessive lethals, chromosome aberrations, dominant lethals and specific-locus mutations.

418. In *Drosophila* spermatozoa, the yields of sex-linked lethals and of autosomal translocations are reduced after post-treatment with nitrogen whereas the yield of dominant lethals shows no differential response to the contrasting post-treatments. In the same germ-cell stage, pre-treatment with sodium fluoride (a known inhibitor of glycolysis) leads to an enhancement of mutation frequencies. In immature (stage 7) oöcytes of the same insect, dominant lethal frequencies are significantly lower after oxygen post-treatment (relative to that with nitrogen), after fractionated exposures (relative to single exposures) and after low dose rates. The response of mature (stage 14) oöcytes to post-treatments (nitrogen or oxygen) and to fractionated exposures is qualitatively similar when measured by dominant lethals and/or chromatid interchanges, but the causal mechanisms that might underlie these effects in mature oöcytes are not sufficiently understood.

419. The existence of an oxygen-dependent repair process in *Drosophila* spermatids is documented but it seems to operate only when radiation is delivered at

a high exposure rate (2,700 R min^{-1}). At rates of 1,000 R min^{-1} and below, no repair of sex-linked recessive lethal damage can be demonstrated.

420. Chromosome breaks induced in *Drosophila* spermatids and spermatozoa stay open until fertilization.

421. Treatment of *Drosophila* females with actinomycin-D prior to their mating with irradiated males results in an increment of the frequency of dominant lethals and a decrement in those of autosomal translocations and sex-linked recessive lethals recovered from irradiated spermatozoa.

422. In silkworm spermatids, oxygen post-treatment following irradiation under anoxia leads only to a slight and non-significant decrease in the frequency of specific-locus mutations. An opposite effect is found in the air-irradiated group, contrary to what has been found in *Drosophila*. Whereas fractionation of the exposure results in a decrement of mutation frequencies in spermatids of normal and near-normal silkworm strains, no such effect is observed in highly radiosensitive strains, suggesting that they may lack the ability to repair radiation damage.

IV. Effects of radiation at the cellular and molecular levels and their implication with regard to genetic risks

423. For a comprehensive assessment of the genetic risks of radiations, it is necessary to take into account all processes from the induction of initial lesions to their final fixation as genetic changes. The extensive body of data now available in radiobiology documents the fact that DNA is one of the main, and perhaps the principal, target, damage to which sets in train a series of biochemical events leading to such visible effects as cell death, mutations, chromosome aberrations and so on.

424. Information on the changes brought about by irradiation at the level of the DNA and the consequences of these changes is not readily obtainable at higher levels of biological complexity. For that reason radiation studies with cell-free and microbial systems, and in recent years with mammalian cell systems as well, are of great value since they are likely to provide the necessary links between the purely chemical studies on DNA and radiobiological effects.

425. The past decade has witnessed an almost explosive growth of molecular radiobiology; a great deal is now known about the kinds of damage produced in the DNA by ultraviolet (UV) irradiation and, to a lesser extent, by ionizing radiations. Advances in UV mutagenesis have led to a greater insight into the effects of ionizing radiations, reinforcing the idea that these approaches to the study of the dynamics of radiation action and of repair processes must be viewed as complementary rather than mutually exclusive.

426. Impressive as these developments are, molecular biology has not yet provided information on the relationship between the damage from ionizing radiation at the DNA level and mutational events to explain or to predict the array of mutational responses observed with different dose rates, cell stages, etc., in mammals. However, since there is the prospect for understanding these phenomena at the molecular level in the future, it seems appropriate to review the present state of knowledge in this field. What follows is a brief survey of the kinds of damage produced in the DNA by UV and ionizing radiations and of repair processes. Exhaustive treatments of these subjects are given in several recent papers (49, 55, 177, 196, 376, 397, 408, 420, 518, 532, 538, 598, 600).

A. Ultraviolet radiation

1. *Nature of damage*

427. Several lines of evidence indicate that the biological inactivation of cells by UV-irradiation is mainly due to DNA damage. Most of the photochemical alterations induced by UV rays in DNA have been studied in micro-organisms and, in recent years, also in mammalian cell systems. The main UV-induced photochemical changes which are now considered to contribute to biological inactivation are: formation of pyrimidine dimers, hydration of cytosine and uracil, single-strand breaks, DNA cross-linking (intra- and inter-molecular), local denaturation and DNA-protein cross-links (516, 517).

428. A major class of photoproducts formed in UV-irradiated DNA is represented by the cyclobutane-type pyrimidine dimers between two adjacent pyrimidine residues in the same DNA strand. Pyrimidine dimers have been found in many organisms after UV-irradiation, e.g., in viruses, bacteria and cells of higher organisms. Thymine-thymine (TT) dimers are formed more readily than other types of pyrimidine dimers. In bacteria, at low UV exposures (up to a few hundred erg mm^{-2}), TT, TC (thymine-cytosine) and CC (cytosine-cytosine) dimers are produced in the relative ratio of 5:2:1 (504). In mouse *L* cells, Klímek (221, 222) has estimated that the ratio of TT to UT dimers is 4:1.

429. Klímek (221, 222) and Trosko *et al.* (569) first demonstrated the UV-induction of thymine dimers in mouse *L* cells and Chinese-hamster cells, respectively. These findings were later extended to other mammalian cell lines. Within the range of incident UV-exposures tested (0.1 10^3 to 20 10^3 erg mm^{-2}) thymine dimers were found to be induced as a linear function of the dose (221, 222, 224, 569).

430. The biological significance of the pyrimidine dimers was deduced from the increased biological activity observed after various treatments by which dimers are either reconverted to the original monomeric state or eliminated from the DNA. All known strains of *Escherichia coli* are equally susceptible to the production of pyrimidine dimers in their DNA (about 6 dimers erg^{-1} mm^2) although, because of differences in repair mechanisms, some strains are more than 2,000 times as resistant to UV-irradiation as others (177, 422).

431. Hydrates of cytosine and of uracil are formed by the additions of a molecule of water at the 5-6 double bond. There is at present not sufficient evidence to show that these photoproducts have biological significance, although cytosine hydrate could have a mutagenic effect (516).

432. Single-strand breaks, which are disruptions of the linear continuity of a single polynucleotide chain in the DNA double helix, occur too infrequently at low UV doses to be of biological significance. The same is true of UV-induced local denaturation of the DNA

strands. DNA cross-links are produced mainly upon irradiation of dry DNA or bacterial spores. They probably have biological significance only at very high UV doses in the inactivation of very UV-resistant organisms and of transforming DNA.

433. DNA-protein cross-links are detected as a dose-dependent decrease in the amount of DNA extracted free of proteins by detergents. Only in the inactivation of very UV-resistant organisms such as *Micrococcus radiodurans* are they probably important lethal factors (516).

2. *Repair mechanisms*

(a) *Procaryotes*

(i) *Photo-enzymatic repair*

434. The lethal and mutagenic effects of UV light on bacteria or viruses can be partially or nearly completely reversed (repaired) by different mechanisms. One of the well-characterized mechanisms is direct photoreactivation or photo-enzymatic repair (PER) (421, 500). PER causes the splitting or monomerization of the pyrimidine dimers *in situ* in the presence of visible light (wavelengths between 0.31 and 0.44 μm) leading to the restoration of the normal helical DNA structure. The process is mediated by an enzyme, the photoreactivating enzyme, the presence of which has been ascertained in *Escherichia coli, Saccharomyces cerevisiae* and some other organisms. The enzyme is very specific in its substrate i.e., pyrimidine dimers. Because of its relative simplicity, photoreactivation is least likely to introduce errors into the DNA in the course of repair (600).

435. The involvement of pyrimidine dimers in UV killing and mutation induction has been examined by comparing photoreversibility in *Phr*$^+$ and *Phr*$^-$ strains of *Escherichia coli* which, respectively, possess or lack the photoreactivating enzyme. Any effect of UV light that is photoreversible in the *Phr*$^+$ but not in the *Phr*$^-$ strain therefore depends more or less on the persistence of pyrimidine dimers in the DNA. On the basis of these studies, it has been concluded that in the *Phr*$^-$ strain, essentially all of the killing caused by UV doses of up to 200 erg mm^{-2} and most of that due to up to about 600 erg mm^{-2} can be attributed to pyrimidine dimers. Similarly, at least 90 per cent of the mutations to the streptomycin resistance (at doses of up to 900 erg mm^{-2}) and 90 per cent of the suppressor mutations (at doses below 100 erg mm^{-2}) in some strains of *Escherichia coli* are due to pyrimidine dimers (596, 602). The gene locus responsible for photoreactivation has been mapped at a position closely linked to the *gal* locus (578).

436. Drake (117) found that nearly 64 per cent of the UV-induced *r* mutations (rapid lysis) in phage *T4* were photoreversible. Since *r* mutations are known to be base-pair substitutions (mostly from GC $\rightarrow$ AT) or frame-shift mutations, their photoreversibility indicates that pyrimidine dimers are important in the induction of phage mutations. Evidence for photoreversibility of the *c* mutations (clear plaque) in the phage *kappa* was obtained by Winkler (595).

(ii) *Excision repair*

437. Excision repair which does not require light is an alternative mechanism whereby pyrimidine dimers are eliminated from DNA, with the resultant gap being mended by "repair synthesis" i.e. by re-polymerization of the missing nucleotides, the bases opposite to the excised segment serving as a template (45, 171, 394, 503). The first steps in this repair process are the recognition of the damage and the introduction of a break in the DNA chain near the lesion (incision step); this is followed by the complete removal of the lesion from the DNA (excision step) and a further widening of the gap. The gap is then filled by the action of DNA polymerase (repair replication) using the opposite strand as the template. When the excised region is filled with undamaged nucleotides, the single-strand interruption is closed enzymatically (probably by polynucleotide ligase (373)).

438. Thus, at least four different enzymatic activities seem to be involved in excision repair: an endonuclease (incision), an exonuclease (excision), a DNA polymerase (repair synthesis) and a DNA ligase (sealing of the backbone). Enzymes of these steps have been found in bacteria (147, 165, 166, 203, 213, 534, 540) and more recently in higher organisms (paragraph 465).

439. In contrast to photoreactivation, excision repair is not specific for pyrimidine dimers, since lesions produced by such diverse agents as 4-nitroquinoline oxide (a carcinogen), mitomycin C, nitrogen mustard (DNA cross-linking agents) etc. may be repaired by the same mechanism or at least by a mechanism sharing some of the same steps (44, 54, 227, 228). This suggests that the enzymes associated with the excision repair mechanism recognize certain distortions of the phosphodiester backbone rather than the precise chemical form of the defective bases (44, 170).

440. As discussed in paragraph 437, repair by excision depends on the presence of an intact complementary strand of the DNA double helix. It would therefore seem that the double-stranded nature of the DNA is a requisite for excision repair. This expectation has been verified by Jansz, Pouwels and Rotterdam (192) and by Yarus and Sinsheimer (613) who UV-irradiated, and used for infecting spheroplasts (bacteria without cell walls), single- and double-stranded DNA (the so-called replicating (RF) form) from mature phage ΦX174. On spheroplasts of wild-type cells (which possess excision ability) the plaque-forming ability of RF-DNA was about 10 times higher than that of single-stranded DNA. However, on spheroplasts of mutants which lack the excision ability, only the survival rate of the RF decreased and to such an extent that both forms of DNA had equal sensitivities to UV light.

441. Strains of bacteria deficient in excision repair are UV-sensitive. Such strains have been isolated in *Escherichia coli, Bacillus subtilis, Serratia marcescens, Salmonella typhimurium* and several other species. A comparison of UV mutagenesis in strains of *Escherichia coli* differing in excision ability (*Hcr*$^+$ possessing excision ability; *Hcr*$^-$ without excision ability) has shown that the different kinds of mutations studied (auxotrophy $\rightarrow$ prototrophy; streptomycin sensitivity $\rightarrow$ resistance; inability to ferment lactose etc.) were induced at much higher rates in *Hcr*$^-$ strains (54, 175, 229, 596-600). On the basis of these and other studies Witkin (600) concludes that *Hcr*$^+$ strains are able to excise at least 99.9 per cent of the pyrimidine dimers produced at low doses.

442. Howard-Flanders *et al.* (178), van de Putte *et al.* (578) and Howard-Flanders, Boyce and Theriot (179) isolated UV-sensitive mutants of *Escherichia coli K12* that were also *Hcr*⁻ and found that the mutations lay in three widely-spaced genetic loci, designated as *uvrA*, *uvrB*, and *uvrC*. A mutation at any of the three *uvr* loci can cause the loss of capacity to reactivate DNA containing UV photoproducts. The UV sensitivity of a given *Hcr*⁻ mutant could not be increased by recombinational incorporation of a second *Hcr*⁻ mutation in the same bacterial choromosome, but at a different site (180).

443. The discovery that the *uvr* genes control the excision enzymes in the host bacterium explains the drastic reduction in the survival of UV-irradiated phages *T1* or *lambda* as the hosts' excision enzymes are presumably necessary for the release of pyrimidine dimers from the phage DNA. However, the *uvr* genes are without effect on the survival of UV-irradiated *T2* or *T4* phages. Setlow (501) showed that the excision of pyrimidine dimers in the DNA of the *T4* phage is controlled by a gene designated as *v*. The *T4* phage excision enzymes are able to release pyrimidine dimers from either phage or bacterial DNA, while the *Escherichia coli* enzymes are without effect upon the *T4* phage DNA (497, 502, 614). The product of the *v* gene has been purified and has been found to be an UV-specific endonuclease (144).

444. Ogawa *et al.* (372) reported the isolation and characterization of yet another class of *uvr* mutants, the genetic locus for which has been designated as *uvrD*. These mutants have an intermediate sensitivity to UV-irradiation and higher sensitivity than others to gamma irradiation. Even at a relatively low dose of UV (110 erg mm^{-2}), the DNA of *uvrD* is rapidly and extensively degraded. In contrast to the other *uvr* mutants, the *uvrD* cells are able to reactivate the UV-irradiated lambda phage (514). A double mutant, *uvrB-uvrD*, in which DNA degradation proceeds at a much lower rate than in *uvrD*, is about three times as sensitive to UV-irradiation as the *uvrB* mutant. These results suggest that the *uvrD* gene participates in the repair synthesis at a step subsequent to that performed by *uvrB*, and that there is a functional relationship between these two genes.

445. There is evidence that the *Escherichia coli* DNA polymerase can perform both the excision step (but not the incision step) and the polymerase function (213). The recent isolation of a mutant deficient in polymerase activity (108, 164) has considerably advanced our understanding of the role of DNA polymerase in repair. The mutation designated as *pol A1* is probably located in the structural gene for DNA polymerase and confers increased (nearly five-fold) sensitivity to UV light and to methylmethane sulphonate. The mutant shows essentially normal genetic recombination.

446. Preliminary experiments indicate that the amount of repair replication in UV-irradiated *pol A1* is similar to that of the parental strain (197). The mutant degrades more of its DNA after low doses of UV-irradiation than does the parental strain. This nuclease activity appears to be exonucleolytic (47).

447. Boyle *et al.* (47) found that the *pol A* mutant cells can excise UV-induced pyrimidine dimers. This property, coupled with the increased exonuclease activity observed in these strains, have led these authors to conclude (*a*) that the increased UV sensitivity of the mutant cells is not the result of a failure to excise dimers and (*b*) that the increased exonuclease activity "leads to the degradation of UV-irradiated DNA, masks the excision of dimers and interferes with the final step in excision-repair, that of restoring the integrity of the phosphodiester backbone of the DNA duplex".

448. The above thesis is confirmed by the observation that the DNA of *pol A1* cells contain more single-stranded breaks than *pol*⁺ when incubated for the same time after UV-irradiation (47, 197). If functional polymerase is truly absent *in vivo* as it is *in vitro* (i.e., has less than 1-2 per cent residual activity), the question arises as to how the gaps in the DNA produced by excision are repaired in *pol A1*. The suggestion has been made that the *rec A* repair system (paragraph 454) presumably substitutes for DNA polymerase in repairing the gaps. The increased UV-sensitivity of *pol A1* cells would thus be explained by assuming that the *rec A* system is slightly less efficient in repairing the gaps produced by excision than is DNA polymerase. It is of interest therefore that attempts to construct a *rec A-pol A1* double mutant have been unsuccessful (80, 163).

449. A ligase-deficient mutant has been isolated and has been shown to be sensitive to UV-irradiation (392) and to x-irradiation (105).

(iii) *Post-replication repair*

450. Post-replication repair of gaps opposite to pyrimidine dimers, discovered in *Escherichia coli* by Rupp and Howard-Flanders (422), is the most recently described and the least understood of the repair mechanisms. The present state of knowledge in this area has recently been reviewed by Smith (518). Rupp and Howard-Flanders studied the replication of DNA containing pyrimidine dimers in an excision-defective strain after UV-irradiation. It was found that in the first DNA replication after irradiation, the daughter strands contained gaps or discontinuities, the number of these defects being similar to the number of pyrimidine dimers in an equivalent length of parental DNA. In recent studies it has been found that these gaps were 800-1,000-nucleotides wide (423). The discontinuities, however, gradually disappeared during incubation (the hour following the first post-radiation DNA replication). As excision-defective cells surviving UV-irradiation usually produce normal rather than mutant daughter cells, it is unlikely that the gaps in the daughter strands are filled by the random insertion of bases.

451. Rupp and Howard-Flanders (422) have suggested that the repair of daughter-strand gaps is effected by a series of recombination-like events after DNA replication. In each of these events, the strand containing a gap at a given level pairs with its complementary sister strand which may contain gaps, but never at the same level. Such pairing would permit repair synthesis to restore the correct sequence of the region within each gap by utilizing the corresponding intact region of the other strand as template. The occurrence of a series of recombinational events at the level of each gap (with or without actual physical exchange) could reconstitute an intact DNA molecule capable of further replication.

452. From the foregoing, it follows that post-replication repair cannot occur unless both daughter mole-

cules of DNA produced by the first post-UV replication are present. Evidence indicating that this is true has been obtained (181).

453. A post-replication repair mechanism of this type could complement the excision repair process in wild-type cells. While excision repair would be effective before replication, the post-replication repair mechanism would act on abnormalities in the daughter strands.

454. Willets, Clark and Low (592) showed that there are three distinct loci in *Escherichia coli K12*, namely, *recA*, *recB* and *recC* which control recombination ability. The *recA* locus lies between the *pheA* and *cysC* loci on the genetic map, *recB* and *recC* loci between *argA* and *thyA* (28). Mutations at the *recA* locus result in a drastic reduction in recombination, high sensitivity to UV-irradiation and increased amount of DNA breakdown following exposure to UV light. On the other hand, mutations at either the *recB* or the *recC* locus lead to reduced but detectable recombination, increased sensitivity to UV light and reduced breakdown of DNA (relative to the *recA* mutations) following UV-irradiation. The product of the *recB* and *recC* genes has been shown to be an ATP-dependent exonuclease (28, 57, 593). The recombination-deficient mutants are collectively referred to as *Rec*$^-$ mutants, in contrast to the wild type which is designated as *Rec*+.

455. There is compelling evidence for the association of the two characteristics, namely, recombination deficiency and high sensitivity to the lethal effects of UV light (81, 177). Moreover, Howard-Flanders, Boyce and Theriot (179) and Howard-Flanders and Boyce (177) found that mutants of *Escherichia coli K12* isolated for their sensitivity to x rays were also recombination-deficient and vice versa. The *exr* mutation (phenotypic symbol Exr) isolated from *Escherichia coli* B_{s-1} confers increased sensitivity to UV light as well as to x rays (296), besides reducing recombination by a factor of two to three (598).

456. Since defects in excision repair or recombination can each cause a large increase in UV sensitivity, studies have been made to assess the relative contribution of these defects to the UV-sensitivity of bacteria. Howard-Flanders (176) investigated the magnitude of mean lethal doses of UV light (37 per cent survival) in strains of *Escherichia coli K12* which were (*a*) *uvrA*+ *recA*+ (wild type); (*b*) *uvrA*$^-$ *recA*+ (excision-defective); (*c*) *uvrA*+ *recA*$^-$ (recombination-deficient); and (*d*) *uvrA*$^-$ *recA*$^-$ (excision-defective *and* recombination-deficient). The mean lethal doses were, respectively, 500, 8, 3 and 0.2 erg mm^{-2} for groups (*a*) to (*d*). It hardly needs to be emphasized that the *recA*+ gene plays a very decisive role in determining UV sensitivity. This observation coupled with the estimated rate of formation of pyrimidine dimers in the bacterial genome (paragraph 430) suggests that the product of the *recA*+ gene is required to tolerate the passage of one pyrimidine dimer through the replication point.

457. Witkin (597) made similar studies in *Escherichia coli B* but used the criterion of mutation induction; she found that UV mutability is absent in any strain carrying an *exr*$^-$ mutation (paragraph 455). The failure of *exr*$^-$ strains to produce UV-induced mutations establishes that the product of the *exr*+ gene is necessary for UV mutability. These results were later extended to the *recA*$^-$ and *recC*$^-$ mutations (601).

458. The observations that (*a*) pyrimidine dimers induced in the DNA of an excision-defective strain cause the formation of daughter-strand gaps at the first DNA replication, that are then slowly repaired by a mechanism presumably involving a series of recombinational events; (*b*) UV mutability and recombination ability are both affected by mutations at four distinct loci (*exr, recA, recB* and *recC*); and (*c*) *exr*$^-$ strains are refractory to mutation induction by UV light whereas *exr*+ strains are not, have led Witkin (598) to suggest that "UV-induced mutations are actually recombination-induced mutations produced as a consequence of inaccurate recombinational repair of secondary UV damage (gaps opposite to pyrimidine dimers) in *exr*+ strains". Bridges, Dennis and Munson (50) and Kondo (227) have also suggested that inaccurate recombinational repair could generate UV-induced mutations.

459. In *Escherichia coli* nearly 80 per cent of all UV-induced mutations of the *tryptophane synthetase A* gene are single-base substitutions, the remainder being frame shifts (612). Since UV-induced mutations are thought to originate from inaccurate recombinational repair (paragraph 458), it may be concluded that the recombinational repair process generates these molecular alterations—transitions, transversions and frame shifts.

(b) *Eucaryotes*

460. Sutherland, Carrier and Setlow (537) showed that UV-irradiation produces pyrimidine dimers in the DNA *Paramecium aurelia* and that these photoproducts can be monomerized *in vivo* by photoreactivating light. Kimball (215) found that the mutational yield was reduced to one half when UV-irradiation was followed by photoreactivating light, which suggests that pyrimidine dimers play a role in UV mutagenesis in *Paramecium aurelia.* In the same study, the mutational yield under normal conditions (in the dark) was found to be maximal when *Paramecia* were exposed to UV light just before, or perhaps in, the *S* period (period of DNA synthesis) and was less when the interval between irradiation and *S* was longer. Kimball interprets this finding as suggesting that UV-induced premutational damage can undergo dark repair until *S* and that this repair is nearly error-free (by analogy with the *exr*$^-$ condition in *Escherichia coli*) since the yield drops to nearly zero. The variation in mutational yields during cell cycle is roughly similar to that found for x rays and triethylene melamine.

461. *Neurospora crassa* studies have demonstrated the ability of this organism to exhibit photoreversal of both lethal and mutagenic damage induced by UV-irradiation (56). Terry, Kilbey and Howe (553) showed that extracts of *Neurospora crassa* in conjunction with light of the proper wavelength can reactivate *in vitro* the UV-irradiated transforming DNA of *Haemophilus influenzae.* The results of Kilbey and de Serres (215) indicate that photoreversal reduces the frequency of all *ad-3B* mutations induced by UV light, including those suspected of being base-pair substitutions and deletions or additions (frame shifts). It therefore seems likely that pyrimidine dimers are capable of giving rise to these molecular alterations in *Neurospora.* The non-photoreactivable mutations constitute about 30 to 40 per cent.

462. Pyrimidine dimers have also been found to play a major role in UV mutagenesis of several other eucaryotic organisms (see 49 for a recent review). Mutations which increase or decrease UV sensitivities have been isolated in *Chlamydomonas reinhardi* (102), *Aspergillus nidulans* (13, 74), *Saccharomyces cerevisiae* (519) and several other organisms. While many of their properties seem to resemble those of comparable bacterial mutants, their biochemical characterization has not proceeded far enough to permit generalizations.

(c) *Mammalian cells in culture*

(i) *Photo-enzymatic repair*

463. Attempts to demonstrate photo-enzymatic repair of normal growth or of DNA synthesis in mammalian cells have been unsuccessful (221, 569, 570) except in marsupial cells in which Cook and Regan (90) demonstrated the existence of this process. The activity was found in all tissues tested, namely, liver, brain, kidney, testis, heart and lung. The activity was also found in an established cell line of rat kangaroo that had been in culture for more than four years. In view of the fact that photoreactivation is found only in marsupials and because it is restricted to UV damage only, it is of marginal interest in the present context.

(ii) *Unscheduled DNA synthesis and repair replication*

464. One of the key steps in excision repair in UV-irradiated bacteria (paragraph 437) is the synthesis of new DNA which is inserted into sites from which the damaged nucleotides have been removed. Experimental evidence for this kind of synthesis called repair replication was first obtained by Pettijohn and Hanawalt (394). Although pyrimidine dimers are formed in mammalian cells after exposure to UV light, the level of excision repair seems to vary widely between different cell lines (see 223, 376, 408 for reviews). Excision of dimers is not easily detectable in mouse (221, 222) or in Chinese-hamster cells (571) whereas excision of 50 per cent or more of the dimers in the DNA of Syrian hamster and from several sources of human cells can occur (411, 505, 618). Painter (376) has pointed out that labelling procedures are required to determine dimer excision, in which the materials of interest (dimers) represent only a very small fraction of the total radio-activity in the system so that up to 10 per cent dimer removal is not detectable by the method employed.

465. It was pointed out in paragraph 438 that, in bacteria, at least four different enzymatic activities (endonuclease, exonuclease, DNA polymerase and ligase) are involved in the excision-repair process. Enzymes of these types have also been found in mammalian cells and the properties of purified DNA polymerases, DNA ligase and DNAse IV (an exonuclease) are very similar in many respects to those of related microbial enzyme activities (265, 266). An endonuclease that attacks alkylated DNA but not normal or UV-irradiated DNA is present in human lymphocytes (534). The observation (paragraph 515) that cells from *Xeroderma pigmentosum*[10] patients lack the normal ability to produce chain-breaks in their DNA after UV-irradiation implies that a different endonuclease that recognizes regions containing pyrimidine dimers is also present in human cells (84, 505). These findings suggest that such enzymatic activities are presumably used for the same purposes in mammalian cells as in micro-organisms and that the process of dimer excision and repair proceeds by similar biochemical mechanisms in both types of cells.

466. The problem of excision repair in mammalian cells has been approached by means of autoradiographic and density-labelling procedures, the latter being based on the technique used by Pettijohn and Hanawalt (394). Rasmussen and Painter (405) reported that if *HeLa* cells were UV-irradiated prior to incubation with ^{3}H-thymidine, all of the cells in the culture became labelled as determined by auto-radiography, instead of just the cells in the *S* phase as was the case in unirradiated cells. These authors subsequently extended the study and found that of 12 different kinds of cells tested (in addition to *HeLa*) all but three showed this phenomenon. The three that did not show the phenomenon were two mouse lines and one Chinese-hamster line (406). Moreover, these showed the effect if they were grown in the presence of 5-bromodeoxyuridine (5-BUDR) prior to irradiation. Recent results have indicated that the effect could be demonstrated in the mouse and Chinese hamster cells if the autoradiographic exposure was greatly extended (378). Therefore this phenomenon, which was also observed by Djordjevic and Tolmach (116) in *HeLa* cells and is called "unscheduled DNA synthesis" occurs to a much lesser extent in some cells than in others.

467. Rasmussen and Painter (406) also demonstrated the occurrence of repair replication in *HeLa* cells after UV-irradiation (using essentially the same technique employed by Pettijohn and Hanawalt (394) for bacteria) and conjectured that repair replication and unscheduled DNA synthesis might reflect the same molecular process (but see paragraph 514).

468. This possibility received strong support from the work of Cleaver (83) who compared normal human skin cells with those from patients suffering from the de Sanctis Cacchione syndrome of *Xeroderma pigmentosum* with respect to the ability of these cells to effect repair replication and unscheduled DNA synthesis after UV-irradiation. Cleaver found that while irradiated cells from normal humans showed both repair replication and unscheduled DNA synthesis, those from the *Xeroderma pigmentosum* patients did not show either. Cleaver's work was the first demonstration of a genetically determined defect in a radiation-repair process in human cells.

469. In further studies, Painter and Cleaver (378) examined repair replication in cells showing extensive unscheduled DNA synthesis (those of human origin) and in those showing very little unscheduled DNA synthesis (mouse and Chinese hamster cells) and found that the former cells always showed extensive repair replication while it was possible to demonstrate repair replication only with difficulty in the latter cells. These

[10] *Xeroderma pigmentosum* is a rare autosomal recessive disorder in which the skin is extremely sensitive to UV and sunlight. The striking clinical feature of homozygotes is a very high incidence of actinic skin cancer on exposed regions of the face and arms. Two clinical forms are known both of which show the skin symptoms, but the rare form shows additional neurological disorders and is known as the de Sanctis Cacchione syndrome. There is no ready way to diagnose heterozygotes, and repair replication in these is near normal (85, 128).

correlations together with the results of comparisons of the amount of repair replication and unscheduled DNA synthesis in *HeLa* cells strengthen the hypothesis that these two phenomena are manifestations of the same molecular process.

470. In bacteria, strong correlations exist between the ability to carry out repair replication and resistance to UV-irradiation (597). But such a correlation is not easy to make between cell survival and repair replication for mammalian cell lines (376, 408). The UV sensitivities of *HeLa*, *L* and Chinese-hamster cells do not appear to differ by more than a factor of two to three, but the amount of repair replication in Chinese-hamster cells and mouse *L* cells is much less than in *HeLa* cells (378).

471. The possibility that repair replication may enhance survival finds support in the recent work of Cleaver (85) and of Goldstein (151). Cleaver (85) found that *Xeroderma pigmentosum* cells (which show greatly reduced levels of repair replication) also show reduced survival in terms of colony formation; both normal and *Xeroderma pigmentosum* fibroblasts have exponential survival curves with a D_0[11] of 29 and 9 erg mm^{-2}, respectively.

472. Goldstein's results are similar to those of Cleaver in that they show that in the *Xeroderma pigmentosum* cell lines that he investigated, exponential survival curves were claimed with a D_0 of 2 erg mm^{-2}. Painter (376) believes that if these observations are confirmed, a case can be made for repair replication having a function in maintaining the reproductive integrity of human and presumably of other mammalian cells.

473. In recent studies evidence has been obtained showing that *Xeroderma pigmentosum* fibroblasts from different patients show different levels of repair replication; these range from zero to 25 per cent in the studies of Cleaver (85) and from zero (extreme case) to 70 per cent (a "light" case) in those of Bootsma *et al.* (42).

474. Regan *et al.* (410) have recently reported the development of a sensitive technique which utilizes the photolysis of bromodeoxyuridine to study the extent of repair of UV-irradiation damage to DNA in human cells. The authors point out that (i) the quantitative aspects of this assay for repair and its sensitivity should make it applicable to the study of repair of damage induced by agents other than UV and (ii) the method can also be used as a rapid, sensitive pre-natal assay for *Xeroderma pigmentosum*.

475. Repair replication and/or unscheduled DNA synthesis occurs in mammalian cells after treatment with nitrogen mustard and methylmethane sulphonate (18, 168, 416).

476. If the DNA that has undergone repair replication is functional, then it must be able to participate in semiconservative replication. Rasmussen *et al.* (407) and Painter *et al.* (379) have shown this to be true for human diploid and aneuploid cells.

(iii) *Recombinational repair*

477. Studies seeking evidence for the occurrence of recombinational repair in mammalian cell systems (similar to that observed in *Escherichia coli*) are only just beginning. Since only a part of the dimers are excised from some mammalian cells and almost not at all from others, such repair systems may be of great importance. Cleaver and Thomas (86), Klímek and Zemanova (225, 226) and Rupp *et al.* (424) have published some evidence for this kind of repair in Chinese-hamster and in mouse cells.

[11] The dose that reduces survival to 37 per cent of an initial survival level on the exponential region of a dose-response survival curve.

B. Ionizing radiation

478. In contrast to the wealth of information available on the nature of the damage induced by UV light and its possible repair mechanisms, our knowledge regarding the effects of ionizing radiations is still meagre. Ionizing radiations produce different types of alterations in the DNA among which are: base changes, base destruction, sugar-phosphate bond cleavage, chain-breakage (single- and double-strand breaks), cross-linking of the strands and degradation (196).

479. In spite of the fact that DNA-strand breakage is an intensively studied phenomenon, the exact chemical changes that occur during the formation of breaks are not known (196, 376). Studies on the irradiation of DNA in aqueous solutions have shown that inorganic phosphate is liberated (472) and that phosphomonoester groups are formed (89). Such studies suggest that chain breakage occurs at the phosphodiester bond when DNA is irradiated in aqueous media. Significant damage to the deoxyribose moiety has also been reported (209) suggesting another site of chain breakage at the C3′-C4′-bonds. The x-ray-induced breaks have sometimes been classified as "clean breaks" (e.g., phosphate-ester break) or "dirty breaks" (e.g., sugar damage and/or base loss). It is presumed that clean breaks can be more quickly repaired than dirty breaks (518).

480. The failure of the polynucleotide-joining enzyme (which is known (373) to act on 3′ hydroxyl-5′ phosphoryl termini in double-stranded DNA) to repair in one step the single-strand breaks produced in DNA by x-irradiation in aqueous media implies that chain breakage involves a more complicated mechanism than a simple rupture of the phosphodiester bond producing polynucleotide chains with 3′-hydroxyl and 5′-phosphoryl groups in juxtaposition (207). After reviewing some other additional lines of evidence, Painter (376) also concluded that after x-irradiation, the single-strand breaks can terminate in several kinds of end groups.

481. The above studies, designed to identify the end groups of irradiated DNA, have been done in solutions of DNA in which the bulk of the damage is probably caused by indirect action (i.e. free radicals formed in water). Within the cell, however, direct action plays a much greater role. Which of the effects described in paragraph 478 is important and to what extent there are mechanisms in the cell to repair or by-pass this damage and the role of oxygen and other agents in modifying the yield are problems that have been intensively pursued.

482. The studies on ionizing radiation-induced damage and repair mechanisms can be broadly divided into two categories, namely, (*a*) those performed by means of physico-chemical techniques at the level of the primary damage induced in the DNA and concerned with the induction of single- and double-strand breaks, base damage, etc. and (*b*) those that apply genetic

techniques to the assessment of the mutational damage.

1. *Primary DNA damage and associated repair mechanisms*

(a) *Single- and double-strand breaks*

483. Freifelder (141) measured the number of x-ray-induced single- and double-strand breaks per phage (*T7*) at high survival levels (20 to 100 per cent) by ultracentrifugal analysis of the DNA, and correlated the inactivation of phages with the yield of double-strand breaks and with possible base damage (thymine?). Single-strand breaks are not lethal and this is consistent with the fact that viable phages contain natural single-strand breaks. While the technique of ultracentrifugation analysis used by Freifelder is simple and direct, it is strictly limited to situations in which the unirradiated DNA molecules, as isolated, are homogeneous.

484. In bacteriophage ΦX174 (single-stranded DNA) every chain break leads to inactivation (136). Lytle and Ginoza (290) estimate that the frequency of sugar-phosphate-backbone breaks induced by gamma rays in this single-stranded phage under conditions of direct action is 0.20 ± 0.03 per lethal event and per primary ionization in the DNA. These results are in contrast with the observation that there are 0.75 single-strand breaks per primary ionization in the double-stranded replicating form of DNA of the same phage, also irradiated under conditions of direct action (544).

485. McGrath and Williams (297) developed a method applicable to the study of whole cells in which the cells are lysed directly on top of an alkaline sucrose gradient. The DNA is released with minimal shearing and sediments through the gradient, the distance being dependent upon the molecular weight.

486. Using this method, these workers analysed the DNA of x-irradiated *Escherichia coli B/r* (radio-resistant) and B_{s-1} (radio-sensitive) strains and observed that the decrease in sedimentation rate of the alkali-denatured DNA of both strains are similar. However, re-incubation of the irradiated cells restored the sedimentation rate essentially to the pre-irradiation level in the *B/r* strain, but not in the B_{s-1} strain. They concluded that the increase in sedimentation rate reflects a repair process that joins broken pieces of the DNA (in *B/r*) with alkali-stable bonds. Single-strand breaks are thus reparable in the *B/r* strain.

487. Calculations showed that single-strand scissions could quantitatively account for lethality in the B_{s-1} strain, although double-strand breaks produced in lesser yield would also be expected to contribute to some extent to lethality.

488. Freifelder (143) has recently reported the results of some experiments with the *Escherichia coli* B_{s-1} strain in which he compared the rate of strand-breakage with the inactivation rate. His data suggest that the ratio of single-strand breaks to lethal hits is about seven "from which one cannot make a very firm statement about the role of single-strand breakage in x-ray inactivation". However, as Freifelder has pointed out, if single-strand breaks are not lethal, this raises the question of the cause of the greater sensitivity of strain B_{s-1}. A study of the role and possible repair of base damage may lead to an answer, although it is not clear at present how to investigate base damage using biologically meaningful doses.

489. Using techniques similar to those employed by McGrath and Williams (297), Kaplan (201) reported that x-irradiation of *Escherichia coli K12* induced a decrease in sedimentation rate of alkali-denatured and of native DNA attributable to single- and double-strand scissions, respectively. Single-strand scissions were repaired during re-incubation of the irradiated cells whereas double-strand scissions were not. BUDR (the incorporation of which in DNA is associated with a 2-3 fold increase in x-ray sensitivity (202)) increased the yield of double-strand scissions per unit dose to an extent proportional to its effect on radiation-induced lethality. These correlations suggest that even in radio-resistant bacteria, double-strand scissions are the major radio-chemical lesions leading to loss of viability.

490. The studies of Munson *et al.* (333) on the sensitivity of *Escherichia coli* to radiations of different LET led to the suggestion that potentially lethal damage may be of two kinds, double-stranded damage, which is largely irreparable, and single-stranded damage, which is reparable to different degrees in different strains.

491. Kapp and Smith (208) showed that a correlation exists between the inability to repair single-strand breaks and the radio-sensitivity of bacteria. These investigators used strains of *Escherichia coli K12* mutant in genes controlling excision repair (*uvr*) and genetic recombination (*rec*) to study their x-ray sensitivity and their ability to repair x-ray-induced single-strand breaks in the DNA. It was found that mutations in the *rec* genes appreciably increased radio-sensitivity (see also paragraphs 454, 518) whereas *uvr* mutations produced little, if any, increase. For a given dose of x rays, the yield of single-strand breaks was largely independent of the presence of *rec* or *uvr* mutations. The *rec*+ cells (including those carrying the *uvr B5* mutation) could efficiently rejoin x-ray-induced single-strand breaks in DNA, whereas *rec A56* mutants could not repair these breaks to any great extent. The *rec B21* and *rec C22* mutants showed some indication of repair capacity. These observations suggest that unrepaired single-strand breaks may be lethal in *Escherichia coli*.

492. This correlation between the inability to repair single-strand breaks and the radio-sensitivity of bacteria is further documented by studies using drugs that appear to selectively inhibit (in *rec*+ strains) the recombinational repair of x-ray-induced single-strand breaks in DNA (518).

493. In *Micrococcus radiodurans*, in contrast to what has been discussed above, both single- and double-strand breaks are effectively rejoined. However, the mechanism by which double-strand breaks are rejoined has not been resolved (10, 106).

494. Alexander *et al.* (11) have shown that in *Micrococcus radiodurans*, approximately 90 per cent of the single-strand breaks produced by x-irradiation in oxygen are repaired rapidly (within minutes) in buffer at 30° C but not at 0° C. The remainder of the breaks not repaired in buffer are restituted slowly (hours) when the cells are incubated in growth medium. However, after irradiation in oxygen, cells are still capable

of repairing rapidly at 0° C single breaks induced by a subsequent anoxic irradiation suggesting that the repair system itself is not especially vulnerable to irradiation in the presence of oxygen. Ligases capable of linking 5′-P . . .3′-OH breaks have been shown to be active at 0° C and the authors have speculated that the majority of breaks produced by x rays under anoxic radiation are of this type, since in *Micrococcus radiodurans* they are restored so rapidly.

495. The finding in the above study that there may be both a fast and slow enzymatic process operating in the repair of single-strand breaks in DNA has led to the suggestion that many of the single-strand breaks in DNA are rapidly repaired in *Escherichia coli* before the samples can be analysed by sedimentation. Such a situation would be consistent with an apparent requirement of about 500 eV to produce a DNA chain break in *Escherichia coli* (5, 150, 201, 209).

496. The DNA polymerase deficient mutant *pol A1* (paragraphs 445-446) of *Escherichia coli* is very sensitive to killing by x-irradiation. In fact it is as sensitive as *rec A*. This property prompted an investigation of the ability of this mutant to repair x-ray-induced single-strand breaks in DNA. These studies revealed an unexpectedly high yield of breaks per dose of radiation compared to *pol*+ (518) and led to the speculation that *pol A1* might be defective in a rapid repair system for chain breaks which had not been previously detected in *Escherichia coli*. This has been confirmed by finding conditions which inhibit this process in *pol*+. In *pol A1* and in "completely" inhibited *pol*+ the energy required to produce single-chain breaks is approximately 75 eV per break (554).

497. The nature or possible extent of interaction between the repair systems controlled by the *rec*+ and *pol*+ genes is not known. Preliminary data indicate that *pol*+ *rec*+ cells can repair more chain breaks than the sum of the efforts of *rec*+ *pol*− and *rec*− *pol*+ cells, suggesting that the two systems may be somewhat interdependent (554).

498. Using the technique of McGrath and Williams (297), Lett *et al.* (262) showed that the x-ray sensitivities of the DNA in murine leukæmic cells (D_0 = 38 rad) and *Micrococcus radiodurans* (D_0 = 70 krad) to the induction of single-strand breaks are very similar. They estimated that, under irradiation in an oxygen atmosphere, one single-strand break was produced for approximately 50 eV with *Micrococcus* and 70 eV with murine lymphoma, suggesting that variations in radio-sensitivity are not determined by the magnitude of the primary DNA lesion. The efficiency of strand breakage in *Micrococcus* is the same as the recently corrected value for "fully protected bacteriophage systems" (142, 143).

499. In general it may be said that the average energy expended per single-strand breakage of DNA irradiated within a cell (for low-LET radiations) is around 50-100 eV and that single-strand breaks are some 7-10 times more numerous than double-strand breaks (91, 142); Neary *et al.* (350) have indicated that the ratio of single-strand to double-strand breaks may be of the order of 10-20 to 1.

500. Lett *et al.* (262) also found that irradiation of *Micrococcus* and of murine lymphoma cells under anoxia gave fewer single-strand breaks (one third to one half the number observed in oxygen) leading to an Oxygen Enhancement Ratio (OER) of between two and three. Dean *et al.* (107) subsequently established that the OER for the induction of single-strand breaks in *Micrococcus* DNA was not significantly different from unity if an inhibitor of repair was present, whereas a value of about three was obtained if repair operated. They also re-examined the earlier data of Lett *et al.* (262) on oxygen effect for mouse-lymphoma cells and considered this to be spurious and to result from the peculiarities of the molecular weight distribution after irradiation in nitrogen. When this factor was taken into consideration, the OER was close to unity.

501. The lack of oxygen effect in the production of single-strand breaks discussed above is in agreement with the result of Freifelder (142) with the DNA of phage *B3* and also with those of Neary *et al.* (350) with the DNA of phage *T7*. It must however be pointed out that there are other reports in the literature in which OER values higher than one have been found (5, 46).

502. Dean *et al.* (107) consider that the initial production of single-strand breaks is uninfluenced by oxygen but that there may be a chemical difference between the breaks produced in the presence or absence of oxygen, which causes a difference in the reparability of the two classes of break. They suggest that the variability in OER values for single-strand breaks of DNA in cells may be accounted for by the extent to which repair has proceeded in the conditions of any particular experiment.

503. In the same study mentioned in paragraph 501 Neary *et al.* (350) found that oxygen did not significantly increase the effectiveness of radiation-induced double-strand breakage in *T7* DNA, a finding which is in line with those reported by Lett *et al.* (262), Lett and Alexander (261), Alexander *et al.* (12), Freifelder (141) and others, but at variance with that of van der Schans and Blok (579).

504. Lett *et al.* (262) were the first to observe the rejoining of single-strand breaks in mammalian cells. They found that rejoining occurred very rapidly in a radio-sensitive strain of mouse lymphoblasts after 30,000 rads, an obviously supra-lethal dose.

505. Lohman (268) and Humphrey *et al.* (183) studied by means of a modified alkaline-sucrose-gradient technique the x-ray (or gamma) induction and rejoining of single-strand breaks in the DNA of human kidney (*T*) cells and in Chinese-hamster (*Don C*) cells. They obtained results similar to those of Lett *et al.* (262) and extended the data to lower doses. While Lohman (268) found that strand-rejoining was most effective in early *S* and minimal in *G2* (after 20 kR), Humphrey *et al.* (183) found no evidence of a difference in ability to repair single-strand breaks during the cell cycle. Results similar to those of the latter authors were obtained by Sawada and Okada (464) with mouse lymphoblasts.

506. Elkind and Kamper (129) were also able to show repair of x-ray induced single-strand breaks in Chinese hamster cells at doses of 1,440 rads and higher.

507. Using a biochemical method (the use of polynucleotide kinase which catalyses the reaction of a polynucleotide chain terminating in 5′-hydroxyl group with the gamma phosphate of ATP to form polynucleotide-5′ phosphate) Dalrymple *et al.* (100) demonstrated

the repair of radiation-induced DNA breaks in mouse liver DNA and in mouse *L* cells. Their work suggests that breaks exposing the 5′ phosphate are metabolically formed within one minute after x-irradiation and then rapidly "healed" within the next 10 minutes. This finding is at variance with the results obtained by Kapp and Smith (207) in their *in vitro* studies (paragraph 480).

508. The question whether double-strand breaks in the DNA can rejoin has been the subject of considerable controversy. Double-strand breaks do occur after irradiation (paragraph 499) but at present there is no direct evidence that they are rejoined. Painter (376) has argued that if double-strand breaks did not rejoin, then it should be possible to detect a small percentage of DNA as a fraction remaining at low sedimentation values in alkaline-sucrose gradients, since 1 in 7 to 10 strand breaks must have been derived from double-strand breaks. This has not been the case however because most (certainly more than 90 per cent) of the broken DNA appears at control sedimentation values. On the basis of these results it may be inferred that many double-strand breaks are rejoined.

509. The other line of reasoning used by Painter is based on the consideration of the number of double-strand breaks that must occur in cells surviving x-irradiation. Since 1 rad produces about 10 single-strand breaks per mammalian genome, a D_0 of 100 rads would produce 1,000 breaks, of which at least 100 must actually be double-strand breaks. Survivors must be able to cope with these in some fashion; it must therefore be presumed that they are rejoined at some time. Possible mechanisms that might play a role in the rejoining of double-strand breaks have been suggested.

(b) *Unscheduled DNA synthesis and repair replication*

510. Unscheduled DNA synthesis and/or repair replication have been demonstrated to occur in mammalian cells after x-irradiation. Rasmussen and Painter (406) observed unscheduled DNA synthesis in *HeLa* cells and Painter and Cleaver (377) reported repair replication in them, but only after a very high exposure (100,000 R). Later, Painter (375) reported repair replication in *HeLa* cells after low doses, and also in unirradiated controls. However, the amount of repair replication measured as specific tritium activity (from ^{3}HBUDR) in normal density DNA did not exceed that in controls until the exposure to the cells exceeded 1,000 roentgens.

511. In a further study of repair replication in mammalian cells after x-irradiation, Painter and Young (380) examined the quantitative and qualitative characters of repair replication in Chinese hamster cells (B14FAF), mouse cells (P388F) and human diploid cells (WI-38) and found them to be similar. Calculations of the amount of DNA damage per cell and number of bases inserted per damaged site indicate that degradation at each damaged site does not exceed three bases; this small amount of base insertion cannot be detected in the presence of the nonconservative synthesis occurring in controls until the damage to DNA is extensive—more than that caused by 1,000 rads (paragraph 510).

512. In contrast, Ayad and Fox (17) and Fox *et al.* (140) reported that repair replication occurred in mouse cells (P388F) after exposures to as low as 150 roentgens and not in controls; the amount of repair replication occurring in these cells after 150 roentgens, however, was extremely large; the incorporation of isotope was 15 to 20 per cent of that occurring by means of semiconservative replication in controls. For higher exposures, the relative amount of repair synthesis was even greater.

513. It is obvious that the results of Ayad and Fox (17) and Fox *et al.* (140) are at variance with those of Painter and Young (paragraph 511). The latter authors have re-examined the data of Ayad and Fox (17) and Fox *et al.* (140) and point out that "the extensive synthesis reported by these workers is not restricted to damaged sites in the DNA and therefore must not be related to repair".

514. Shaeffer and Menz (506) compared unscheduled DNA synthesis, D_0, cell recovery and chromosome number in several x-irradiated mammalian cell lines. If unscheduled DNA synthesis represents a biologically significant repair system, cell lines showing greater extents of unscheduled DNA synthesis should exhibit a correspondingly lower radio-sensitivity (higher D_0) and/or a higher recovery ratio. However, the data of these authors suggest that there was no such correlation. These observations are consistent with the conclusion that cell survival after x-irradiation is not solely, if at all, dependent on unscheduled DNA synthesis.

515. Perhaps one of the most interesting findings in mammalian cells is the occurrence of unscheduled DNA synthesis and repair replication in *Xeroderma pigmentosum* cells after x-irradiation: Cleaver (84) found that unscheduled DNA synthesis occurred in these cells to the same extent as in normal diploids; Kleijer *et al.* (220) found this to be true for both unscheduled DNA synthesis and repair replication. Since x-irradiation is known to produce single-strand breaks, these findings have led to the proposal that *Xeroderma pigmentosum* cells are defective in the initial incision-step (and consequently unable to effect repair replication after UV-irradiation, paragraph 468). *Xeroderma pigmentosum* cells apparently have normal levels of the other enzymes in the sequence involved in repair replication.

2. *Mutational damage and its repair*

(a) *Procaryotes*

516. Munson and Bridges (332) found that the mutagenic damage in *Escherichia coli* is largely single-stranded and considered it likely that this might consist of the scission of the sugar-phosphate backbone of the DNA.

517. The lack of photoreversibility of x-ray-induced mutational damage in *Escherichia coli* indicates that pyrimidine dimers are not involved (194). Excision-defective strains (*Hcr*−) are no more sensitive to x rays than their *Hcr*+ counterparts suggesting that the damage is not reparable by excision (54, 177, 331).

518. It has been mentioned earlier (paragraph 455) that, in *Escherichia coli*, sensitivity to UV-killing is significantly increased by *exr*− or *rec*− mutations. The same is true for the killing effects of x rays (177, 296). Since both these loci affect genetic recombination, the suggestion has been made that there might be a common pathway for UV and ionizing-radiation mutageneses and that some potentially lethal primary or

secondary x-ray damage may be reparable by recombination (50, 598, 600). The findings that one *Rec*⁻ strain is refractory to the induction of mutations by x rays (227, 228) and that, in an *Exr*⁻ strain, the yield of gamma-ray-induced mutations is only 5 per cent of that observed in the *Exr*+ strain (53) are entirely in line with the expectation based on the postulated role of recombination in the induction of mutations by x rays (see paragraph 457 for UV mutability in similar strains).

519. In contrast to UV-induced mutations which seem to arise *after* replication of DNA (paragraph 458), x-ray-induced mutations are produced *before* replication. Unlike UV mutations, the x-ray-induced mutations can be transferred by conjugation immediately after irradiation of the donor (195) and appear on both daughter chromosomes at the next DNA replication (55, 330). It is thus obvious that, if recombination is the primary mechanism that generates x-ray-induced mutations as well, it should operate before DNA replication. Witkin (598) has postulated that, if the single-strand breaks in the DNA induced by x rays are located in parts of DNA which have replicated before x-irradiation, then these may be subject to recombinational repair and may thus be the only breaks capable of giving rise to x-ray-induced mutations.

520. It should here be pointed out that it has not yet been demonstrated that a complete recombinational event is required for the repair of x-ray-induced single-strand breaks. It is possible that only a few of the enzymes normally required for genetic recombination are used in the repair of x-ray-induced single-strand breaks (518).

(b) *Eucaryotes*

521. The x-ray induction of forward mutations at the *ad*-3*A* and *ad*-3*B* loci in *Neurospora crassa* has been extensively investigated by de Serres *et al.* (110, 112, 113, 114, 293, 590). Although *Neurospora* is a haploid organism, by using a two-component heterokaryon this system can be made to mimic a diploid organism. The heterokaryon is heterozygous for markers at the two closely linked loci, *ad*-3*A* and *ad*-3*B*, which control different but sequential steps in purine biosynthesis. Inactivation of either of these genes results in the accumulation of a reddish-purple pigment in the mycelium and a requirement for adenine.

522. It has been shown that the x-ray-induced mutations at these specific loci fall into two classes designated as *ad*-3^{R} and *ad*-3^{IR} (114). The first class consists of reparable mutants that will grow as homokaryons on adenine-supplemented medium and the second consists of irreparable mutants that will not grow as homokaryons either on adenine-supplemented or on complete medium.

523. Genetic analysis has shown that the *ad*-3^{R} mutants have only the *ad*-3*A* or *ad*-3*B* locus inactivated whereas, in the *ad*-3^{IR} mutants, the inactivation covers other loci in the immediately adjacent regions (110). Moreover, the *ad*-3^{R} mutants predominate at low doses and show a linear relationship with dose whereas the *ad*-3^{IR} class predominates at high doses and the rate of induction is proportional to the square of the dose (590).

524. These results are consistent with the interpretation that *ad*-3^{R} mutations are essentially point mutations and that the *ad*-3^{IR} mutations are multilocus deletions. In line with this interpretation are the results of dose-rate studies in which it was found that the *ad*-3^{R} class showed no dose-rate effect whereas lowering the dose rate (1,000 R min^{-1} to 10 R min^{-1}) brought about a significant reduction of the frequencies in the *ad*-3^{IR} class (113). These results are taken as evidence for the occurrence of repair of the *ad*-3^{IR} class of lesions.

525. The molecular alterations that lead to point mutations (*ad*-3^{R}) have been characterized (293). The results of allelic complementation and specific revertibility tests (with chemical mutagens) conducted on a sample of sixty-eight x-ray-induced *ad*-3*B* mutations (*ad*-3^{R} type) revealed that nearly more than one third and possibly up to one half of these mutations could be due to base-pair changes and deletions.

C. Summary

526. Our knowledge concerning the effects of radiations on DNA and repair processes has rapidly expanded during the past several years. A variety of systems from procaryotes to mammalian-cell cultures have been used to examine damage induction and to elucidate the operation of repair processes of the primary damage in the DNA (by physico-chemical and biochemical techniques) and of mutations (genetic techniques).

527. Cyclobutane-type pyrimidine dimers are among the most studied photoproducts formed in the DNA after UV-irradiation. These have been identified in micro-organisms as well as in mammalian cells. They act as at least a temporary block to DNA synthesis in micro-organisms, but not in certain mammalian cells.

528. In bacteria, there are at least three repair processes—photo-enzymatic repair, excision repair and post-replication (recombinational) repair—which operate to eliminate these lesions and restore the normal DNA structure.

529. Photo-enzymatic repair and excision repair operate before DNA replication whereas post-replication repair, as the name implies, operates after DNA replication. Strains of bacteria deficient in one or more of these processes are significantly more sensitive to the killing effects of UV light. In addition, excision-defective and recombination-deficient strains are also more sensitive to x-ray killing. The genetic loci that control these processes have been identified.

530. Photo-enzymatic repair and excision repair are considered to be very much less likely to introduce errors into the DNA in the course of repair than post-replication repair. The striking correlation between recombination-deficiency and increased sensitivity to UV-killing, and the absence or near-absence of UV mutability in strains of bacteria such as *exr*⁻ or *rec*⁻ which are recombination-deficient have led to the hypothesis that UV mutability is intimately related to recombination and that UV light induces mutations in normal wild-type bacteria through inaccuracies introduced into the DNA by the recombinational repair process.

531. The results of studies of the tryptophane synthetase *A* gene in *Escherichia coli* indicate that about 80 per cent of the mutations at this locus are single-base substitutions, the remainder being frame shifts. If the hypothesis of recombinational origin of muta-

tions in *Escherichia coli* is accepted, then it follows that the errors introduced into the DNA during recombinational repair relate mainly to the alteration of pairing specificities in single bases.

532. Among mammals, the photo-enzymatic repair system exists only in marsupials. The ability to excise dimers varies markedly among mammalian cell lines and ranges from nearly no detectable excision (mouse and Chinese hamster cells) to excision of up to 50 per cent or more (human cells), still much less than in bacteria where over 90 per cent of the dimers are removed from the DNA.

533. One of the essential steps in excision repair — synthesis of new DNA to fill up gaps produced by the excision of dimers — has been demonstrated to occur by autoradiographic techniques (unscheduled DNA synthesis) and by density labelling procedures (repair replication) in several mammalian cell lines.

534. It has been shown that repair replication is functional i.e. repaired DNA can undergo normal semi-conservative replication.

535. After UV-irradiation, cells from patients suffering from *Xeroderma pigmentosum* are either unable to effect unscheduled DNA synthesis and repair replication or are able to do so only at low rates.

536. The amount of repair replication occurring after UV-irradiation in several mammalian cell lines does not appear to be strongly correlated with cell-survival data; however, the possibility that repair replication may enhance survival follows from the demonstration that *Xeroderma pigmentosum* cells show reduced survival levels (relative to normal cells) in terms of colony formation.

537. The fact that repair replication and unscheduled DNA synthesis in *Xeroderma pigmentosum* cells occur at normal rates after x-irradiation (which is known to produce single-strand breaks) but not after UV-irradiation, shows that these cells are probably lacking, or deficient in, the incision enzyme(s), the operation of which precedes the excision of dimers.

538. Evidence showing the occurrence of recombinational repair after UV-irradiation has been obtained in mammalian cells.

539. The identification and isolation of certain enzymes in mammalian cell systems the properties of which are similar to those controlling the excision repair process in microbial systems suggest that such enzyme activities are probably used for the same purposes in mammalian cells as in microbial systems and that the process of dimer excision and repair proceeds by similar biochemical mechanisms in both types of cells.

540. Among the different kinds of damage produced by ionizing radiations, the formation and repair of single- and double-strand breaks in the DNA have been extensively studied in bacteriophages, bacteria and mammalian cells. It has been shown that single- and double-strand breaks occur in a ratio of about 10-20 to 1 in DNA after exposure to ionizing radiation. Their production, at least in the systems studied, is unaffected by the presence or absence of oxygen during irradiation.

541. Single-strand breaks are not normally lethal since they may be effectively repaired, whereas double-strand breaks are lethal in phages and bacteria (except in *Micrococcus radiodurans* in which double-strand breaks are also repaired). In mammalian cells, although there is no direct evidence demonstrating the rejoining of double-strand breaks, there are grounds to believe that they may undergo repair.

542. There is not yet enough evidence for repair synthesis in bacterial DNA following exposure to ionizing radiation. In mammalian cells, however, both repair replication and unscheduled DNA synthesis do occur following exposures to ionizing radiation.

543. The mutagenic damage produced by ionizing radiation in bacteria is not photoreversible, suggesting that these lesions are not likely to be pyrimidine dimers. In addition they are not exciseable either. The parallelism between recombination-deficiency and enhanced sensitivity to the killing effects of UV light and x rays on the one hand, and the refractoriness of the recombination-deficient strains to mutation induction by UV light as well as by x rays on the other, have led to the suggestion that x-ray-induced mutations may also arise via a recombinational repair mechanism. However, whereas UV-induced mutations are expressed after DNA replication, the x-ray-induced ones are expressed before it.

544. In *Neurospora*, evidence is available indicating that x-ray-induced mutations at the *ad*-3 loci may be either point mutations (intragenic alterations) or chromosome deletions, the former type predominating at low doses and the latter type at high doses. Nearly one third and possibly one half of the point mutations involving the *ad*-3 loci may be due to base-pair changes and deletions.

V. Risk estimates

545. In the 1966 report, risks of genetic effects were expressed in terms of expected frequencies of genetic changes (point mutations or chromosome aberrations) induced per unit dose; this procedure will also be followed in the present report. The following paragraphs will be devoted to an updating of some of the estimates reached in the 1966 and 1969 reports of the Committee in the light of recent advances in radiation genetics and human population cytogenetics (48, 189, 249, 353, 448, 461, 480, 481).

546. Estimates of the genetic damage for the mouse will first be reviewed and the meaning and the significance of such estimates for man will then be discussed. An estimate of the risks in terms which may be related to the incidence of genetic disorders in man will also be given. Attention will be focused on the germ-cell stages most at risk, namely, spermatogonia and oöcytes. For the mouse, unfractioned x-ray exposures at high doses and dose rates are taken as the standard condition and the effects of other types of treatment are considered in relation to this. For man the risk estimates are based on expected rates at low doses and under conditions of chronic exposure (see paragraph 579).[12]

A. Rates of induction of different kinds of genetic damage in the mouse

1. *Dominant lethals*

547. The rate of induction of dominant lethals following acute x-irradiation of spermatogonia can be

[12] The terms "acute" and "chronic" will be used to denote irradiation at high and low dose rates, respectively.

estimated from four sets of data (288, 474, 507, 510). Each set gives a different estimate of post-implantation mortality (used here as an index of dominant lethality)[13] ranging from 4.0 10^{-5} per rad (507) to slightly more than three times this figure (474)[14] with a mean value of 8.6 10^{-5} per rad.

548. In making these estimates, three assumptions have been made, namely, (*a*) the dose-response curve for the induction of events leading to dominant lethality is linear. This seems fairly reasonable since it has been demonstrated that almost all dominant lethality induced in spermatogonia is due to secondary causes arising from induced translocations and that the dose-response curve for the latter is linear; (*b*) the frequency of cells carrying 0, 1, 2, etc. transmitted lethal effects follows a Poisson distribution; and (*c*) the post-implantation losses observed in the controls are due to dominant lethals, although the relative proportions of these losses that are due to genetic and non-genetic causes are not known.

549. Data are insufficient to determine risks of induction of dominant lethals under other conditions of irradiation (low dose rate, fractionation procedures, high LET etc.) but it can be presumed that the response of the dominant lethals will be similar to that of translocations (described in paragraphs 552-556). The study of Sheridan (510) in which a total exposure of 275 roentgens was delivered in 55 daily fractions of 5 rads each (spermatogonial irradiation) shows that the frequency of induced post-implantation losses is less than one tenth of that obtaining after acute irradiation. This observation suggests that the risk may be considerably reduced with such fractionation procedures and is supported by the findings with respect to translocations (paragraph 72).

550. No new data are available for estimating the rate of induction of dominant lethals in female mice. Based on the results given in table 3 the dominant lethal rate for oöcytes can be estimated to be about 0.9 10^{-3} per rad of acute irradiation. This estimate is in line with the conclusion drawn in the 1966 report from the data of Bateman (37) for spermatogonial rate and those of Edwards and Searle (122) for the rate in dictyate oöcytes, namely, that the dictyate oöcytes are more sensitive than spermatogonia by a factor of about 10 to 20.

551. The above difference between oöcytes and spermatogonia may well result from the fact that chromosomes damaged in oöcytes, i.e. during meiosis, have a much higher probability of being transmitted than those damaged at a premeiotic stage, as in spermatogonia. As discussed in paragraph 11, unbalanced chromosome changes induced in spermatogonia are practically all eliminated before meiosis; in metaphase-I oöcytes of irradiated female mice, on the other hand, chromatid breaks and acentric fragments (changes that may result in dominant lethality) have been observed (482).

[13] Strictly speaking, the total mortality due to both pre- and post-implantation losses should be used as an index of dominant lethality; however, only the latter can be compared in the four sets of data. Furthermore, the actual extent of pre-implantation mortality is difficult to assess though its magnitude is known to be small (275). Moreover, pre-implantation mortality is of no consequence from the standpoint of genetic risks. Consequently, in the present section, only post-implantation losses are used to compute dominant lethal rates.

[14] Experiments 5 and 6 of Schröder (474) have been omitted since a different mouse strain had been used. In addition, in these experiments, control and irradiated males were mated at different ages.

2. *Translocations*

552. The rate of induction of translocations can be estimated for the mouse using two kinds of data, namely, those from semi-sterility tests and those from cytogenetic studies of spermatocytes. For purposes of risk estimation, the most pertinent data are the confirmed cases of inherited semi-sterility. The spontaneous frequency of semi-sterility is 10.4 10^{-4} per gamete (275). For the radiation-induced rates, the most relevant data are those obtained by experiments in which heritable semi-sterility is recorded and confirmed cytologically in the offspring of males given two 600-roentgen exposures eight weeks apart. The rate that can be estimated from these data after correction for controls is 0.33 10^{-4} per gamete per rad (139, 288, 477).

553. The frequency of spontaneous reciprocal translocations detected cytologically in primary spermatocytes is very much lower than the frequency of spontaneous semi-steriles mentioned in the previous paragraph (258, 283, 488, 492). This suggests that most of the reciprocal translocations identified as spontaneous semi-steriles must arise in the male germ-cell line subsequent to meiosis or in the female germ line (137). Consequently, the frequency of translocations observed in spermatocytes cannot be used in the computation of risks.

554. On the other hand, the induction rate can be used since the expected frequency of semi-steriles can be computed from the frequencies observed in spermatocytes. The data presented in paragraphs 45-47 would indicate that in the 25-600 roentgens range the frequency of induction in spermatogonia is linearly related to the exposure mean rates, as measured in spermatocytes, being 2.0 10^{-4} per rad. From this, the expected reduced rate of translocations (semi-steriles) among live-born can be estimated to be 0.5 10^{-4} per rad. In the experiments of Ford *et al.* (139) involving two 600-roentgen exposures, the observed frequency was only about one half of the expected value (paragraph 94). This leads to an estimate of 0.25 10^{-4} per rad and is in good agreement with that of 0.33 10^{-4} per rad from genetic experiments.

555. Translocation frequencies after chronic gamma-irradiation are only about one ninth of those after acute x-irradiation (491). Although Léonard and Deknudt found no divergence from linearity in the relationship between translocations yield and x-ray exposure down to 25 roentgens, some of the evidence from fractionation experiments (paragraph 72) suggests that the rate of induction may be reduced after a small single dose. At low exposure levels, fission neutrons are nearly four times as effective as acute x rays for translocation induction (492).

556. Although observations on sons of x-irradiated females (435, 482) suggest a very low frequency of translocation-induction (1/705 with semi-sterility after 300 R or 400 R) those on daughters present a very different picture (8/293 with proven or presumptive semi-sterility). The over-all rate is about 0.3 10^{-4} per rad, which is very similar to that for spermatogonial x-irradiation. No estimates of relative rates under other conditions are possible at present.

3. *Sex-chromosome loss*

557. As L. B. Russell (428) has shown, the highest frequency of X-chromosome loss is found after

irradiation of the fertilized egg at the pronuclear stage. The frequency after spermatogonial irradiation does not differ significantly from control values (paragraph 138). For acute x-ray exposures of late dictyate oöcytes, the induced rate is 15 10^{-6} per rad; for gamma-ray exposures at 0.6 R min^{-1}, the figure is 6.5 10^{-6} per rad (449, 452).

558. Little information is available on X-chromosome loss after exposure of female mice to fission neutrons but a high RBE is indicated.

4. *Point mutations*

(a) *Specific-locus mutations*

559. Five sets of data are available for estimating the rate of induction of recessive mutations in adult spermatogonia at exposures of 300 and 600 roentgens (283, 395, 440, 446). An over-all estimate of 1.7 10^{-7} per locus per rad is obtained by giving equal weight to each locus in the calculations. With chronic gamma irradiation, the rate is reduced by a factor of three to four. Although there are no direct data as yet on rates in spermatogonia at low x-ray exposures, the results of fractionation experiments (285)[15] suggest that these will be reduced by a factor of about three under these conditions as well. However, with acute (up to 100 rad) and chronic (220 rad) fission-neutron-irradiation, the rates are increased by a factor of about six, there being no dose-rate effect at low doses (e.g. $\sim$ 60 rad) and a reverse dose-rate effect at high doses.

560. The induced rate at high acute x-ray exposures (400 R) in mature mouse oöcytes can be estimated at 5.4 10^{-7} per locus per rad or 5.5 10^{-7} per locus per rad, depending on which control frequency is used for correction[16] (paragraphs 144-146). At an exposure of 50 roentgens, the rate is either one third or one fifth of this, again depending on the assumption regarding the control frequency.[17] These rates apply to oöcytes sampled within seven weeks after irradiation; in later samplings, hardly any mutation is induced. With high doses at low dose rates, the rate is reduced by a factor of about 20.

561. It should be kept in mind that specific-locus mutations may involve more than one functional unit. With x- and gamma-irradiation of oöcytes and post-spermatogonial stages, and with neutron-irradiation of all stages, there are clear and not infrequent examples of the mutation consisting of a small deficiency affecting both of the closely linked *d* and *se* loci. With x- and gamma-irradiation of spermatogonia, deficiencies of even this small size are rare. Nevertheless, even under these conditions there is evidence from complementation tests that at least some of the mutations involve more than one functional unit (425).

562. The results of various experiments, with both male and female mice on the effect of age at irradiation, indicate no marked increase in mutational hazard over that determined for young adult animals. In fact, in males, all ages tested (namely, older adults, infants, new-born, two fœtal stages and embryos) give mutation frequencies below that for young adults, although only in new-born and 13½-day-old fœtuses is the reduction statistically significant.[18]

563. In new-born females and 17½-day-old female fœtuses, there is a marked and statistically significant reduction compared with the mutation frequencies in young adults. In the former, the rate is reduced by a factor of about six, in the latter by a factor of nearly eight.

564. The data from experiments involving protracted fast-neutron-irradiation of embryos suggest that the risk might be reduced by a factor of about two, relative to that after similar irradiation of adults but at a higher dose rate (0.17 rad min^{-1}).

565. There are, however, two striking qualitative differences between the results from adult females on the one hand, and new-born and fœtal females on the other. Firstly, whereas fertility persists after acute exposures of 300 roentgens to new-born and 200 roentgens to fœtal females, adults given these exposures become sterile after one or two litters. Secondly, whereas adults given doses or dose rates low enough to permit extended fertility have zero or near-zero mutation rates in offspring conceived more than seven weeks after irradiation, the mutations from the new-born and fœtal females come from conceptions occurring at much longer intervals. This is also true in the case of protracted neutron-irradiation of the embryos discussed in the preceding paragraph (448).

(b) *Sex-linked lethals*

566. The results of Grahn *et al.* (153) on sex-ratio changes at birth (following 500 R to P_1 spermatogonia) were discussed in paragraphs 204-207. He interpreted his results as being due to the induction of sex-linked lethal equivalents. However, similar significant changes in sex-proportion which were observed by Searle (477) and Lüning and Sheridan (279) seemed to result mainly from the action of factors other than sex-linked lethals. These and other uncertainties preclude the use of the data cited above to make reliable risk estimates for sex-linked lethals.

(c) *Autosomal recessive lethals*

(i) *Spermatogonial x-irradiation in one generation*

567. The best data currently available from which risk estimates for the induction of autosomal recessive lethals in mouse spermatogonia can be obtained are those summarized by Lüning and Searle (275) who estimated the spontaneous rate for lethals acting *in utero* as 29 10^{-4} per gamete with an upper 95 per cent confidence limit of 65 10^{-4} per gamete. Averaging results from the four sets of data presented, the authors have estimated the induced rate as 0.9 10^{-4} per gamete per rad (see paragraph 213).

568. Since autosomal lethals are included among specific-locus mutations, it can probably be assumed that the response of the former group (see preceding

[15] 600 rads (gamma) delivered in 60 daily fractions at 17 rad min^{-1} to spermatogonia.

[16] The figure of 5.4 10^{-7} is obtained assuming a control frequency of 7 mutations in 202,812 offspring; that of 5.5 10^{-7} is obtained if, instead, the control frequency is assumed to be 2 in 202,812 offspring. For details see paragraphs 144-146.

[17] A figure of 1.8 10^{-7} is obtained by using the lowest control frequency and of 1.1 10^{-7} is obtained by using the highest control frequency.

[18] For new-born irradiated on day of birth, the rate is less than one half of that in similarly irradiated adult males (paragraph 174). For 13½-day-old fœtuses the estimated low rate of 4.7 10^{-8} per locus per rad, however, might have been due to strong germinal selection (paragraph 179).

paragraph) to the various modifying factors will not differ greatly from that of the specific-locus mutations.

569. No data are available as yet for estimating the rate of induction of recessive lethals in females.

(ii) *Spermatogonial x-irradiation over several generations*

570. In their paper, Lüning and Searle (275) did not consider data from population studies involving irradiation of mice or rats over several generations on the valid grounds that (*a*) there were no precautions to exclude semi-sterile animals, with the consequence that the results may show considerable variation and (*b*) consecutive generations are not independent of each other. Nevertheless it is worth noting that the estimates derived from the study of these irradiated populations (table 22) are of the same order of magnitude as the upper limits discussed in the previous paragraphs.

(d) *Dominant mutations*

571. A limited amount of data is available on the induction of dominant visible mutations after acute irradiation of mouse spermatogonia (275). Among 184,972 control mice examined, three dominant visible mutations were observed, giving a spontaneous frequency of about 81 10^{-7} per gamete (the number of tested gametes is taken to be twice the number of mice). The data from radiation experiments after correction for the above control rate give an induced rate of 5 10^{-7} per rad per gamete for this type of mutation. This value is an obvious underestimate of the total dominant mutation rate because it includes only easily visible traits.

572. Dominant mutations affecting the skeletal system have been studied by Ehling (124, 125) whose data on the effects of spermatogonial x-irradiation yield an estimated rate of 1.1 10^{-5} per gamete per rad, the control frequency being 2.9 10^{-4} per gamete.

573. Whenever it has been possible to compare the effects of varying the conditions of irradiation on the incidence of specific-locus and dominant visible mutations, the responses of these two categories of genetic damage have been very similar. Therefore, the risks associated with dominant mutations are likely to be similar to those for specific-locus mutations.

574. These and other estimates discussed in the preceding paragraphs are set forth in table 28.

B. Applicability of the mouse estimates to other mammals

575. The applicability of the mouse estimates discussed in the preceding paragraphs to other mammalian species including man depends on the validity of the assumption that the radiation response of the latter is similar to that of the mouse, or at least not strikingly different from it, an assumption that has been used in the Committee's earlier reports. There still appears to be no obvious reason for rejecting the applicability of the results in mouse spermatogonia. For oöcytes, however, there may be a serious problem.

576. Studies on radiation effects on monkey, human and mouse oöcytes have clearly shown that both the monkey and human oöcytes are far less sensitive to cell killing than the mouse oöcytes (paragraphs 35, 37). The female mouse is sterilized, as a result of oöcyte killing, by doses that have no effect on the fertility in women.

577. These findings might be taken to imply that human oöcytes are also far less sensitive than mouse oöcytes to mutation induction. However, other evidence shows that no simple deduction of this kind is possible. In the mouse, irradiated at high doses and high dose rates, the mature dictyate oöcytes are resistant to killing, but sensitive to mutation-induction whereas the reverse appears to be true for immature dictyate oöcytes under these conditions. However, at low dose rates (which are particularly relevant from the stand-point of genetic risks to irradiated women) the mature dictyate oöcytes are not only resistant to killing, but also show extremely low mutational sensitivity.

578. These findings thus underline the need for caution in extrapolating from one species to another and from one measured end-point of radiation damage to another; however, the use of data from the genetically most sensitive stage in mouse females to estimate risks in human females should not lead to any underestimate of the hazards.

C. Risk estimates for man

579. Individuals in human populations generally receive low total doses of radiation during their reproductive life. These are either delivered at high dose rates (e.g., for diagnostic medical purposes) or are greatly protracted (e.g., continuous exposures from natural and man-made environmental sources). Under these exposure conditions, the rate of induction of mutations or chromosome aberrations per rad received is expected to be several times less than with high acute doses. The extent of the reduction depends partly on the kind of genetic damage and germ-cell stage involved.

1. *Point mutations*

580. In the 1966 report the risk of gene mutations for the human genome was obtained on the basis of the rate of induction per locus in the mouse (12 loci) and the number of genes that were estimated to make up the human genome. Basic to the latter estimate was the rate of spontaneous sex-linked recessive lethals in man as derived from sex-ratio changes with age over three generations (230). However, the Committee is unwilling at present to use sex-ratio changes as a basis for estimating the size of the human genome (see paragraphs 208, 209). As a consequence, there is a need to consider alternative approaches to estimate the size of the human genome. One such approach detailed below makes use of published data on the number of functional units in a defined chromosome segment of the mouse.

(a) *Size of the human genome*

581. From the stand-point of genetic fine-structure analysis, the most intensively studied chromosomal region in the mouse is the one between the *dilute* (*d*) and *short-ear* (*se*) loci of linkage group II (see paragraph 183). Two functional units — l_2 and l_3 (each lethal when homozygous) and possibly a third one affecting the size of the animal — have been identified in this region (425, 430). Since the *d* and *se* loci are

0.16 cross-over unit apart, under the assumption that this sector is fairly representative of the mouse chromosome, it would appear that there are about 20 functional units per cross-over unit.

582. It should be pointed out here that the number of functional units that are identified within a certain map length may vary depending on the segment of the chromosome analysed, as indicated by extensive data from similar studies in *Drosophila* (75, 211, 263, 264, 409). For example, in the best-studied section of the X chromosome between the loci *white* (*w*) and *zeste* (*ze*) spanning a distance of 0.5 cross-over unit, 12 functional units have been mapped; in the region surrounding *rosy* (*ry*: chromosome III) with 0.5 map unit, 17 functional units have been defined; there appears to be 34 such units in the vicinity of *maroon-like* (*ma-l*) with a recombinational span of slightly longer than 1.5 cross-over units.

583. The entire *Drosophila* genome is 280 (cross-over) units long (267) and the total number of bands in salivary chromosomes is 5,161 (41). Since, at least in the chromosomal regions intensively studied (41, 211, 237, 409), there is a one-to-one relationship between functional units and salivary chromosome bands, it can be estimated that in *Drosophila* too, the number of functional units per cross-over unit is around 20. A consideration of the above sets of information in conjunction with that available for the *d-se* region of the mouse makes us feel reasonably confident that the figure of about 20 functional units per cross-over unit is probably not an unrealistic estimate for the mouse.

584. From the recent linkage map of the mouse published by Green *et al.* (159) it appears that the total number of cross-over units between end-markers in known linkage groups is 1,054. This figure is clearly an underestimate since linkage group XIX has not yet been found and XV is represented only by two very closely linked markers. Allowing for these, it can be presumed that the genetic length of the mouse genome is of the order of about 1,250 map units. Multiplying 1,250 by 20 (the latter being the number of functional units per cross-over unit) one gets a figure of 25,000 as the number of functional units capable of mutating.

585. The estimated number of nucleotide pairs per diploid cell is $4.7\ 10^9$ in the mouse and $5.6\ 10^9$ in man (581).[19] When this difference is taken into account, one arrives at a figure of about 30,000 functional units as the size of the human genome.

586. The figure of 30,000 functional units, estimated as the size of the human genome, is in agreement with that of Muller (328) who arrived at the same figure using other data, is within the range obtained by the Committee in its 1966 report, and one and a half times that used there for computing the total risk from the induction of point mutations.

(b) *Total rate of induction of recessive point mutations*

587. There are at least two ways to estimate the total rate of induction of recessive mutations. If the rate of induction of specific-locus mutations in male mice (spermatogonial rate) assumed to apply to man is multiplied by the estimated size of the human genome, the resulting estimate of total risk of point mutations in the male is $0.5\ 10^{-7} \times 30{,}000 = 1{,}500$ per million gametes per rad under conditions of chronic x-irradiation. Since, as pointed out earlier (paragraph 561), specific-locus mutations may involve more than one functional unit the total rate given above may be an over-estimate.

588. The estimated rate for recessive lethals (acting *in utero*) per gamete per rad in mice (spermatogonia) is 30 per million. Correcting for the 20 per cent greater size of the human genome and assuming that the corresponding rate will apply to man, one arrives at the figure of 36 per million. As studies of specific-locus mutations indicate that the proportion of prenatal lethals averaged over the loci is less than one half of the total mutations (449), this estimate must be considered as an underestimate of the total risk of point mutations.

589. In females the risk is expected to be very low under conditions of chronic irradiation at low-dose levels.

590. The nature of the damage measured by the total rate of induction of recessive mutations is difficult to assess, or to express in terms of individual or collective hardship. Data from *Drosophila* would suggest that induced "recessive" mutations have a considerable degree of semi-dominance, adversely affecting the fitness of heterozygotes in terms of fertility, viability, etc. to the extent of 2 to 5 per cent. However, many of the types of adverse effect likely to be important in man can hardly be studied in experimental animals. So an accurate measure of the heterozygous effects on human fitness of newly arisen recessive mutations can only be obtained from studies on man himself. In the mouse, the evidence accumulated so far suggests that these heterozygous effects are smaller than in *Drosophila* (paragraph 219); the same may be true of man. However, it is possible, as Green (155) has remarked, that the right indicator traits have not yet been found. If, for computational purposes, the 2-5 per cent range is accepted as applying to man, at least as an upper limit, it can be expected that 30-75 or 1-2 mutations per million male gametes per rad will be expressed in the first generation after exposure, depending upon whether 1,500 or 36 mutations per male gamete per rad are induced (paragraphs 587, 588).

(c) *Dominant mutations*

591. In the 1966 report it was assumed that the part of the human genome responsible for some 50 dominant traits most commonly observed and easily detected consists of at least 50 loci and is unlikely to consist of as many as 500. However, McKusick's compendium (301) of Mendelian traits in man now lists over 400 well demonstrated dominant traits and over 500 more for which the evidence is incomplete. There is good reason to predict that the number will not be less than 1,000 based on the progress of research in this area.

592. The rate of induction at high acute doses of dominant visible mutations in mouse spermatogonia has been estimated as $4.96\ 10^{-7}$ per gamete per rad (275). At low doses and dose rates it is probably one third of this (on the basis of specific-locus findings). About 75 loci are now known in the mouse which have mutated to visible dominant traits. Therefore an upper estimate of the mutation rate per locus to dominant

[19] Vogel (582) has assumed that the haploid chromosome set of man contains about $3\ 10^9$ nucleotide pairs.

visibles is $\frac{4.96\ 10^{-7}}{3 \times 75} = 2.2\ 10^{-9}$. If this rate is multiplied by the assumed number of loci that determine dominant traits in man, an over-all rate of two dominants per rad per million is obtained.

593. A presumed class of dominant mutations is constituted by those that cause dominant skeletal damage in the mouse (paragraphs 197-198). The data of Ehling (124, 125) show that at high doses and high dose rates, the rate of induction is 1.1 10^{-5} per rad per gamete (spermatogonial irradiation). Proceeding on the empirical assumption that the response of the skeletal mutations to low doses and dose rates will be similar to that of specific-locus mutations, one can presume that the rate may be 4 per million under these conditions.

594. So far the transmission of only a few skeletal mutations has been studied (paragraph 198). It seems probable that most of the presumed dominant skeletal mutations may be heterozygous manifestations of recessive mutations. Therefore, they have been placed in the appropriate category (recessive mutations with heterozygous effects) for considering risks.

2. *Chromosome aberrations*

(a) *Translocations*

595. The rapid progress of human cytogenetics since the publication of the 1966 and 1969 reports of the Committee has increased our knowledge on the spontaneous incidence and genetic properties of structural rearrangements, especially on translocations (119, 138, 169, 189). Since information on these is quite relevant for the assessment of the over-all risk due to induced translocations in terms of (*a*) the likelihood of transmission to first generation progeny; (*b*) the risk of transmission to subsequent generations and (*c*) the risk of abortion and of birth of congenitally-malformed children, it is necessary to review the recent advances in this field.

596. Almost all of the data on the incidence of translocations in man have been obtained from studies on somatic cells (lymphocytes). Since only those translocations involving the exchanges of parts of chromosomes of very different lengths (unequal exchanges) are detectable in this type of material, such rearrangements may well represent only a small proportion of the total "translocation load". Exchanges of approximately equal chromosome segments would be undetected and it would appear that depending on the techniques employed, a smaller or a larger proportion of them are missed.[20] Although this limitation is likely to be overcome in the near future,[21] it should be stressed that the data currently available on the frequencies of translocations in human populations can only provide lower limits of the estimates.

597. The majority of spontaneously-occurring translocations recorded in man are Robertsonian translocations (combination of two acrocentric chromosomes resulting in one metacentric chromosome so that the chromosome number in the heterozygote is reduced by one), the remainder being reciprocal translocations identified in the somatic chromosomes through the observation of one chromosome shorter than normal and of a second chromosome longer by the same amount.

598. Because of the nature of the rearrangement, the number of possible types of Robertsonian translocations is limited and these types can all be detected in somatic cells. In contrast, the breaks leading to the production of reciprocal translocations can occur at many points on any of the chromosomes, with the result that there are a large number of theoretically possible types. Because of this, each reciprocal translocation may be considered for all practical purposes as being unique in terms of the kind and amount of chromosome material involved and may therefore also be unique in terms of its behaviour at meiosis (190). However, because of the relatively small number of families thus far studied, it is not realistic at present to treat any particular translocation separately.

599. Robertsonian translocations occur with an over-all frequency of about 8 per 10,000 births (paragraph 602). Some rare types of Robertsonian translocations (between homologues) carry a 100 per cent risk of producing unbalanced progeny; some others—t(Dq 21q), t(21q 22q)[22]—produce trisomy 21 with a frequency that varies with the sex of the carrier. The more frequent type t(13q 14q) is associated with a relatively low risk ($\sim$ 5 per cent) of producing unbalanced progeny (119). Data from population surveys (190) suggest that a certain proportion of those Robertsonian translocations between non-homologous chromosomes may be transmitted with a low or even a zero risk of producing unbalanced progeny.

600. Robertsonian translocations have been found in the mouse (133, 253) and the recent discovery of a wild population (*Mus poschiavinus*; the tobacco mouse) with no less than seven pairs of metacentrics (162) shows that these translocations may have evolutionary importance (137). However, all the above-mentioned Robertsonian translocations are of spontaneous origin and there is no evidence so far for their induction in mouse germ cells (481).

601. In the mouse it has been established that the predominant type of radiation-induced structural change is represented by reciprocal translocation. If this reflects a general property of chromosomes rather than a species peculiarity the same is likely to obtain in man also.

(i) *Rates of incidence and origin of structural rearrangements*

602. Surveys of the chromosomal constitution of consecutive live-born hospital births have been undertaken in several laboratories (for recent summaries of the data, see references 169 and 189). The chromosomes of peripheral blood leucocytes from a total of 21,996 babies have been examined and 114 of them (0.52 per cent) found to have an abnormal constitution.[23] A total of 37 babies (0.17 per cent) were found

[20] Evans has estimated that the efficiency of scoring symmetrical rearrangements in cultured human lymphocytes following irradiation may be as low as 20 per cent (92). Jacobs *et al.* (191) consider that the efficiency is about 25 per cent.

[21] The recent technical advances in identifying chromosomes from banding patterns produced with fluorescent dyes (67) or by one of a variety of Giemsa techniques (118) holds a great deal of promise of making possible the identification with a high degree of precision of the chromosomes involved in translocations.

[22] t = translocation; the numbers of 13, 14, 21, 22 denote the chromosome involved; D refers to a chromosome of group D; q denotes a long arm.

[23] Calculations based on Jacobs (189) and Hamerton (169).

to have a structural abnormality of the autosomes, namely, 13 (0.06 per cent) had reciprocal translocations, 17 (0.08 per cent) had Robertsonian translocations (14 D/D and 3 D/G) and 7 (0.03 per cent) had unbalanced rearrangements.

603. Jacobs *et al.* (191) have recently estimated that the mutation rate for all structural rearrangements of the autosomes which result in live-births is about $4\ 10^{-4}$ per gamete per generation composed of about $2.8\ 10^{-4}$ balanced and $1.2\ 10^{-4}$ unbalanced rearrangements. They consider that the figure of $4\ 10^{-4}$ must be a serious underestimate of the true rate for at least two reasons: the first is that only a fraction of all chromosome rearrangements in man is detectable in preparations of somatic cells; the second is that many aberrations may be selected against before birth.

604. As mentioned earlier (paragraph 602) unbalanced structural rearrangements of the autosomes are infrequent in neonatal surveys (only 7 in 21,996 babies). An examination of the transmission data obtained from these surveys and from other sources summarized by Jacobs (189) and by Dutrillaux (119) suggest that between one half to two thirds of the non-mosaic unbalanced structural rearrangements arise *de novo*, the remainder being familial. In those with an affected parent, the mother is about two to three times more likely to have an abnormal constitution than the father.

(ii) *Genetics of reciprocal translocations*

605. The great majority of families with a reciprocal translocation have been ascertained through an index case who carried an unbalanced form of the translocation. In the two earlier analyses (138, 244), it was found that the ratio of zygotes with normal genomes and with balanced translocations to those presumed to be carrying the unbalanced form of the translocation departed from 1 : 1 with a significant deficit in the latter class. More recent and extensive analysis involving much larger material (comprising 200 families, conception histories of 330 couples, 903 live-born and 246 abortions) confirmed the above observation (119).

606. One hundred and fifty of these families were ascertained through abnormal probands with unbalanced karyotype; in 105 of these, the translocation was transmitted through the mother and in the rest, through the father. The calculated frequency of unbalanced children is 19 per cent in the progeny of male as well as in those of female carriers.

607. Among the phenotypically normal children, one half had normal karyotype and the rest carried the translocation in the balanced form. The frequency of abortions is 22 and 16 per cent in the progeny of female and male carriers, respectively. Although, at face value, the abortion frequencies recorded above do not represent striking increases over the level in the general population (around 15 per cent, see reference 606) they are significantly higher than the 10 per cent for control samples (the progeny of normal people related to these families).

608. When the carrier parent is female, the mean number of children is 2.77 whereas with the male carrier, it is reduced to 1.96. The latter figure is also lower relative to the mean number of children (2.92) in control samples (individuals related to the families under study but with normal karyotypes). The sex-dependent difference in selective values may, at least in part, explain the relatively lower ascertainment through an abnormal proband born to male carriers (paragraph 606).

609. When a familial translocation is ascertained through a balanced proband, it is found that (*a*) the ratio of balanced carriers to normals among the progeny of carriers does not differ significantly from unity (as in the situation outlined in paragraph 607); (*b*) the risk of producing unbalanced progeny must be close to zero for both male and female heterozygotes since no individual has been found with an unbalanced form of the translocation, in spite of the substantial number of individuals studied (thus differing from the situation when ascertainment is through an unbalanced proband).

610. It thus appears that the method of ascertainment of the majority of reported translocations is biased in favour of detecting those translocations which give rise to genetically unbalanced but viable offspring. Therefore any estimate of future risks to carriers based on families ascertained through an unbalanced proband is not applicable to translocations detected via a balanced carrier. It may be that the two methods of approach (ascertainment through unbalanced and balanced probands) tend to detect different types of translocations. This hypothesis seems to be supported by the observation of differential risks depending on the method of ascertainment.

611. The virtual absence of progeny with unbalanced products of segregating translocations where there is ascertainment through a balanced proband has raised the question of whether unbalanced products are generated at all and, if they are, whether the resultant gametes are selected against. The significant deficit of abortuses plus congenitally abnormal children where ascertainment is through an unbalanced proband raises similar problems. However, a consideration of the behaviour of mouse translocations helps to elucidate them.

612. Data from the mouse suggest that unbalanced products of balanced translocations do arise in meiosis at expected frequencies and show normal transmission. However, most of them produce lethality around the time of implantation although in some this occurs a little later and only a very small minority survive to produce viable progeny (481).

613. If the situation in man is similar and if most unbalanced products cause death of the resulting zygotes around implantation, this would at most result in a missed menstrual period for the mother and consequently would not be diagnosed as pregnancy. This means that no striking increase in the frequency of abortions would be expected.

614. If the zygotes resulting from unbalanced gametes are eliminated before pregnancy is identifiable, a slightly larger mean interval between births would be expected in the case of matings between translocation heterozygotes and normals.[24] The evidence of Jacobs (188) of no difference in mean birth interval is hardly sufficient to rule out this possibility.

615. Notwithstanding these considerations, those unbalanced products that produce viable but abnormal

[24] To what extent the adoption of birth-control measures may mask or distort this difference cannot be estimated.

children (which may be in a small minority) constitute a group associated with the greatest social load. The frequency of such translocations cannot be estimated with any accuracy at present.

616. An upper estimate of the number of viable but chromosomally unbalanced live-born relative to the total unbalanced zygotes conceived may be derived from the data on spontaneous abortions (43, 59). Firstly, Carr (61) suggests that 45 per cent of all conceptions spontaneously terminate before birth. Only one third of these (15 per cent) are recognized as abortions. The remaining two thirds occur so early as to go undetected.

617. It has been shown (43, 59) that 8 out of a total of 747 abortions analysed cytologically, or 1.07 per cent, were unbalanced or aneuploid as a consequence of structural rearrangement. Assuming that this frequency will also be found in the undetected class, it can then be estimated that 0.48 per cent of all conceptions (0.0107×0.45) end as a result of unbalanced structural rearrangements. Since, as mentioned in paragraph 602, 0.03 per cent of all live-born carry unbalanced translocations, it follows that about 6 per cent of all conceptions with a structurally unbalanced chromosome complement will survive birth (i.e., $\frac{0.0003}{0.0048 + 0.0003} \times 100$).

(iii) *Risks from radiation exposure*

618. While there is no direct information on the induction of translocations in human germ-cells, the recent data of Brewen *et al.* (48) on five different species of mammals demonstrate that the rate of induction of dicentrics in lymphocytes is proportional to the number of chromosome arms. Their data show that there are twice as many dicentrics in human (arm number = 81) as compared to mouse (arm number = 40) lymphocytes at each of the six x-ray levels studied (50, 100, 150, 200, 300 and 400 rad).

619. The above data permit the inference that the induced translocation frequency in human gametes will be twice that obtained for the mouse. With acute irradiation at high doses, the rate of induction in mouse spermatogonia and dictyate oöcytes is of the order of $0.3\ 10^{-4}$ per gamete per rad.[25] Therefore for man, the expected value under these conditions is $0.6\ 10^{-4}$ per gamete per rad. For low-dose acute x-irradiation, the rate is likely to be one quarter of this (i.e., $1.5\ 10^{-5}$) and for chronic gamma-irradiation about one ninth (i.e. $0.7\ 10^{-5}$). The rates in females under both conditions is expected to be very low, but no estimates can be given.

620. It follows from this that if males are exposed to low-dose acute x-irradiation, the expected number (per million progeny per rad) of balanced and unbalanced translocation-carrying zygotes in the F_1 will be 15 and 30, respectively. The corresponding figures for chronic gamma-irradiation will be 7 balanced and 14 unbalanced zygotes per million per rad.[26]

621. Assuming further that only about 6 per cent of the unbalanced products (and this is likely to be an over-estimate) results in children with multiple congenital anomalies (paragraph 617) about two malformed children per million would be expected from males exposed to low-dose acute x-irradiation. After chronic gamma-irradiation, however, only one malformed child will be expected. One third of the remaining unbalanced zygotes after either of these two types of exposure would fall into the recognized abortion category whilst the other two thirds would die so early as to go undetected.

622. In all the above considerations the "load" due to spontaneously-occurring translocations and their unbalanced products has not been considered. The toll due to the induced translocations will be over and above that occurring spontaneously and consequently the figures for multiple congenital anomalies and abortions given above are to be considered as "increment" over the spontaneous level.

623. Assuming that translocation carriers contribute an equal number of zygotes to the next generation as non-translocation carriers, then the 15 balanced carriers of translocations per million resulting from paternal exposure to low acute x-ray doses will give rise to 7.5 zygotes per million with balanced translocations and to 15 zygotes per million with unbalanced translocation products in the next generation.[27] For chronic gamma-irradiation the frequency of zygotes with balanced translocation products will be 3.5 and 7 per million, respectively. However, the carriers may well contribute more zygotes than normal to the next generation because of early losses of unbalanced genomes, in which case their numbers would be increased. The unbalanced F_1 zygotes will, of course, not contribute to the next generation.

624. Assuming as before that 6 per cent of the unbalanced genomes survive to produce congenitally abnormal children, there will be about one such child per million after low-dose acute irradiation,[28] or one per 2 million zygotes (chronic gamma-irradiation) that can be attributed to causes stemming from reciprocal translocations.

625. The risks outlined above may be influenced by the selective values of the different translocations and by those depending on the sex of the carrier parent.

626. The formulation of risks to generations beyond the second is considered premature at this time.

627. It has been assumed that translocation induction per rad in human spermatogonia and oöcytes is twice that of the same stages in the mouse. Particularly needed is information (currently not available) on the question as to whether the human oöcyte more closely resembles the mature dictyate oöcyte (as has been assumed hitherto) or the immature dictyate oöcyte from which virtually no mutations have been recovered.

(b) *Loss of X chromosome*

628. The available mouse data (paragraph 557) suggest that the frequency of induction of X-chromo-

[25] Estimate based on semi-sterility data in mice.

[26] The contribution from exposed males to the F_1 translocation load is estimated on the assumption of 1 : 1 : 2 ratio of normal to balanced to unbalanced gametes. Therefore, if the rate of induction in the parental generation under acute irradiation at low dose is $1.5\ 10^{-5}$ per rad, there should be 15 balanced carriers per million progeny and twice this number would have unbalanced genomes. The comparable figures for chronic gamma-irradiation would be 7 balanced carriers and 14 unbalanced genomes.

[27] Each balanced translocation heterozygote irrespective of sex produces gametes in the ratio of 1 normal : 1 balanced : 2 unbalanced. If 15 per million is taken as the figure for carrier gametes, the frequency of unbalanced gametes will be 30 per million. The figure for the zygotes will be 7.5 million balanced, and 15 per million unbalanced.

[28] $6\ 10^{-2} \times 15\ 10^{-6}$.

some losses in spermatogonia is not significantly above that in controls, although in dictyate oöcytes the risk is higher (15 10^{-6} per rad per gamete) at high dose rates and reduced by a factor of at least two at lower dose rates.

629. Since about 7 per cent of spontaneous abortions in man are associated with the loss of the X chromosomes (189), and since the normal level of spontaneous abortions in man is about 15 per cent (paragraph 607) it can be concluded that about 1 per cent of all recognized conceptions terminate as abortions due to loss of the X chromosome. The data from neonatal surveys indicate that the frequency of individuals with Turner's syndrome due to the 45,X karyotype is very low, suggesting that a predominant majority of XOs are inviable (189, 575).

630. On the basis of mouse data it can be assumed that low dose-rate irradiation of human spermatogonia and oöcytes will result in the production of about eight additional XO zygotes per rad per million progeny. If almost all of them are lost as abortions, then they should be added to those resulting from the induction of reciprocal translocations.

631. So far, there is no evidence that XXY, XYY or other types of sex-chromosomal aneuploidy have been induced by irradiation of mouse germ cells.

(c) *Other chromosomal anomalies*

632. Most, if not all, types of autosomal aneuploidy seem to act as dominant lethals in the mouse, since very few possible examples have been reported from examination of juvenile or adult individuals. The same is probably true of polyploids and of large duplications and deficiencies. Since the dominant lethality arising after spermatogonial irradiation seems to be largely, if not entirely, accounted for by the induction of reciprocal translocations (paragraphs 9-11) the extra risk of induction of these other types of gross chromosomal aberration is probably small.

633. In the present state of our knowledge, however, it is not possible to give individual risk estimates for these different categories of chromosomal change. This is also true for small deletions and duplications. It is known that both of these categories can lead to the production of viable heterozygotes (282, 453), although known duplications in the mouse usually cause sterility. Known small deletions (i.e., those involving the *d* and *se* loci) are lethal in the homozygotes and are therefore included in the category of autosomal recessive lethals. The proportion of recessive lethals falling into this category is unknown, although it is known that *d-se* mutations are very rarely recovered from x- or gamma-irradiation of spermatogonia. There is a greater probability of transmission of autosomal aneuploidy and other types of chromosomal anomaly after irradiation of maturing dictyate oöcytes. Again, they will mainly be expressed as dominant lethals.

634. The incidence of dominant lethality after x-irradiation of maturing dictyate oöcytes is much higher than after spermatogonial irradiation and it seems likely that a substantial part of this is due to causes other than translocation induction. Since the rate of induction of X-chromosome loss in such dictyate oöcytes is estimated to be 15 10^{-6} per rad per gamete, it seems probable that the rate of induction of autosomal loss in the same germ-cell stage will be about 19 times this, i.e. 28.5 10^{-6} per rad per gamete. The rate of induction of other types of chromosomal change cannot be individually estimated at present. However, it is interesting to note that L. B. Russell (430) found that the proportion of *d-se* deficiency events among mutations at the dilute and short-ear loci was over nine times as high after unfractionated x- or gamma-irradiation of oöcytes as after similar irradiation of spermatogonia (41.7 per cent against 4.4 per cent).

D. Relation to natural incidence of genetic ill-health in man

635. This report, so far, has presented revised estimates of genetic risks as given in the 1966 report. These are expressed in terms of the number of new mutations induced per gamete per rad. Information of this kind cannot, at present, be translated directly into socially meaningful terms. It is possible, however, to express the risk in terms which relate to the observed incidence of genetic disorders now present in man. This involves knowledge of the extent to which the genetic load is maintained by recurring spontaneous mutation, as well as information on the induced mutation rates. The advantages of this approach are obvious, and it has been used by this Committee in earlier reports (573, 574). However, it is important to realize that the estimates so obtained are fraught with considerable uncertainties, particularly with regard to spontaneous mutation frequencies in man and mouse and would imply that the absolute risk of induction of genetic effects will be different in populations with different spontaneous mutation rates. In this report, the relative risk per unit dose is applied to the assumed average spontaneous incidence of genetic disorders in the world population.

636. The interpretation in terms of an actual increase of ill-health and human suffering as expressed in future generations depends on various assumptions concerning (*a*) the comparability of the nature of spontaneous and radiation-induced mutations, and (*b*) the rate at which the newly arisen mutant genes are eliminated from the population.

637. In a recent review of mutation studies in mice, Lüning and Searle (275) summarized a number of quantitative estimates by calculating the doses which would double the natural incidence of five different kinds of radiation-induced genetic damage (i.e. semi-sterility, specific-locus mutations, dominant visibles, mutations affecting the skeleton and recessive lethals). These all fall within a range of 16-51 rads averaging about 30 rads for spermatogonia exposed to high acute x-ray doses. Some of the individual estimates have very wide confidence limits.

638. With chronic exposures or with acute x-irradiation at very low doses, it can be expected that the rate of induction will be reduced by a factor of 3-4. Hence, the doubling dose under these conditions could be estimated at approximately 100 rads for males. The authors gave no doubling dose estimates for oöcytes, since very little information on spontaneous rates in females has been obtained.

639. As has been pointed out in previous reports of this Committee, about 1 per cent of all live-born suffer from conditions determined by single Mendelian factors of which a substantial proportion is dominant. The incidence of these traits is believed to be essen-

tially supported by recurring mutation. In addition another 2 per cent developing serious physical or mental abnormality is presumably also genetic in origin, but their mode of transmission is not yet clearly understood. For that reason it cannot be said with certainty to what extent these traits are maintained by mutation. A further 0.5 to 1.0 per cent result from chromosomal anomalies. In consequence the total frequency of disease maintained by mutation or resulting from chromosomal anomalies ranges from 2 to 4 per cent.

640. For computational purposes, it will be assumed that 30,000 live-born per million are affected by deleterious traits maintained by mutation. If the population is in equilibrium with respect to spontaneously-occurring mutations, this will correspond to a rate of 30,000 gene and chromosome mutations per million zygotes per generation.

641. This rate of mutations will be increased by 300 per million for each rad of low-dose or low-dose-rate radiation to the males in a parental generation, if a doubling dose of 100 rads is accepted. The great majority of these will be gene mutations with an unknown degree of dominance. If, however, the range observed in *Drosophila* (2-5 per cent) is used as an upper limit to the average dominance in man as expressed by the frequency of deleterious traits among live-born, then 6-15 affected individuals per million live-born would be expected in the first generation following irradiation, the rest of the damage being expressed in subsequent generations.

642. The fragility of the estimates obtained in this section, as well as that of the direct estimates given earlier, must be emphasized, but it is encouraging to note that the two sets are not too widely at variance if the fact is taken into account that direct estimates apply to genetic damage expressed through the whole period from conception to the end of reproductive life whereas the doubling dose has been used in such a way as to apply only to damage expressed post-natally. On the other hand, results with *Drosophila* show that mutations resulting in minor deleterious effects grossly outnumber those with severe effects (paragraphs 383-387). The calculations given here for estimating the total radiation-induced genetic damage by either of the methods employed do not take into account this class of mutations which lead to minor disability and disease. Because of the greater frequency of occurrence of these mutations, their total effects in terms of genetic burden to the population could be greater than that of a smaller number of relatively more serious conditions. There is, however, no way at present to assess their contribution to the genetic burden in man.

E. Summary and conclusions

643. This section has been devoted to an updating of the earlier conclusions of the Committee (1966 and 1969 reports) regarding genetic risk estimates for man, in the light of progress that has been made in recent years in radiation genetics and human population cytogenetics.

644. Risk estimates for the mouse are first given and those for man are discussed in this context. While those for the mouse are expressed per rad of acute x-irradiation at high doses and possible modifications expressed under other conditions, those for man are based on the conditions of radiation exposure most relevant for our species, namely, low doses and prolonged exposures. Risk estimates are summarized in table 29.

645. The estimate of the total risk from recessive point mutations has been arrived at in two ways: (*a*) using the specific-locus rate in the mouse and multiplying it by the estimated size of the human genome in terms of the number of functional units at which detectable recessive mutations arise, and (*b*) using the per genome mutation rate for recessive lethals in mice and multiplying by a factor to correct for the 20 per cent greater size of the human genome.

646. The first method gives a figure of 15 10^{-4} mutations per gamete per rad under conditions of chronic x-irradiation (0.5 10^{-7}, the rate after chronic irradiation multiplied by 30,000, the estimated number of functional units). With the second method, the total risk of recessive point mutations is 0.3 10^{-4} per gamete per rad under similar conditions (i.e. 0.25 10^{-4} multiplied by 1.2 to correct for the genome size in man). The estimate arrived at by the first method is to be considered as an upper limit in that it is based on specific-locus mutations some of which may include more than one functional unit; the second estimate is a lower limit based on recessive pre-natal lethals which are only a part of all mutations.

647. The estimate of 30,000 loci is based on that for the mouse genome (25,000) and the fact that the number of nucleotide pairs per diploid cell in man (5.6 10^9) is slightly higher than that in the mouse (4.7 10^9). The size of the mouse genome was estimated using the results of fine-structure analysis carried out on a section of the mouse chromosome—results which are consistent with those from similar studies on chromosomal regions in *Drosophila*.

648. The rate of induction of dominant visible mutations has now been estimated at about two per rad per million at low doses and dose rates. This estimate is based on the expected rate of induction of such mutations in mouse spermatogonia under similar conditions (2.2 10^{-9} per rad) multiplied by the number (1,000) of loci likely to determine dominant traits in man. Regarding dominant skeletal mutations, there have not been substantial additions to our knowledge that would warrant revising the risk estimates made in the 1966 report.

649. The predominant risk from radiation-induced chromosome aberrations in the mouse is constituted by reciprocal translocations. It is possible that in man the hazards from other types of chromosome aberrations are greater than in the mouse.

650. Under the assumption that the risk of induction of reciprocal translocations in human germ cells is twice that in those of the mouse, the expected frequencies of abortions and congenitally malformed children in the first and second generation progeny have been calculated; to estimate the relative proportions of unbalanced genomes (resulting from unbalanced products of translocations) that will result in either abortions or malformed children, the extensive data available from surveys on abortions and similar data from neonatal surveys have been used.

651. With this procedure, an exposure to low-dose x-irradiation of human spermatogonia can be estimated to result in an increment of 30 zygotes per million per

rad carrying unbalanced products. Of these, about two will result in live-born, but congenitally malformed, children in the generation following that irradiated. One third of the remaining unbalanced zygotes will be lost as a result of abortions after pregnancy is identified, while the other two thirds will be lost so early as to go unrecognized. After chronic gamma-irradiation, the frequencies will be half of those just given. In the second generation, the expected frequencies of abortions and malformed live-born are 15 and 1, respectively, per million zygotes.

652. It appears that the method of ascertainment is crucial to the estimation of risk of unbalanced products of translocations. Thus, when a reciprocal translocation is ascertained through an unbalanced proband, the proportion of multiple congenital anomalies and abortions in the progeny of carriers of balanced translocations is much higher than when ascertainment is through a balanced proband. It seems likely that different types of reciprocal translocations are involved in the two methods. Further research may shed light on this problem.

653. The risk of inducing X-chromosome losses in irradiated spermatogonia appears to be very low. It is somewhat higher in irradiated oöcytes where, however, a dose-rate effect has also been found. Again these inferences are based on mouse data. The available human data indicated that about 7 per cent of all spontaneous abortions are due to X-chromosome losses, corresponding to a frequency of 1 per cent of X-chromosome losses among all conceptions. In newborn, the frequency of 45,X individuals (Turner's syndrome) is very low. If the above situation obtains in the case of radiation-induced X-chromosome losses too, then virtually all the 45,X conceptions will be lost as abortions and only a very small fraction will survive to produce individuals with Turner's syndrome. It can be calculated that chronic exposures to both sexes will produce an increment of about eight abortions per million zygotes, a frequency to be added to that resulting from reciprocal translocations.

654. Rates of induction of point mutations per unit dose of radiation have also been related to the observed incidence of genetic disorders in man. This approach has advantages but depends on a number of unproven assumptions and at present can only be applied to exposures of males.

655. Estimates of doubling doses obtained from acute x-irradiation of mouse spermatogonia all fall within a range of 16-51 rads, with a mean of about 30 rads. Under chronic exposure a value of about 100 rads would be expected, corresponding to a 1 per cent increase in mutation frequency per rad. If this figure applies to man, it can be estimated that low dose or low-dose-rate exposure of males will result in the induction of 300 new mutations per million zygotes per rad. These mutations would be expressed over several generations, with perhaps 6-15 of them becoming manifest in the first generation after exposure.

VI. Suggestions for future research in the field of radiation genetics

656. A considerable body of new information has been presented in the report on genetic effects of radiation, but the Committee feels that, for the more accurate assessment of genetic risks, further work is desirable in the following areas:

(*a*) Spontaneous frequencies of gene mutations and chromosome aberrations (especially reciprocal translocations) in human populations: more accurate estimates by the exploitation of existing methods and the development and use of new ones;

(*b*) The rates of elimination of deleterious mutations (especially of recessive lethal and detrimental mutations) from human populations; in particular, studies on the expression of these mutations in heterozygous condition are considered of importance;

(*c*) Spontaneous and induced rates of chromosome rearrangements in mammalian oöcytes;

(*d*) The over-all frequency and genetic behaviour of human reciprocal translocations, especially the extent to which their unbalanced products lead to social harm by causing death in late pregnancy or malformations at birth;

(*e*) The development of new "bridges" or points of comparison between experimental animals and man, which will allow more confident estimates of relative genetic radio-sensitivity to be made. Thus, information on the induction of mutations and chromosome aberrations in germ cells of the mouse could be used for risk estimates with greater confidence when comparative studies on the induction of similar mutational changes have been made *in vivo* and *in vitro*;

(*f*) Studies on the mechanism of induction of non-disjunction (leading to gains and losses of chromosomes) by irradiation of germ cells in experimental organisms, and on its frequency of occurrence under different conditions of irradiation of the germ-cell stages most at risk;

(*g*) Comparative radio-genetic studies on female mammals to discover whether the "interval effect" (in which mutation frequencies fall to virtually zero when the interval between irradiation and conception is more than a few weeks) is likely to apply to man or is more restricted in its occurrence;

(*h*) Rates of induction of mutations in germ cells and somatic cells at very low doses, and the development of new techniques to facilitate such studies;

(*i*) Molecular approaches to basic phenomena of mutation and chromosome breakage and further elucidation of the role of heterochromatin in chromosome breakage.

TABLE 1. DOMINANT LETHALS AND TRANSLOCATIONS IN MICE FOLLOWING SPERMATOGONIAL X-RAY EXPOSURE OF 1,200 R IN TWO EQUAL FRACTIONS SEPARATED BY EIGHT WEEKS (139)

Experiment	*Percentage of zygotic classes*		
	Dominant lethals	*Semi-steriles*	*Total translocation heterozygotes*
Expected frequencies from cytological observations of fathers			
Pilot experiment	23.5 ± 1.70	11.6 ± 1.47	12.5 ± 1.51
Main experiment	18.1 ± 0.99	8.6 ± 0.45	9.2 ± 0.48
Observed frequencies in sons of irradiated males			
Main experiment		4.0[a] ± 1.60	3.3 ± 1.46
Observed frequencies in genetic experiments			
Lyon *et al.* (288)	10.6 ± 3.8	3.5 ± 0.88	3.5 ± 0.88
Searle (477)		6.7 ± 2.45	6.7[b] ± 2.45

[a] One semi-sterile son was cytologically normal but gave some semi-sterile progeny that were also cytologically normal.
[b] One semi-sterile daughter with sterile sons that were not examined cytologically was presumed to be a translocation heterozygote.

TABLE 2. PRE- AND POST-IMPLANTATION LOSSES AND TOTAL DOMINANT LETHALITY IN DIFFERENT MAMMALIAN SPECIES IN SUCCESSIVE WEEKS FOLLOWING X-IRRADIATION OF MALES

Based on Lyon (281)

Species	*Dose (rad)*	*Dose rate (rad min^{-1})*	*Week*	Corpora lutea *(C)*	*Implants (I)*	*Live embryos (E)*	*Induced pre-implantation losses*[a]	*Induced post-implantation losses*[b]	*Total dominant lethality*[c]
Mouse	0			725	587	529			
	100	229	1	122	90	77	0.09	0.05	0.14
			2	104	78	66	0.07	0.06	0.13
			3	160	113	94	0.13	0.08	0.20
	500	200	1	181	123	76	0.16	0.30	0.42
			2	239	155	93	0.20	0.33	0.47
			3	145	87	24	0.26	0.69	0.77
			Post-sterile	560	422	354	0.07	0.07	0.13
Guinea-pig	0			98	89	87			
	500	200	1	39	31	25	0.12	0.18	0.28
			2	34	32	21		0.33	0.30
			3	32	29	17		0.40	0.40
			4 and 5	32	30	9		0.69	0.68
			Post-sterile	94	83	80	0.03	0.01	0.04
Rabbit	0			114	89	74			
	500	200	1	71	39	32	0.30	0.01	0.31
			2	79	43	35	0.30	0.02	0.32
			3	59	27	19	0.41	0.15	0.50
			4 and 5	37	15	13	0.48		0.46
			Post-sterile	28	15	14	0.31		0.23
Hamster	0			64	62	50			
	100	87	1	56	50	32	0.08	0.21	0.27
	200	87	1	65	46	30	0.27	0.19	0.41
	300	87	1	69	43	21	0.36	0.39	0.61
	0			148	139	111			
	200	87	1	213	187	106	0.07	0.29	0.34
			2	178	172	118		0.14	0.12
			3	154	135	80	0.07	0.26	0.31
			4	128	106	65	0.12	0.23	0.32
			Post-sterile	129	108	85	0.11	0.01	0.12

[a] $1 - \frac{\text{I/C in the irradiated}}{\text{I/C in controls}}$. [b] $1 - \frac{\text{E/I in the irradiated}}{\text{E/I in controls}}$. [c] $1 - \frac{\text{E/C in the irradiated}}{\text{E/C in controls}}$.

TABLE 3. X-RAY INDUCED DOMINANT LETHALS IN MATURE DIPLOTENE OÖCYTES OF THE GUINEA-PIG, THE GOLDEN HAMSTER AND THE MOUSE

Species	*Dose*[a] *(rad)*	*Dose rate*[a] *(rad min^{-1})*	*No. of females*	Corpora lutea *(C)*	*Implants (I)*	*Live embryos (E)*	Corpora lutea *per female*	*Live embryos per female*	*Induced*[b] *pre-implantation losses*	*Induced*[b] *post-implantation losses*	*Total*[b] *dominant lethality*	*Reference*
Guinea-pig	0		17	61	51	50	3.6	2.9				289
	70±5	32±2	15	60	56	52	4.0	3.5	−0.12	0.05	−0.06	289
	130±10	32±2	18	65	54	49	3.6	2.7	0.01	0.07	0.08	289
	270±20	32±2	13	52	42	33	4.0	2.5	0.04	0.20	0.23	289
	370±25	32±2	6	25	17	12	4.2	2.0	0.19	0.28	0.41	289
Golden hamster	0		5	69	54	43	13.8	8.6				289
	100±10	63±3	8	121	93	66	15.1	8.3	0.02	0.11	0.13	289
	200	63±3	6	109	101	73	18.2	12.2	−0.18	0.09	−0.08	289
	400±40	63±3	9	144	111	37	16.0	4.1	0.02	0.58	0.59	289
Mouse	0		8	158	99	81	19.8	10.1				122
	100[c]	48[c]	13	250	128	103	19.2	7.9	0.18	0.02	0.20	122
	0		14	196	70	90	14.0	6.4				122
	200[c]	48[c]	8	111	53	42	13.9	5.3	−0.04	0.10	0.07	122
	0		16	314	150	142	19.6	8.9				122
	200[c]	48[c]	10	168	82	65	16.8	6.5	−0.02	0.16	0.14	122
	0		?	119		108						434
	400[c]	?	?	100		76					0.16	434

[a] The recorded doses and dose rates varied appreciably according to the exact position and size of the animal's body (guinea-pigs and hamsters). Hence, not only the mean but also the possible range of doses and dose rates are indicated. Anterior third of the body was shielded during irradiation.

[b] See foot-notes to table 2.

[c] Roentgens; whole-body irradiation from beneath.

TABLE 4. TRANSLOCATIONS IN SPERMATOGONIA OF MICE FOLLOWING X-RAY EXPOSURES OF SHORT DURATION

Experiment[a]	Exposure (R)	Whole body (WB) or local (L)	Exposure rate (R min^{-1})	Mice	When examined (days after irradiation)	Scored metaphases	Abnormal metaphases	Frequency[h] per cent	Reference
1 ..	25	WB	100	12	70	2,400	10	0.4±0.1	254
2 ..	50	WB	100	12	70	2,400	16	0.7±0.2	254
3 ..	50	L	88	5	77–210[g]	1,000	14	1.4±1.0	132
4 ..	50	WB	85	5	70	1,000	12	1.2±0.3	347
5 ..	75	WB	100	12	70	2,400	37	1.5±0.3	254
6 ..	100	WB	100	12	70	2,400	45	2.0±0.3	254
7 ..	100	WB	100	9	70	1,500	55	3.7±0.6	253
8 ..	100	L	88	6	77–210[g]	1,200	31	2.6±0.4	132
9 ..	100	WB	85	5	70	1,000	12	3.7±0.6	347
10 ..	200	WB	100	9	70	1,800	77	4.3±0.6	253
11 ..	200	L	88	6	77–210[g]	1,200	96	8.0±0.7	132
12 ..	200	WB	85	10	70	2,000	144	7.2±0.6	347
13 ..	250	L	100	8	210	1,600	72	4.5±0.8	256
14 ..	300	WB	100	9	70	1,350	82	6.1±0.6	253
15 ..	300	WB, L	217	3+3	98	1,200+1,200	95+88	7.6[c]	132
16 ..	300[b]	WB	16.8	8	Not precise; post-fertile period	1,620	103	6.3±0.6	284
17 ..	300	WB	85	8	70	1,600	135	8.4±0.7	347
18 ..	300	WB	93	10	56–77	950	68	7.2±0.8	484
19 ..	400	WB	100	9	70	1,750	110	6.3±0.7	253
20 ..	400	WB	100	10+10+7+8+2	60	6,900	518	7.5[d]	250
21 ..	400	L	88	6	77–210[g]	1,200	141	11.8±0.9	132
22 ..	400	WB	85	7	70	1,400	125	8.9±0.8	347
23 ..	500	WB	100	7	70	1,300	104	8.0±0.8	253
24 ..	500	L	100	9	210	1,700	124	7.3±1.3	256
25 ..	500[b]	L	88.3[b]	7	56+	1,400	171	12.2±0.9	463
26 ..	500	WB	85	5	70	1,000	103	10.3±1.0	347
27 ..	600	WB	100	5	70	640	64	10.0±1.7	253
28 ..	600	L	100	8	60	1,600	135	8.4±1.5	258
29 ..	600	L	100	9	100	1,800	227	12.6±1.1	258
30 ..	600	L	100	9	150	1,500	197	13.1±1.7	258
31 ..	600	L	100	8	200	1,600	208	13.0±2.5	258
32 ..	600[b]	WB	234[b]	3	231–259	2,400	417	17.4[e]	15
33 ..	600[b]	WB	0.8–913[b]	22	84–98	11,600	1,478	12.8[f]	491
34 ..	600[b]	L	88.3[b]	7	56+	1,400	196	14.0±0.9	463
35 ..	600	WB	85	5	70	1,000	135	13.5±1.1	347
36 ..	600	WB	60–70	6	At least 84	1,200	123	10.3±1.6	287
37 ..	700[b]	L	88.3[b]	7	56+	1,400	199	14.2±0.9	463
38 ..	700	WB	85	4	70	800	113	14.1±1.2	347
39 ..	750	L	100	10	210	1,925	89	4.6±1.0	256
40 ..	800	L	88	6	77–210[g]	1,200	198	16.5±2.2	132
41 ..	800[b]	L	88.3[b]	7	56+	1,400	95	6.8±0.7	463
42 ..	1,000	L	100	8	210	1,600	25	1.6±0.6	256
43 ..	1,000[b]	L	88.3[b]	10	Not stated; presumably as in experiment 16	2,000	106	5.3±0.9	283
44 ..	1,250	L	100	7	210	1,275	22	1.7±1.3	256

[a] Although several exposure levels were used at the same time in a single experiment, they have been given serial numbers for easy reference.

[b] Rads instead of roentgens.

[c] Pooled data of part-body and whole-body irradiation (no significant difference between the two groups).

[d] Pooled data of five different strains of inbred mice used in the experiments (no significant inter-strain variation).

[e] Pooled data of three different mice; 800 spermatocytes per mouse scored. Frequencies for the individual mice are: 14.38±1.24; 23.38±1.50; 14.38±1.24.

[f] In view of the lack of dose-rate effect the data obtained at eight different dose rates (ranging from 0.8 R min^{-1} to 913 R min^{-1}) are combined to give an average estimate.

[g] Since length of time between irradiation and examination (77 days, 140 days, 210 days) did not give rise to a significant trend in the observed translocation frequencies, the data at each exposure level have been considered together.

[h] Not corrected for controls.

TABLE 5. TRANSLOCATIONS IN SPERMATOGONIA OF MICE FOLLOWING GAMMA-RAY (^{60}Co) EXPOSURES OF SHORT DURATION (483)[a]

Exposure (R)	*Mice*	*Spermatocytes scored*	*Cells with translocations*	*Frequency*
56	4	800	9	1.1 ± 0.4
112	4	800	11	1.4 ± 0.4
214	4	800	17	2.1 ± 0.5
402	4	800	71	8.9 ± 1.5
816	4	800	105	13.5 ± 2.5

[a] All exposures were at 95 R min^{-1}; with all but the 56-R exposure, the front part of the body was shielded with lead. The mice were killed 12-17 weeks after irradiation for making meiotic preparations.

TABLE 6. TRANSLOCATIONS IN SPERMATOGONIA OF MICE FOLLOWING FAST-NEUTRON EXPOSURES OF SHORT DURATION (492)[a]

Dose (rad)	*Mice*	*Spermatocytes scored*	*Cells with translocations*	*Frequency*
25	2	1,600	37	2.3 ± 0.9
50	2	1,600	89	5.6 ± 3.3
100	2	1,600	139	8.7 ± 1.7
140	3	2,200	103	4.7 ± 1.3
188	3	2,609	91	3.5 ± 0.7
220	3	1,800	29	1.6 ± 0.3

[a] All doses were delivered at 49-55 rad min^{-1}.

TABLE 7. TRANSLOCATIONS IN SPERMATOGONIA OF MICE FOLLOWING X-, GAMMA- OR NEUTRON-IRRADIATION AT DIFFERENT RATES

Type of radiation	*Exposure[a] or dose*	*Exposure[b] or dose rate*	*Mice*	*Spermatocytes scored*	*Cells with translocations*	*Frequency*	*Reference*
X rays	300	93	10	950	68	7.2 ± 0.8	484
	300	0.87	10	1,000	30	3.0 ± 0.5	484
	300	0.09	10	1,000	30	3.0 ± 0.5	484
	600	913	2	1,600	205	12.8 ± 0.8	491
	600	89	3	2,400	291	12.1 ± 0.7	491
	600	87	3	1,200	159	13.3 ± 1.0	491
	600	9.8	3	1,200	162	13.5 ± 1.0	491
	600	9.7	2	1,600	204	12.8 ± 0.8	491
	600	5.0	3	1,200	181	15.1 ± 1.0	491
	600	2.4	3	1,200	147	12.3 ± 1.0	491
	600	0.8	3	1,200	129	10.7 ± 1.6	491
Gamma rays	600	83	3	1,200	145	12.1 ± 0.9	491
	600	11	3	1,200	123	10.3 ± 0.9	491
	600	0.86	3	2,400	120	5.0 ± 1.0	491
	600	0.09	2	1,600	47	2.9 ± 0.4	491
	600	0.02	3	2,400	33	1.4 ± 0.2	491
Neutrons	50	49–55	2	1,600	89	5.6 ± 3.3	492
	62	0.0005–0.0008	3[c]	1,200	28 2.3	3.3 ± 0.4	492
	62	0.0005–0.0008	6[d]	3,200	118 3.7		
	220	49–55	3	1,800	29	1.6 ± 0.3	492
	214	0.0014–0.0024	3	2,932	635	21.7 ± 2.1	492

[a] Roentgens or rads.
[b] Roentgens or rads per minute.
[c] Killed 17 weeks after irradiation.
[d] Killed 63-66 weeks after irradiation.

TABLE 8. TRANSLOCATIONS IN SPERMATOCYTES AFTER FRACTIONATED IRRADIATION OF SPERMATOGONIA

Experiment	Dose (rad)	Interval between fractions	Animals	Days after irradiation[a]	Scored cells	Abnormal cells	Per cent abnormal	Translocations per cell	Reference
1A	50 + 50	24 h	6	84	1,462	31	2.1 ± 0.4	0.021	309
1B	100		6	77–210	1,200	31	2.6	0.026	132
2A	150 + 150	24 h	6	84	1,324	72	5.4 ± 0.6	0.059	309
2B	5 fractions[d] of 60 rad	24 h	7	84	1,723	58	3.4 ± 0.4	[b]	284
2C	30 fractions[d] of 10 rad	24 h	8	84	1,600	21	1.3 ± 0.3	[b]	284
2D	60 fractions[d] of 5 rad	24 h	9	84	1,629	28	1.7 ± 0.3	[b]	284
2E	300		8		1,620	103	6.3 ± 0.6	[b]	284
3A	250 + 250	24 h	11	84	2,998	312	10.4 ± 0.6	0.114	309
3B	500		7	56	1,400	171	12.2 ± 0.9	0.129	463
4A	300 + 300	24 h	6	84	1,335	163	12.2 ± 0.9	0.132	309
4B	600		7	56	1,400	196	14.0 ± 0.9	0.161	463
5A	400 + 400	24 h	9	84	2,617	529	20.2 ± 0.8	0.229	309
5B	800		7	56	1,400	95	6.8 ± 0.7	0.078	463
6A	500 + 500	24 h	10	84	2,000	497	24.9 ± 1.6	0.294	283
6B	1,000		10	?	2,000	106	5.3 ±0.9	0.058	283
7A	600 + 600	24 h	16	84	2,162	510	23.6 ± 0.9	0.276	309
7B	600 + 600	56 d	5	91–126	623	259	41.6	0.531	139
7C	600 + 600	56 d	5	413	4,000	1,300	32.5	0.411	139
7D	1,250[c]		7	210	1,275	22	1.7 ± 1.3	[b]	256
8A	700 + 700	24 h	3	84	311	117	37.6 ± 2.8	0.473	309

[a] Days after the second or the last dose (fractionated exposures) or after the unfractionated dose.
[b] Cannot be estimated from the data.
[c] Roentgens.
[d] Daily fractions.

TABLE 9. YIELD OF TRANSLOCATIONS AFTER REPEATED DAILY DOSES OF GAMMA RAYS TO MOUSE SPERMATOGONIA (286)

Weeks	No. of doses (10.4 rad each)	No. of mice	No. of spermatocytes scored	No. of affected spermatocytes	Frequency (per cent)	Translocations per cell (per cent)
3	15	11	2,200	22	1.0	1.0
6	30	9	1,800	29	1.6	1.6
9	45	10	2,000	34	1.7	1.8
12	60	10	2,000	47	2.4	2.5
		Single dose of 620 rads				
		10	2,000	231	11.5	13.2

TABLE 10. COMPARISON OF THE YIELD OF TRANSLOCATIONS AFTER SINGLE OR REPEATED RADIATION DOSES OF X OR GAMMA RAYS TO MOUSE SPERMATOGONIA

(After Lyon, Phillips and Glenister (287))

Type of radiation	Dose[a]	No. of mice	No. of spermatocytes scored	No. of affected spermatocytes	Frequency (per cent)
X rays	12 × 50 rad; daily	11	2,200	134	6.1 ± 0.7
X rays	12 × 50 rad; weekly	11	2,200	156	7.1 ± 0.9
Gamma rays (^{60}Co)	~600 rad; single	6	1,200	123	10.3 ± 1.6
Gamma rays (^{60}Co)	620 rad;[b] single	10	2,000	231	11.5 ± 1.3

[a] X-ray doses at 60-70 rad min^{-1}; gamma rays at 17 rad min^{-1}.
[b] From table 9.

TABLE 11. FREQUENCIES OF TRANSLOCATIONS INDUCED IN MOUSE SPERMATOGONIA ACCORDING TO INTERVAL BETWEEN ACUTE X-IRRADIATION AND EXAMINATION (132)

Interval (days)	*No. of mice*	*No. of cells examined at each exposure level*	*50 R*	*100 R*	*200 R*	*400 R*	*800 R*
			Frequency (per cent)				
77	2	400	0	3.3	8.0	11.3	20.5
140	2	400	2.3	2.5	6.5	10.0	16.3
210	2	400[a]	2.5	2.0	9.5	14.0	12.8
		Mean frequency	1.4 ± 1.0	2.6 ± 0.4	8.0 ± 0.7	11.8 ± 0.9	16.5 ± 2.2

[a] Only 200 cells examined at 50 roentgens.

TABLE 12. X-RAY-INDUCED TRANSLOCATIONS IN SPERMATOGONIA OF SOME LABORATORY MAMMALS (289)

Species	*Dose (rad)*	*No. of animals*	*No. of slides*[a]	*Total no. of cells*	*Percentage translocations (possible + definite)*	*Percentage translocations (definite)*
Guinea-pig	0	4	70	1,254	0.24	0
	100	1	20	233	1.72	0.85
	200	3	40	541	5.18	4.62
	370	3	45	645	1.24	0.31
	1,000	1	10	101	0	0
	1,845	1	10	73	0	0
Rabbit	0	2	13	655	0.31	0.15
	250	1	28	683	2.34	1.17
	300	1	15	716	6.64	5.79
	500	1	32	436	1.15	0.23
	600	1	10	252	0	0
Hamster	0	1	10	70	0	0
	200	2	37	560	1.61	0.89
Mouse	200	3	31	1,745	4.58	4.47
	500	3	30	1,489	11.21	10.95

[a] Cytological preparations were made according to Meredith (302). In contrast to the method of Evans, Breckon and Ford whereby slides are prepared from a homogeneous cell suspension by macerating the whole testis, in Meredith's method only a small portion of the testis macerated in 60 per cent acetic acid is used to make each slide. To avoid possible heterogeneities between slides, many separate pieces of tubule were used for maceration to make each slide.

TABLE 13. ESTIMATES OF SPONTANEOUS RATES TO VISIBLE MUTATIONS IN MICE AND RATS

Loci	*Nature of mutation studied*	*Tested gametes*	*Mutations*	*Mutation rate per locus per gamete*	*Remarks*	*Reference*
			Mice			
a, b, c, d, ln	Forward: + → recessive allele	2,220,376	25[a]	$11.3\ 10^{-6}$ ($7.3\ 10^{-6}$, $16.6\ 10^{-6}$)[b]	Estimates based on mutations that occurred in both males and females	469
a, bp, fz, ln, pa, pe ..	Forward: + → recessive allele	20,769	0			283
a, b, c, d, se, p, s ...	Forward: + → recessive allele	531,500	28	$7.5\ 10^{-6}$	Mutation rate in males	440
a, b, c, d, se, p, s ...	Forward: + → recessive allele	157,421	11	$10.0\ 10^{-6}$	Mutation rate in males; summary of Harwell data	285
				$8.1\ 10^{-6}$	Over-all rate based on data given in references 285 and 440	285

TABLE 13. ESTIMATES OF SPONTANEOUS RATES TO VISIBLE MUTATIONS IN MICE AND RATS (*continued*)

Loci	*Nature of mutation studied*	*Tested gametes*	*Mutations*	*Mutation rate per locus per gamete*	*Remarks*	*Reference*
a, b, c, d, se, p, s ...	Forward: + → recessive allele	164,999	7	$1.4\ 10^{-6}$	Mutation rate in females[c]	448
a, b, c, d, se, p, s ...	Forward: + → recessive allele	37,813	0	$4.9\ 10^{-6}$	Mutation rate in females[c]	36
a, b, c, d, ln	Reverse: recessive allele → + or dominant allele	17,236,978	43	$2.5\ 10^{-6}$ ($1.8\ 10^{-6}$, $3.4\ 10^{-6}$)[b]	Estimate based on mutations in both males and females. Reverse mutation rate about 1/4 of forward rate, *a* and *d* alleles backmutate at a significantly higher rate ($4.2\ 10^{-6}$ and $3.9\ 10^{-6}$, respectively) than *b* and *c* alleles (no backmutations)	469
Unselected (26 loci)	Forward	83,368,463	28	$0.67\ 10^{-6}$ ($0.51\ 10^{-6}$, $0.87\ 10^{-6}$)[b]	Forward rate at unselected loci is about 1/17 of the forward rate at the five specific loci. The number of mutations actually observed was multiplied by 2 to estimate mutation frequency since the breeding system permitted detection of only half of the mutations that occurred	467
Unselected (12 loci)	Dominant visibles	14,021,464	54	$0.44\ 10^{-6}$	The rate given is the average unweighted rate for the 12 loci (rates for individual loci range from $2.20\ 10^{-6}$ to $0.07\ 10^{-6}$). The average rate is much lower than $2.5\ 10^{-6}$ estimated for *a, b, c, d, ln*	469
Unselected	Dominant skeletal	1,739[g]	1[h]	$2.9\ 10^{-4}$[a, i] ($0.7\ 10^{-4}$, $16.0\ 10^{-4}$)[b]		124
Unselected	Dominant skeletal	438[g]	0			572
Unselected	Dominant visibles	117,727	2			65
		854	0			64
		4,290	0			288
		3,519	0			395
		37,813	0			36
		20,769	1			283
	TOTAL	184,972	3	$0.81\ 10^{-5}$[e, i] ($0.3\ 10^{-5}$, $2.4\ 10^{-5}$)[b]		
			Rats			
Unselected	Forward: → recessive visibles		3	$(0.75 \pm 0.38)\ 10^{-3}$[f]		541

[a] Includes mutations to dominant alleles at the *a* locus.

[b] 95 per cent confidence limits.

[c] Six of the seven mutations represent a cluster; the rate $1.4\ 10^{-6}$ assumes two independent mutational events among 202,812 progeny; the rate $4.9\ 10^{-6}$ also assumes two mutational events, but involves an adjustment for sample size. For full details, see paragraphs 144-146.

[d] Sex-linked.

[e] Rate per gamete.

[f] Rate per gamete per generation.

[g] Number of F_1 skeletons examined.

[h] Presumed mutation.

[i] In calculating the mutation rate, the number of tested gametes is taken to be twice the number given to take into account the possible origin of the mutations in either the male or the female germ line.

TABLE 14. MUTATION RATES AT 12 SPECIFIC LOCI IN ADULT AND NEONATAL MOUSE SPERMATOGONIA

Loci involved	*Effect studied*	*Type of radiation*	*Exposure or dose*[a]	*Exposure or dose rate*[b]	*No. of offspring tested*	*No. of mutations observed*	*Mutation rate per locus per gamete per* $R \times 10^{7}$[c]	*Reference*
Seven	Dose response	X rays	300	80–90	65,548	40	2.9	440
		X rays	300[l]	80–90	55,126[d]	16	1.4	498
		X rays	300[m]	80–90	77,429[d]	43	2.6	498
		X rays	300	1,000	38,207[d]	24	3.0	446
		X rays	600	80–90	119,326	111	2.2	440
		X rays	600	60–70	11,138	12	2.57	285[n]
		X rays	1,000	80–90	44,649	29	0.85	442
Six	Dose response	X rays	600	88	24,834[d]	7	0.78	283
Seven	Dose rate	^{60}Co gamma rays	600	24	44,352	33	1.77	437a
		X rays	600	9	40,326	23	1.35	440
		^{137}Cs gamma rays	600	0.8	28,059	10	0.85	440
		^{137}Cs gamma rays	861	0.009	24,281	12	0.82	440
		^{137}Cs gamma rays	516	0.009	26,325	5	0.52	440
		^{137}Cs gamma rays	300	0.009	58,457	10	0.80	440
		^{60}Co gamma rays	603	0.007–0.009	22,682	5	0.53	285[n]
		^{60}Co gamma rays	606[f]	0.005	58,795	16	0.44[e]	34
		^{60}Co gamma rays	37.5	0.0011–0.0078	63,322	6	3.6	62
		^{137}Cs gamma rays	86	0.001	59,810	6	1.63	440
		^{137}Cs gamma rays	300	0.001	49,569	15	1.43	440
		^{137}Cs gamma rays	600	0.001	31,652	13	0.98	440
Seven	Dose fractionation	X rays	1,000 (unfractionated)	80–90	44,649	29	0.85	442
		X rays	2 fractions; 500+500 2-hr interval	80–90	14,879	12	1.15	439
		X rays	2 fractions; 500+500 24-hr interval	80–90	11,164	39	4.92	442
		X rays	2 fractions; 500+500 24-hour interval	88	5,462[d]	16	4.2	283
		X rays	5 fractions of 200 each; 24-hr intervals	80–90	8,588	16	2.66	439
		X rays	5 fractions of 200 each; weekly intervals	80–90	10,968	15	1.88	439
		X rays	2 fractions; 600+400 >15 week interval	80–90	4,904	10	2.84	437a
			600 (unfractionated)	80–90	119,326	111	2.2	440
		X rays	2 fractions; 100+500 24-hr interval	80–90	24,811	42	3.9	442

TABLE 14. MUTATION RATES AT 12 SPECIFIC LOCI IN ADULT AND NEONATAL MOUSE SPERMATOGONIA *(continued)*

Loci involved	*Effect studied*	*Type of radiation*	*Exposure or dose*[a]	*Exposure or dose rate*[b]	*No. of offspring tested*	*No. of mutations observed*	*Mutation rate per locus per gamete per* $R \times 10^{7}$[e]	*Reference*
Six	Dose fractionation	X rays	1,000 (unfractionated)	—	—	—	0.28[k]	
			2 fractions; 500+500 24-hr interval	88	17,301	14	1.40	285
Seven	Dose response and dose rate	1–2 MeV neutrons	59[g]	79	16,758	10	14.4	440
			59[g]	0.79	17,041	12	17.1	440
			63[g]	0.17	18,194	13	16.2	440
			101[g]	0.13	19,506	20	14.4	440
		0.7 MeV neutrons	62[h]	0.001	39,083	27	9.5	35
			214[i]	0.002–0.003	41,875	67	10.1[e]	34
			188[j]	55–60	39,028	8	1.42	35

[a] Roentgens or rads.
[b] Roentgens per minute or rads per minute.
[c] Not corrected for controls; while the lack of correction at higher doses will make little difference, at lower doses, the induced rates will be lower than those given.
[d] New data.
[e] Corrected for control rate.
[f] Plus 2.5 rad neutron contamination.
[g] Includes a gamma component equal to approximately one seventh of the neutron component.
[h] Plus 42 rad gamma contamination.
[i] Plus 93 rad gamma contamination.
[j] Plus 18 rad gamma contamination.
[k] Estimated on the assumption that the six loci are about one third as mutable as the seven loci.
[l] Irradiation of young adults on day of birth.
[m] Irradiation at ages from 2 to 35 days.
[n] Data of Phillips reported in this reference.

TABLE 15. MUTATION RATES AT SEVEN SPECIFIC LOCI IN ADULT SPERMATOGONIA AFTER ~600 RAD OF X RAYS OR GAMMA RAYS TO MALE MICE IN SINGLE OR REPEATED DOSES (285)

Treatment no.	*Radiation type*	*Interval and no. of exposures*	*Dose rate (rad min*$^{-1}$*)*	*Total offspring*	*Mutants*	*Mutation rate per locus* $\times 10^{5}$	*95 per cent confidence limits of mutation rate* $\times 10^{5}$
1	Gamma rays (^{60}Co)	Single dose	17	12,021	11	13.1	7.6, 24.7
2	Gamma rays (^{60}Co)	Daily[a]	17	23,982	7	4.2	1.6, 8.6
3	Gamma rays (^{60}Co)	Daily[c]	0.008	22,682	5[b]	3.2	1.0, 7.4
4	Gamma rays (^{60}Co)	Weekly[d]	0.05–0.07	22,816	10	6.3	3.0, 11.5
5	X rays	Weekly[e]	60–70	18,119	16	12.6	7.9, 21.3
6	X rays	Single dose	60–70	11,138	12[b]	15.4	9.1, 28.2

[a] Five consecutive days a week for 12 weeks.
[b] Includes data of Phillips (395).
[c] Ninety consecutive daily exposures.
[d] One night each week (12-16 hours).
[e] Twelve consecutive weeks.

TABLE 16. MUTATION RATES AT SEVEN SPECIFIC LOCI IN OÖCYTES OF ADULT AND NEONATAL MICE

Effect studied	*Type of radiation*	*Exposure or dose*[a]	*Exposure or dose rate*[b]	*No. of offspring tested*	*No. of mutations observed*	*Mutations per locus per gamete per R × 10⁷*[c]	*Reference*
Dose response and interval effect	X rays	50	90	180,472[d]	13	2.06	448
	X rays	50	90	78,191[e]	0	—	448
	X rays	200	90	37,297[d]	21	4.02	442
	X rays	300[f]	90	14,259	3	1.0	499
	X rays	400	90	14,842[d]	23	5.53	446
Dose-rate and interval effect	^{137}Cs gamma rays	400	0.8	20,827	7	1.2	440
	^{60}Co gamma rays	600	0.05	10,117	1	0.23	62
	^{137}Cs gamma rays	258	0.009	8,373[d]	1	0.67	448
	^{137}Cs gamma rays	258	0.009	18,684[e]	0	—	448
	^{137}Cs gamma rays	400	0.009	15,195[d]	1	0.24	448
	^{137}Cs gamma rays	400	0.009	21,854[e]	1	—	448
	^{137}Cs gamma rays	400[g]	0.009	14,130[d]	2	—	448
	^{137}Cs gamma rays	400[g]	0.009	953[e]	0	—	448
	^{60}Co gamma rays	412	0.0034	34,263	0	—	36, 493
Dose fractionation ..	X rays	400 (unfractionated)	90	14,591[h]	21	5.15	446
	X rays	2 fractions 200 + 200 24-hr interval	90	6,086[h]	9	5.28	442
	X rays	8 fractions of 50 each; 75 min intervals	90	27,906[d]	19	2.43	443
Dose response and interval effect	1-2 MeV neutrons	63	79	43,000[d]	37	19.4	448
		63	79	40,096[e]	0	—	448
		120	79	6,058[d]	7	13.8	448
		120	79	33[e]	0	—	448
Dose-rate and interval effect	1-2 MeV neutrons	30	8	5,870[d]	1	8.1	448
		30	8	19,477[e]	1	2.4	448
		63	0.17	46,301[d]	22	10.8	448
		63	0.17	80,395[e]	1	0.29	448
	0.7 MeV neutrons	79.7[i]	0.0007	32,221	1	0.3	36, 493

[a] Roentgens or rads.
[b] Roentgens or rads per minute.
[c] Not corrected for controls; the lack of correction at higher doses is likely to make little difference to the actual induced rates; at low doses, however, the reduced rates will be lower than those given (see table 13 for spontaneous rates).
[d] Restricted to conceptions occurring within the first seven weeks after irradiation.
[e] Conceptions occurring later than seven weeks after irradiation.
[f] New-born females irradiated within seven hours after birth.
[g] Old adults at time of irradiation.
[h] Restricted to conceptions occurring within the first three weeks after irradiation.
[i] Plus 57.8 rad gamma contamination.

TABLE 17. APPROXIMATE ESTIMATES OF RBE[a] FOR THE INDUCTION OF SPECIFIC-LOCUS MUTATIONS, DOMINANT VISIBLES AND TRANSLOCATIONS IN THE MOUSE

Cell stage	Test radiation			Standard radiation			RBE of test radiation	Reference
	Radiation	Dose or exposure[b]	Dose rate or exposure rate[c]	Radiation	Dose or exposure[b]	Dose rate or exposure rate[c]		
			Specific-locus mutations					
Spermatogonia	1-2 MeV neutrons	59	79	X rays	300–600	60–1,000	5.5	440, 446
	1-2 MeV neutrons	59	0.79	^{137}Cs gamma rays	600	0.8	20	440
	0.7 MeV neutrons	62-214	0.001-0.003	^{60}Co gamma rays	606	0.005	23	34, 35
	X rays	600	9	X rays	300–600	60–1,000	0.5	440
	^{137}Cs gamma rays	300-861	0.001-0.8	X rays	300–600	60–1,000	0.3	440
	1-2 MeV neutrons	59-101	0.13-0.79	1-2 MeV neutrons	59	79	1	440
	0.7 MeV neutrons	214	0.002-0.003	0.7 MeV neutrons	188	55–60	7	34
Oöcytes	1-2 MeV neutrons	63	79	X rays	400	90	3.5[d]	440, 442, 445
	1-2 MeV neutrons	63	0.17	X rays	400	90	2.0[d]	442, 445
	^{137}Cs gamma rays	400	0.8	X rays	400	90	0.25	440, 442
	^{137}Cs gamma rays	258-400	0.009	X rays	400	90	0.04	440
			Dominant visibles					
Spermatogonia	0.7 MeV neutrons	214	0.002-0.003	^{60}Co gamma rays	606	0.005	19.6	34
			Translocations					
Spermatogonia	0.7 MeV neutrons	25-50	49-55	X rays	50–400	80–90	3.7	492
	0.7 MeV neutrons	188	49-55	X rays	50–400	80–90	0.7	492
	0.7 MeV neutrons	220	49-55	X rays	50–400	80–90	0.25	492
	0.7 MeV neutrons	62	0.0005-0.0008	^{60}Co gamma rays	600	0.02	23	491, 492
	^{60}Co gamma rays	56-816	95	X rays	600	89	0.6	483

[a] The term RBE is used here in a broad sense to compare not only the effects of two types of radiation but also to compare the effects of a type of radiation used in one way with the effect of the *same* radiation used in a different way (193).

[b] Roentgens or rads.
[c] Roentgens or rads per minute.
[d] Data restricted to conceptions occurring in the first seven weeks after irradiation.

TABLE 18. DISTRIBUTION OF MUTATIONAL EVENTS AT THE *d-se* REGION (LINKAGE GROUP II) IN MOUSE ACCORDING TO GERM-CELL STAGE AND MODE OF INDUCTION[a] (430)

Source of mutations	*d*	*se*	Df(*d se*)	*Spermatogonia* *d*	*se*	Df(*d se*)	*Post-spermatogonial stages* *d*	*se*	Df(*d se*)	*Oöcytes* *d*	*se*	Df(*d se*)
Control[b]	16	3	1[c]									
X- or gamma-irradiation experiments at exposure rates of:												
<10 R min^{-1}				18	1	0	2	0	0	0	2	2
10-100 R min^{-1}				35	6	2	6	4	7	8	3	6
>100 R min^{-1}				4	1	1	0	1	1	1	0	2
TOTAL				57	8	3	8	5	8	9	5	10
X-irradiation experiments:												
Fractionated exposures (24-hr interval)				11	5	2	1	2	1	0	0	1
Fractionated exposures (others)				12	4	1	0	0	0	4	0	0
Neutron-irradiation experiments				24	10	7	1	4	2	1	1	2

[a] Includes some mutants only partially tested.
[b] All but three events occurred in control males.
[c] Died at 2 months; unknown whether Df (deficiency) or double non-disjunction.

TABLE 19. PROPORTION OF MUTATIONAL EVENTS AT THE *d-se* REGION IN MOUSE INVOLVING MORE THAN ONE FUNCTIONAL UNIT, BASED ON COMPLEMENTATION TESTS (430)

Cell stage	Irradiation	No. of mutants	*Percentage involving >1 functional unit* Total	Cross-over length >2 map units[b] Minimum	Maximum
Control		19	5.6 or 10.5[a]	0	0
Spermatogonia	X or gamma rays excluding 24-hr fractionation	67	13.5	0	0
Spermatogonia	X rays; 24-hr fractionation	18	27.8	5.6	5.6
Spermatogonia	Neutrons	41	31.7	4.9	4.9
Post-spermatogonial stages	All experiments	26	42.3	7.7	23.1
Oöcytes	All experiments	32	65.6	3.1	18.8

[a] Excluding or including, respectively, the questionable *d-se* mutant.
[b] Minimum is based on only 44 presumed aberrations (out of a total of 61) for which the length had been established. Maximum is based on the assumption that all of the 9 Df(*d se*)s not used in complementation tests were longer than 2 cross-over units.

TABLE 20. DISTRIBUTION OF RADIATION-INDUCED SPECIFIC-LOCUS MUTATIONS IN MOUSE SPERMATOGONIA AT VARIOUS EXPOSURE RATES (442)

Exposure rate (R min^{-1})	Radiation		*Locus* *a*	*b*	*c*	*p*	*d*	*se*	*dse*[a]	*s*	Total
90	X		2	32	15	22	24	2		69	166
9	X		1	1	3	7	3		1	10	26
0.8, 0.009, and 0.001	Gamma	Observed	2	12	9	9	15	1		29	77
		Expected[b]	1	15	7	10	11	1		32	77

[a] Simultaneous occurrence of mutations at the *d* and *se* loci.
[b] Number of mutations expected on the basis of results at 90 R min^{-1}.

TABLE 21. FREQUENCIES OF DOMINANT VISIBLE MUTATIONS AFTER IRRADIATION OF MOUSE SPERMATOGONIA OR OF POST-SPERMATOGONIAL STAGES WITH X RAYS, NEUTRONS OR GAMMA RAYS

Germ-cell stage	*Type of radiation*	*Dose or exposure*[a]	*Dose or exposure rate*[b]	*Total no. of offspring*	*No. of mutants*	*Mutations per 10^5 gametes*	*Reference*
Spermatogonia	X rays	600	68	838	0	—	64
	X rays	600	60–70	10,761	2	18.6	395
	X rays	600	88	24,834	9	36.2	283
	X rays	600+600[c]	88	3,612	2	55.4	288
	X rays	500+500[d]	88	17,301	18	104.0	283
	X rays	500+500[d]	88	5,462	6	109.8	283
	Neutrons (0.7 MeV)	188[e]	54–60	39,028	2	5.1	35
	Neutrons (0.7 MeV)	62[f]	0.001	39,083	7	17.9	35
	Neutrons (0.7 MeV)	214[g]	0.001–0.002	41,875	24	57.3	34
	^{60}Co gamma rays	606[h]	0.005	58,795	6	10.2	34
	X rays	600	83	754[j]	5[k]	663	124, 125
	X rays	100+500[d]	83	277[j]	5[k]	1,805	124, 125
	X rays	500+500[i]	83	131[j]	2[k]	1,527	124, 125
	Neutrons (14.1 MeV)	485	47.5	433[j]	1[k]	231	572
Post-spermatogonial	X rays	600	83	569[j]	10[k]	1,757	124
	X rays	222	47.5	154[j]	2[k]	1,299	572
	Neutrons (14.1 MeV)	242.5	47.5	343[j]	4[k]	1,166	572
	Neutrons (14.1 MeV)	485	47.5	157[j]	4[k]	2,548	572

[a] Roentgens or rads.
[b] Roentgens or rads per minute.
[c] Separated by 8 weeks.
[d] Separated by 24 hours.
[e] Plus 18 rad gamma contamination.
[f] Plus 42 rad gamma contamination.
[g] Plus 93 rad gamma contamination.
[h] Plus 2.5 rad neutron contamination.
[i] Separated by 10 weeks.
[j] F_1 skeletons screened.
[k] Presumed mutations.

TABLE 22. MUTATION RATES OF AUTOSOMAL RECESSIVE LETHALS AND VISIBLES IN MICE AND RATS

Experiment	Germ-cell stage	Exposure	Damage measured	Type of mutation	Mutations per gamete × 10^4 [a]	95 per cent confidence limits	Reference
			Mice				
1	Male	Control	Post-implantation losses in backcross and outcross tests	Recessive lethals	156	78, 233	510
2	Male	Control	Post-implantation losses in backcross and outcross tests	Recessive lethals	6.6	<0, 54	419
3	Male	Control	Post-implantation losses in backcross and outcross tests	Recessive lethals	—	<0, 29	274
					29[b]	<0, 65	275
4	Male	Control-14th generation	Post-implantation losses in backcross and outcross tests	Recessive lethals	46	24, 68	513
5 (i)	Spermatogonia	(600+600) R	Pre- and post-implantation losses in backcross and outcross tests	Recessive lethals	2.46	0.6, 4.6	288
(ii)	Spermatogonia	(600+600) R	Post-implantation losses in backcross and outcross tests	Recessive lethals	1.73	1.2, 2.3	288
							275
6	Spermatogonia	1,092 R	Post-implantation losses in backcross and outcross tests	Recessive lethals	1.0[c]	<0, 2.2	279a
7	Spermatogonia	275 R	Post-implantation losses in backcross and outcross tests	Recessive lethals	—	<0, <0	510
					0.9[d]	0.4, 1.5	275
8	Spermatogonia	276 R per generation for 9 generations	Post-implantation losses in backcross and outcross tests	Recessive lethals	0.8-2.1	<0, 6.8	271
9	Spermatogonia	276 R per generation for 14 generations	Post-implantation losses in backcross and outcross tests	Recessive lethals	0.43	<0, 0.9	513
			Rats				
10	Spermatogonia	450 R per generation for 9 generations and 5 subsequent generations without irradiation	Litter-size: (sib and non-sib matings) at	Recessive lethals			541
			(i) birth		0.0±0.6		
			(ii) 1 day		1.0±0.8		
			(iii) 21 days		1.2±1.1		
			(iv) 69 days		1.6±1.3		
11	Spermatogonia	450 R per generation for 9 generations and 5 subsequent generations without irradiation	—	Recessive visibles	0.16±0.07		541
12	Spermatogonia	Two experiments: 600 R and 450 R	Litter-size: (sib and non-sib matings) at one day of age	Recessive lethals	8.4±7.6 to 9.1±3.3		71
13	Oöcytes	Same as in experiment 10	Same as in experiment 10	Recessive lethals			173
			(i) birth		0.5	<0, 2.7	
			(ii) 1 day		1.6	<0, 4.1	
			(iii) 21 days		1.5	<0, 5.0	
			(iv) 69 days		1.4	<0, 5.7	

[a] For irradiated groups, per roentgen.
[b] Mean.
[c] Include spontaneous mutations.
[d] Mean of estimates from experiments 5 (ii), 6 and 7.

TABLE 23. EFFECTS OF RADIATION ON COMPONENTS OF FITNESS

Experiment no.	Founder strain	Problem and brief description	Mating system	Accumulated genetic exposure	Criteria used and major conclusions	Reference
1	Inbred CBA mice	Heterozygous effects of a spontaneous autosomal dominant visible mutant which behaved as a recessive lethal	Sib-matings between heterozygotes and non-mutants	None	(i) Mating prowess: male heterozygotes < normal brothers (ii) Intrauterine death: male heterozygotes > normal brothers (iii) Litter size: female heterozygotes < normal sisters (iv) Implantation rate: female heterozygotes < normal sisters Differences not significant.	509
2	Inbred CBA mice	Heterozygous effects of radiation induced recessive lethals. Material for tests derived from offspring of generations 7, 8, 9 of population in which males in each generation received 276 R of spermatogonial x-irradiation; comparison with appropriate controls	Random non-sib	966, 1,104 and 1,242 R in generations 7, 8 and 9, respectively	(i) The "lethal" group (i.e., heterozygotes for lethals) was significantly superior to "non-lethal" group in the number of females made pregnant in the tests of sons (81.6 per cent *versus* 75.7 per cent) (ii) The "lethal" group was better (not significant) than the other in breeding performance (iii) No evidence for over-all deleterious or heterotic effects of recessive lethals in heterozygotes	277
3	Inbred CBA mice	Same problem as in experiment 2. Material for tests derived from the experiments of Rönnbäck and Sheridan (unpublished) in which female fœtuses were gamma-irradiated, during the 10th to 14th or the 14th to 18th day of gestation, through nine generations; criterion: relative intrauterine deaths	Random non-sib	?	(i) Offspring of one F_1 male showed some deleterious dominant effect (10.9 per cent intrauterine deaths for this "lethal" *versus* 7.9 per cent for the remaining groups) (ii) No over-all indication of dominant deleterious effects	278
4	Inbred CBA mice	Same problem as in experiment 2. Starting material for tests from male offspring of the 14th generation of experiment 2	Random non-sib	1,932 R	No over-all indication of deleterious effects of lethals in the heterozygous condition as measured by relative intrauterine deaths	513
5	Inbred CBA mice	Same problem as in experiment 2. Starting material for tests from male offspring of the 14th generation of experiment 2	Random non-sib	1,932 R	(i) Same as in experiment 4, except one family (out of the 14 that could be tested) showed an increased rate of intrauterine death (ii) Lethal heterozygous males inferior in mating ability to lethal-free males (iii) Finding (ii) is the opposite of that in experiment 2; the author believes that the method of dividing the material into "lethal" and "lethal free" groups was less adequate in the earlier study	272

6 .... Random-bred specific-pathogen free albino mice "Swiss"	Same problem as in experiment 2. Material for tests derived from a population in which males were exposed to 545 R of spermatogonial, gamma irradiation; after one or more generations	Random non-sib	1,090 R (maximum)	(i) Litters born to irradiated fathers showed a decrease in size at weaning between 4-5 per cent (ii) Litters in groups in which irradiation was relaxed for one or more generations showed a small insignificant increase in litter size (iii) Dominant lethals are induced but no other net dominant deleterious effects	217
7 .... Inbred CBA mice	Search for radio-sensitivity differences between offspring of irradiated population and controls (described under experiment 2); offspring for tests derived from 13th and 14th generations (males) or from the 14th (females); in one "male" experiment, the LD_{50} at 30 days was determined for test males, control males and CBA founder males and in the other survival after an exposure to 1,400 R in 10 unequal fractions was studied; in the "female" experiment, the test females and controls were exposed to 65 R or 100 R	Random non-sib	1,794 R (13th generation)	"Male" experiment: (i) No differences in $LD_{50/30}$ or in survival between the males with and without radiation histories (ii) CBA strain showed a greater radio-sensitivity ($\sim$ 10 per cent) than either population	512
			1,932 R (14th generation)	"Female" experiment: (i) No significant differences between the groups at either dose level in numbers of litters or litter size (ii) An increase in length of the gestation period noted in control as well as in those with radiation history	508
8 .... Inbred C57BL mice and "Hybrid" from a 4-way cross of 4 inbred strains	Lifetime reproductive performance: material for tests derived from non-irradiated descendants from "low" (50 R; 100 R/generation to spermatogonia) inbred populations and from "high" (900 R/generation) "hybrid" populations. Irradiation over several generations	Random non-sib	Up to 5,400 R	The suggestive indication obtained in an earlier study for a decrease in the days of reproductive life and in the number of litters produced in the inbred "low" level lines *could not* be confirmed	156
9 .... Inbred CBA mice	Lifetime reproductive performance of offspring of a population in which spermatogonia were exposed to 276 R each generation; material for tests derived from 14th generation progeny	Random non-sib	1,932 R	No significant differences in reproductive capacity between the control and the irradiated populations; however, the offspring of the irradiated population showed a significant tendency towards lower age at first litter. This is interpreted as a sign of earlier sexual maturity	511
10 .... Inbred CBA mice	Effects of cumulative spermatogonial irradiation on life span and body weight. Material for tests derived from the population described under experiment 8	Random non-sib	250-2,700 R depending on the population	(i) No consistent effect of ancestral x-irradiation on life span in either population (ii) A significant reduction of body weight at maturity (89-91 days) with ancestral irradiation in two of the three generations studied in the "high" population	470
11 .... "Hybrid" (see experiment 9 above)	60-day body weights and embryonic mortality in the offspring from the	Random non-sib	4,494 R (14 generations)	(i) Mean body weight of male offspring declined at a rate of 6.8	154

TABLE 23. EFFECTS OF RADIATION ON COMPONENTS OF FITNESS *(continued)*

Experiment no.	*Founder strain*	*Problem and brief description*	*Mating system*	*Accumulated genetic exposure*	*Criteria used and major conclusions*	*Reference*
					10,000 R of accumulated genetic exposure; the former might be due to X-linked mutations having deleterious effects in the hemizygous sex (ii) No apparent effect upon embryonic mortality rate nor upon fœtal abnormality	
12	Inbred RFM mice	Effects of cumulative pre- and post-spermatogonial x-irradiation on body and organ weight, fat deposition etc.; 200 rads/generation for 25-37 generations	Sib matings	~ 2,500-3,700 rads	(i) Body weight, omental and uterine fat, total white blood cell counts less in the irradiated line than in controls (ii) Kidney weight was slightly higher in the irradiated line. (iii) Differences either non-significant or at the borderline of significance at the 5 per cent level	70
13	Inbred RFM mice	Same population as in experiment 12, but life span was studied	Sib matings	~ 2,500 rads	No significant differences between irradiated and control lines	530
14	Mice of FSB/Gn (non-pedigreed) and C57BL/6J strains	Effects, in heterozygous conditions of *fs* (furless) and *shm* (shambling) mutations, which as homozygotes have deleterious effects on viability; *fs*/+ and *shm*/+ mice compared with appropriate +/+ mice in terms of survival to weaning age, body weight from 4 to 15 weeks, life span, reproductive performance and median lethal exposure ($LD_{50/30}$)	Strains maintained by sib-matings of heterozygotes	650-850 R; (whole-body) exposures in the radiation study	No significant differences between heterozygotes and +/+ mice in any of the criteria used	157
15	Albino mice NMRI (Bom SPF)	Comparisons of productivity of males irradiated either with a single acute dose of 570 rads or with a first dose of 95 rads followed by a second one of 475 rads, the two exposures being separated by time intervals ranging from 18 to 30 hr. Acute and first exposures were given between 10 and 11 a.m. (experiment a), or between 5 and 6 p.m. (experiment b); spermatogonia were sampled (9 to 24 weeks after irradiation: 3 females per male per week)	———	570 rads either singly or in two fractions 95 and 475 rads separated by varying time intervals	*Experiment a*: The productivity in the acutely irradiated group (number of live young/male) was around 80 per cent of that of unirradiated controls. In the fractionately irradiated group, the productivity-time interval relationship showed a pattern with a clear peak around the 24-hr interval (productivity exceeding that of unirradiated controls) and lower productivity at other intervals chosen *Experiment b*: there was a gradual increase in productivity with increasing fractionation intervals and there was *no* peak. In either experiment, the acutely irradiated group (single exposure) showed similar productivity irrespective of whether the doses were delivered at 11 a.m. or 5 p.m.	368, 369

				The observations with fractionation are tentatively interpreted as resulting from the interaction between the fractionation schedule and possible diurnal rhythm of sensitivity changes of treated germ cells.	
16 .... Inbred MI_4 rats	Main study designed to estimate the rates of induction of dominant and recessive lethal and visible mutations and the effects of these mutations on fitness in populations of rats, irradiated (male-line, spermatogonial exposures; female-line oöcyte exposures) with 450 R of x rays in every generation up to a maximum of 14 generations	Restricted random non-sib	0-over 3,000 R depending on the test generation	(i) In the female irradiated line, litter sizes at birth, at 21 days and at 69 days tended to be smaller than in the controls (non-significant)	173
				(ii) No measurable detrimental effects of induced recessive lethals in heterozygotes	542
				(iii) In the male-irradiated line, the average heterozygous effects are to increase body weights and decrease age at vaginal opening, while the average homozygous effects are to decrease body weights and increase age at vaginal opening. Overdominance of induced mutations with respect to these quantitative traits (growth and age at sexual maturity) seems indicated	543
				(iv) The results obtained in the female-irradiated line similar to those in (iii) above	103
17 .... Natural populations of South Indian black rat	Comparison of discrete and continuous skeletal traits and pre-natal mortality between populations from "high" and "low" natural radioactivity areas	No experimental breeding possible	~ 500 R ("high" area) ~ 67 R ("low" area)	Differences consistently non-significant	167
18 .... Inbred Duroc and Hampshire pigs	Birth weights of individuals descended from x-irradiated spermatogonia (300 R)	Non-sib ?	150 R	No significant differences	93
19 .... Inbred Duroc and Hampshire pigs	Weight and depth of fat at 164 days; irradiation as in experiment 14	Non-sib ?	150 R	Pigs descended from irradiated spermatogonia weighed less and had less fat than contemporary controls; differences small and non-significant except in Durocs where a shift of 0.85 kg (1 per cent of the average weight) was detected	94
20 .... Inbred Duroc and Hampshire pigs	Effects of paternal x-irradiation on litter size and early post-natal mortality in swine	Non-sib ?	300 R to males	Paternal irradiation increased litter size at birth in the Duroc breed but not in the Hampshire breed; paternal irradiation slightly decreased the probability of survival of Durocs; but this effect was not consistent in the Hampshires	322

TABLE 24. FREQUENCY OF LABELLED A-TYPE MOUSE SPERMATOGONIA SURVIVING DIFFERENT X-IRRADIATION EXPOSURES (362)

Time after irradiation	*Control*	*100 R*	*500 R*	*1,000 R*	*500 + 500 R*[a]
12 hours	0.600	0.349	0.343	0.229	0.387
72 hours	0.159	0.474	0.577	0.557	0.593
5 days	0.134	0.467	0.629	0.590	0.598
8.5 days	0.078	0.156	0.163	0.024	0.391
17 days	0.007	0.016	0.017	0.002	0.031

[a] Fractions given 24 hours apart.

TABLE 25. FORWARD MUTATION RATES AT SPECIFIC LOCI IN VARIOUS CELL SYSTEMS AFTER HIGH-DOSE-RATE X- OR GAMMA-IRRADIATION

Experiment	*Test organism*	*Cell stage*	*No. of loci studied*	*Exposure or dose*[a]	*Mutation rate per locus per roentgen (or rad)*	*Reference*
1 ..	Mouse[b]	Spermatogonia	7	600	$2.2\ 10^{-7}$	440
2 ..	Mouse[b]	Spermatogonia	6	600	$7.8\ 10^{-8}$	283
3 ..	Mouse[b]	Oöcytes	7	400	$5.5\ 10^{-7}$	445
4 ..	Chinese hamster	Somatic cells in culture (from lung); aneuploid cell line	1 ($azg^{r-7.5}$)[c]	200–1,000	$4.1\ 10^{-4}$–$2.1\ 10^{-2}$	51
5 ..	Chinese hamster	Somatic cells in culture (from lung); aneuploid cell line	(azg^{r-30})[d]	450	$9.2\ 10^{-7}$	51
6 ..	Chinese hamster	Somatic cells in culture (from lung); aneuploid cell line	(azg^{r-30})[d]	200-1,200	$4.2\ 10^{-7}$–$1.8\ 10^{-6}$	77
7 ..	Chinese hamster	Somatic cells in culture (from ovary); aneuploid cell line	4 ($gly^{+}\rightarrow gly^{-}$)	600	$4.0\ 10^{-8}$	200
8 ..	*Drosophila*	Spermatogonia	8 on chromosome III	900	$1.5\ 10^{-8}$	8
9 ..	*Drosophila*	Spermatogonia	8 on chromosome III	900	$1.3\ 10^{-8}$	9
10 ..	*Drosophila*	Immature oöcytes (stage 7 and earlier)	10 on X-chromosome	4,000	$6.9\ 10^{-8}$	577
11 ..	*Drosophila*	Oögonia	10 on X-chromosome	4,000	$1.7\ 10^{-8}$	577
12 ..	Silkworm	Spermatogonia in 7-day-old larvæ	2 (*pe*, *re*)	1,000	$7.4\ 10^{-7}$ (*pe*) $3.2\ 10^{-7}$ (*re*)	546
13 ..	Silkworm	Spermatogonia in 7-day-old larvæ	2 (*pe*, *re*)	1,000[e]	$3.5\ 10^{-7}$ (*pe*) $1.3\ 10^{-7}$ (*re*)	550
14 ..	Silkworm	Oögonia in 7-day-old larvæ	2 (*pe*, *re*)	1,000	$3.7\ 10^{-7}$ (*pe*) $3.2\ 10^{-7}$ (*re*)	546
15 ..	*Dahlbominus*	Oögonia	4	1,000	$1.3\ 10^{-7}$	29, 30
		Oögonia	4	1,500	$5.3\ 10^{-8}$	30
16 ..	*Dahlbominus*	Oöcytes in females at ages of 12, 60 and 108 hr	4	250	$3.5\ 10^{-7}$ (12 hr) $5.5\ 10^{-7}$ (60 hr) $7.0\ 10^{-7}$ (108 hr)	31
17 ..	*Dahlbominus*	Oöcytes in females at ages of 12, 60 and 108 hr	4	1,000	$3.0\ 10^{-7}$ (12 hr) $4.6\ 10^{-7}$ (60 hr) $6.6\ 10^{-7}$ (108 hr)	31
18 ..	*Dahlbominus*	Mature oöcytes in females aged 9-13 days	4	500[e]	$18.9\ 10^{-7}$	32
19 ..	*Dahlbominus*	Mature oöcytes in females aged 11 days	4	250[e]	$17.1\ 10^{-7}$	32
20 ..	*Marmoniella*	Oöcytes	5	?	$1.4\ 10^{-7}$	212
21 ..	*Neurospora crassa*		2 ($ad\text{-}3A^{+}\rightarrow ad\text{-}3A^{-}$) ($ad\text{-}3B^{+}\rightarrow ad\text{-}3B^{-}$)		$1.8\ 10^{-9}$ $3.6\ 10^{-9}$	590
22 ..	*Escherichia coli B/r*	—	2 (resistance to T_1 phage)	?	$1.0\ 10^{-9}$	109

[a] Roentgens or rads.
[b] Rates at other exposures are given in tables 13 and 14.
[c] Resistance to 8-AG at a concentration of 7.5 μg ml^{-1}.
[d] Resistance to 8-AG at a concentration of 30 μg ml^{-1}.
[e] Gamma rays.

TABLE 26. APPROXIMATE RBEs OF NEUTRONS IN INDUCING RECESSIVE LETHALS, TRANSLOCATIONS AND DOMINANT LETHALS IN THE GERM CELLS OF *Drosophila*

Experiment no.	Germ-cell stage	Measured end-point of genetic damage	Neutrons: Mean energy	Neutrons: Dose[a]	Neutrons: Dose rate[b]	X rays: Dose or exposure[c]	X rays: Dose or exposure rate[d]	RBE	Reference
1	Late spermatids and spermatogonia	II chromosome recessive lethals	0.7 MeV	200–1,000	50	200–1,000	542	2.2 (late spermatids) 2.1 (spermatogonia)	232
2	Post-meiotic germ cells as sampled in four one-day broods	Sex-linked recessive lethals in rod-X chromosomes	~4 MeV	245–1,460	~10	960–2,800	~10	1.2 (brood-1) 2.2 (brood-2) 1.4 (brood-3) 1.3 (brood-4)	101
3	Mature spermatozoa	Sex-linked recessive lethals in rod-X chromosomes	2.5 MeV	500–3,700	10	930–2,790	100	2.3	349
4	Mature spermatozoa	Sex-linked recessive lethals in rod-X chromosomes	0.68 MeV	250–1,250	2.2 or 9	500–2,500	180	1.8	151a
5	Mature spermatozoa and late spermatids	Sex-linked recessive lethals in ring-X chromosomes	15 MeV	1,200–3,000[e] 1,200–4,000[f]	100	1,600–3,000[e] 1,600–4,000[f]	550	0.8 (mature spermatozoa) 1.2 (late spermatids)	526
6	Oögonia, mature and immature oöcytes	Sex-linked recessive lethals in rod-X chromosomes	0.2-0.3 MeV	267–1,066	45	960–3,840	155	<2	115
7	Post-meiotic germ cells as sampled in four one-day broods	Translocations between chromosomes II and III	~4 MeV	245–1,460	~10	1,000–4,000	~10	2.3 (brood-1) 3.2 (brood-2) 3.3 (brood-3) 1.7 (brood-4)	101
8	Mature spermatozoa	Translocations between chromosomes II and III	0.68 MeV	152–1,362	2.2	940–4,700	180	1.8-3.2[g]	151a
9	Mature spermatozoa	Translocations involving chromosomes II, III and Y	2.5 MeV	500–3,700	10	930–2,790	100	5.6-5.7	349
10	Mature spermatozoa and late spermatids	Translocations between chromosomes II and III	15 MeV	1,200–3,000[e] 1,200–4,000[f]	100	1,600–3,000[e] 1,600–4,000[f]	550	1.0 (mature spermatozoa) 1.1-2.3 (late spermatids)[h]	526
11	Mature spermatozoa	Dominant lethals	2.5 MeV	500–3,700	10	930–2,790	100	2.8-3.6	348
12	Mature spermatozoa	Dominant lethals	2.5 MeV	100–2,500		500–5,000		3.5[i]	349
13	Mature spermatozoa	Dominant lethals	0.68 MeV	250–1,250	9	500–5,000	180	3.5[i]	151a

[a] Rads.
[b] Rads per minute.
[c] Roentgens or rads.
[d] Roentgens or rads per minute.
[e] Range for mature spermatozoa.
[f] Range for late spermatids.
[g] The RBE varied from 3.2 at doses that induced 1.0 per cent translocations to 1.8 at the 18.0 per cent level.
[h] The RBE varied from 2.3 at doses that induced 2.0 per cent translocations to 1.1 at the 8.0 per cent level.
[i] At 50 per cent survival.

TABLE 27. APPROXIMATE RBEs OF NEUTRONS IN INDUCING RECESSIVE VISIBLES AT THE *pe* AND *re* LOCI IN SILKWORM GERM CELLS

Experiment no.	Germ-cell stage	Neutrons			Standard radiation			RBE[d]	Reference
		Mean energy	Dose[a]	Dose rate	Radiation	Dose or exposure[b]	Dose or exposure rate[c]		
1	Primordial spermatogonia in hibernating eggs	14 MeV	320-1,300	8.7	Gamma rays (^{137}Cs)	250–3,000	100	1.8 2.9 2.4	346
2	Primordial spermatogonia in newly hatched larvæ	14 MeV	760-2,240	6.7-19.6	Gamma rays (^{137}Cs)	500–2,000	316–333	0.8 1.0 0.9	342
3	Primordial spermatogonia in newly hatched larvæ	1.5 MeV	202-787	200.7	Gamma rays (^{137}Cs)	500–2,000	316–333	1.7 1.9 1.8	343
4	Late spermatogonia in 7-day old larvæ	14 MeV	860-4,420	7.6-38.7	Gamma rays (^{137}Cs)	1,000–3,500	100	3.2 2.1 2.7	342
5	Late spermatogonia in 7-day old larvæ	1.5 MeV	202-787	200.7	Gamma rays (^{137}Cs)	1,000-3,500	316–333	4.2 3.5 3.9	343
6	Mature sperm in late pupæ	14 MeV	990-5,050	1.2-6.0	Gamma rays (^{137}Cs)	2,000–6,000	100	5.3 6.7 6.0	334, 340
7[e]	Mature sperm in late pupæ	14 MeV	990-5,050	1.2-6.0	Gamma rays (^{137}Cs)	2,000–6,000	100	4.2 4.8 4.5	334
8	Primordial oögonia in newly hatched larvæ	14 MeV	760-2,240	6.7-19.6	Gamma rays (^{137}Cs)	500–2,000	316–333	1.2 1.2 1.2	342
9	Primordial oögonia in newly hatched larvæ	1.5 MeV	202-787	200.7	Gamma rays (^{137}Cs)	500–2,000	316-333	2.1 2.4 2.3	343
10	Late oögonia in 7-day old larvæ	14 MeV	860-4,420	7.6-38.7	Gamma rays (^{137}Cs)	1,000–3,500	100	1.7 2.8 2.3	342
11	Late oögonia in 7-day old larvæ	1.5 MeV	244-949	242.2	Gamma rays (^{137}Cs)	1,000-3,500	100	3.8 3.0 3.4	343
12	Prophase-I oöcytes in pupæ	2.5 MeV	?	?	X rays	?	?	3.0	291

[a] All doses are absorbed doses.
[b] Roentgens or rads.
[c] Roentgens or rads per minute.
[d] Except in experiments 6 and 12, the RBEs were estimated as a ratio of doses at an arbitrarily chosen level of mutational yield of 10^{-3}; this was done because the mutation frequencies increased faster than linearly with dose regardless of the type of radiation used. In experiment 6, because of linearity, the RBE was estimated as a ratio of the two slopes; in experiment 12, the dose-response was again non-linear and the RBE given is for low doses where the responses were approximately linear. Of the three RBEs given for each of experiments 1-11, the first is for the *pe* locus, the second for the *re* locus and the third is the mean value.
[e] Mosaic mutations were scored at the two loci.

TABLE 28. RATES OF INDUCTION OF DIFFERENT KINDS OF GENETIC DAMAGE IN THE MOUSE AND THEIR MODIFICATIONS UNDER VARIOUS CONDITIONS OF IRRADIATION

Scored end-point of genetic damage	*Spermatogonia in adults*				*Spermatogonia in new-born*	*Late dictyate oöcyte in adults*				*Early dictyate oöcytes in adults*	*Dictyate oöcytes in new-born*	*Oögonia and precursors in embryos*
	Mutations per rad 10^7: high dose x-irradiation at high dose rates	*Factor[a] by which mutation rate is modified after*			*Mutations per rad 10^7: high dose x-irradiation at high dose rates*	*Mutations per rad 10^7: high dose x-irradiation at high dose rates*	*Factor[a] by which mutation rate is modified after*			*Mutations per rad 10^7: high dose x-irradiation at high dose rates*		*Mutations per rad 10^7: high-dose fission neutrons at low dose rates*
		Low dose x-irradiation at high dose rates	*High doses of gamma irradiation at low dose rates*	*Low dose fission neutrons at high dose rates*			*Low dose x-irradiation at high dose rates*	*High doses of gamma irradiation at low dose rates*	*Low dose fission neutrons at high dose rates*			
Dominant lethals[b] ..	860	Presumably as for translocations			—	9,000	—	—	—	0	—	—
Translocations[b]	330	1/4[c]	1/9	4	—	300	—	—	—	—	—	—
X-chromosome loss[b]	2	—	—	—	—	150	—	1/2	—	—	—	—
Specific-locus mutations[d]	1.7[e]	1/3?	1/3	6[f]	1.4[g]	5.4[g]	1/3	1/20	3 1/2	—	1.0[g]	7.7[g]
Autosomal recessive lethals[b]	900	—	—	—	—	—	—	—	—	—	—	—
Dominant visibles ...	5	—	—	—	—	—	—	—	—	—	—	—
Dominant skeletal mutations	110	—	—	—	—	—	—	—	—	—	—	—

Note: dashes indicate that no data are available.

[a] The figures given under these columns are to be used to multiply the absolute rates of induction to obtain rates under the conditions specified.

[b] Rate per gamete.

[c] From 25 rad up the factor is 1.

[d] Rate per locus.

[e] Based on 12 loci.

[f] For spermatogonia in embryos the figure is 2.

[g] Based on 7 loci.

TABLE 29. RISKS OF INDUCTION OF DIFFERENT KINDS OF GENETIC DAMAGE IN MAN PER RAD AT LOW DOSES OR AFTER CHRONIC EXPOSURES

End point	*Expected rate of induction per million*		*Expression in F_1 per million conceptions after spermatogonial irradiation*
	Spermatogonia	*Oöcytes*	
1. Recessive point mutations	1,500[a] (36)[b]	Very low —	30-75 (1-2)
2. Dominant visibles	2	—	2
3. Skeletal mutations	4	—	[c]
4. Reciprocal translocations[d]	15[e]	Very low	2 congenitally malformed children, 19 unrecognized early embryonic losses and 9 recognized abortions[f]
5. X-chromosome losses	Very low	8	8 early embryonic losses and/or abortions
6. Other chromosome anomalies	Very low	—	Very low
Total genetic damage	1,521[g] (57)[h]		
Total genetic damage[i]	300		6-15[j]

Note: dashes indicate that inadequate or no information is available.

[a] Estimate based on mouse specific locus data.

[b] Estimate based on the per genome rate for recessive lethals induced in mouse spermatogonia.

[c] Included under (1); see paragraph 594.

[d] Figures apply to low-dose x-irradiation. Estimates for chronic gamma-irradiation are 50 per cent lower.

[e] Balanced products.

[f] For low dose x-irradiation; for chronic gamma-irradiation, figures should be halved (see paragraph 621).

[g] Obtained by adding 1,500+2+4+15 in the column.

[h] Obtained by adding 36+2+4+15 in the column.

[i] Relative to spontaneous incidence of genetic diseases among live-born, based on an estimated "doubling dose" of 100 rad.

[j] In terms of incidence of genetic disease among live-born.

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Annex F

EFFECTS OF RADIATION ON THE IMMUNE RESPONSE

CONTENTS

Introduction

1. For many years it has been realized that whole-body irradiation has profound effects on the immune response of experimental animals, and, more recently, this has also been demonstrated in man. Since many types of radiation are now being frequently used in clinical treatment of patients and in experimental research, it is essential that more detailed information on the effect of irradiation on the different phases of immune responses be obtained. This particularly applies to the effects of single or multiple low doses of radiation, as it is becoming increasingly clear that the complex process of immunity is composed of several distinctly separate events, some of which involve very radio-sensitive cells.

2. The essential aim of a review of this type is to provide a means of estimating the risks to man from radiation-induced lesions in the immune system. At

the present time numerical risk estimates cannot be made in relation to the immune system. This annex will therefore merely attempt to evaluate the order of magnitude of the immune system's radio-sensitivity, on the basis of experimental and clinical observations involving mostly high radiation doses. As much of the experimental work is drawn from animal species other than man, some attention will be paid to species variation in order to evaluate the significance of extrapolation to man.

3. It is essential to realize that an analysis of radiosensitivity of the immune system is not simply a study of the radio-sensitivity of one cell type. The immune response as a whole comprises several distinctly different types of response with different cell types and modes of expression. These will therefore be examined separately and, in the course of this analysis, reference will be made to the possible medical uses of suppressing immunity by radiation to assist organ transplantation, and to the use of immunological methods in tumour therapy. This annex will cover each of the major types of the immune response which are detailed in the following paragraphs.

4. Detailed information on the events leading up to the release of circulating antibody has been obtained in the past few years. In many systems an antigen-processing step is obligatory before the antibody-forming machinery can be brought into action. Furthermore in many antibody responses the early events subsequent to antigen processing also may involve a collaboration between two ontogenically distinct haemopoietic cell lines. Thus at least three different cell types can be involved prior to the development of the actual antibody-forming cell. Since all three types are frequently obligatory for certain antibody responses, suppression of any one by irradiation will profoundly affect the over-all antibody response. As these three components may involve cells of different differentiation stages, it is possible that they may show differential radio-sensitivities. This review will accordingly attempt to analyse the radio-sensitivity of the humoral antibody response in terms of the sensitivities of the different components comprising the response.

5. The time interval between antigen administration and irradiation greatly affects the subsequent changes induced by radiation. Whereas it is more commonly found that radiation suppresses immunity, under some circumstances enhancement of certain aspects can be generated. Stimulation may be related to certain over-corrections in the controlling mechanisms following irradiation. A specific analysis of this point will be made, as it is relevant for consideration of radiation therapy in man.

6. Radiation induction of some animal tumours is thought to be mediated through an activation of latent viruses. As it has been clearly demonstrated that many of these tumours carry strong tumour-specific transplantation antigens, it is possible that a factor in the induction of tumours by radiation is the associated immune depression, which in turn permits a normally suppressable potential malignancy to become expressed. Since these experiments usually involve fractionated doses of radiation of the order of 100-200 rads, it is important to consider this phenomenon in terms of possible relevance to human neoplasia.

7. The effect of radiation on the state of immunological tolerance may also be in either direction, towards breaking the tolerant state or helping in the induction of tolerance. In recent years a new concept of two zones of antigen dosage for the induction of tolerance has emerged. Some studies suggest that low-zone tolerance may be involved in normal immunological homeostasis and that breaks in this mechanism may lead to auto-immune disease. It is therefore relevant for human studies to consider the effects of radiation on the state of tolerance, as radiation-induced alterations in this state may lead to auto-immune phenomena.

8. This annex will attempt to consider the effects of radiation in three main areas: (*a*) the normal immune response, specifically examining the various components of resistance to infection, the antibody-forming mechanism, transplantation immunity and delayed hypersensitivity; (*b*) effects of radiation on experimental tumour induction associated with effects on the immune state; and (*c*) the two zones of immunological tolerance, with specific reference to possible auto-immune consequences after alteration of the normal homeostatic condition. For definitions of immunological terms, the reader is referred to a glossary of immunology (231).

I. The general components of the immune response

A. Resistance to infection

9. Immunity has been associated with resistance to infection. In this context we are considering the ability of the body as a whole to check the large number of infectious agents and parasites that perpetually threaten life and health. The term infection is used here to describe the situation in which an organism enters into a relationship with the host such that the host's cells or tissues are frequently damaged. Resistance describes the relative ability of the animal to counteract the infection and not to succumb to the invading organisms.

10. Resistance has been frequently divided into natural and acquired resistance. Natural resistance generally refers to the resistance of animals not specifically immunized, or exposed, to the infection, whereas acquired resistance refers to the state of resistance which develops in animals following active or passive immunization or following exposure to the infection at a sub-clinical level. In general, acquired resistance is specific for a particular organism while natural resistance may be relatively non-specific. This implies that acquired resistance is therefore mediated by a specific immune response either cellular or humoral in nature. It is at this point that considerable confusion arises within the immunological literature. To students of infectious disease, cellular immunity refers to the form of acquired anti-microbial resistance in which the host's mononuclear phagocytes show increased destructive capacity for ingested organisms. This form of cellular immunity can be transferred with cells but not with serum (15). Although it is evoked by way of a specific immunological reaction, it is frequently non-specific in its anti-microbial effects for the period of a few weeks following antigenic challenge but, once established, it will be specific for the original immunogen (160, 258, 318).

11. Another use of the term cellular immunity refers to those immunological reactions that are mediated directly by lymphocytes and are not dependent on

secreted antibody. This includes most forms of transplantation immunity and delayed hypersensitivity and will be discussed in section I B. Recent studies have tended to bring these two alternative views of cellular immunity closer together, as lymphocytes as well as macrophages have now been shown (319) to have an important role in at least some types of resistance to infection, in that lymphoid cells play an inductive role in the immune response which is then primarily effected by the macrophages. In transplantation immunity, however, both lymphocytes and macrophages can be involved in the actual effector stage of killing target cells.

12. For the purpose of this report, this section and section II will deal with resistance to infection in which the animal as a whole is studied, or in which other processes apart from specific antibody formation or lymphocyte-mediated immunity are involved.

13. Resistance to infection is a broad field and has been the subject of several excellent books and reviews (67, 321, 402, 441, 626). It includes defined cell responses in which the macrophage is the essential cell type, antibody formation, possible role of eosinophils, and various non-specific phenomena. Specific antibodies can neutralize toxins, neutralize viruses, or prevent their entry into susceptible cells. With complement, and possibly lysozyme, lysis of bacteria can occur. Antibodies can promote phagocytosis of microorganisms by polymorphs, as can natural antibodies in natural resistance. A large role in natural resistance may be played by such non-immunological factors as unbroken cutaneous or mucous surfaces; free fatty acids with antibacterial properties on the skin; the sweeping action of cilia in the bronchial tree and by lysozyme and other humoral factors (204, 267, 513).

14. From the time of Metchnikoff (360), it was strongly felt that acquired resistance to infection resulted from "the perfecting of the phagocytic and digestive powers of the leucocytes". The important role of the macrophage has indeed been well documented (402), and little more need be said in this introductory section about the importance of this cell type, other than to stress one point concerning its heightened activity in acquired resistance. Although the formation of specific antibody can be an important factor in acquired resistance, it is also clear that macrophages from infected animals can show an intrinsic elevated functional activity, although non-specific methods of stimulating increased lysozymal activity of macrophages will not lead to increased functional activity against specific organisms. This is indicated by the fact that cells from infected mice will completely inactivate *Salmonella typhimurium* organisms within 15 minutes, whereas normal cells only partially inactivate, and do so in a much slower time (51). Furthermore, cells from animals infected with *Listeria monocytogenes* or *Salmonella typhimurium* are equally microbicidal for *Salmonella typhimurium*, despite the absence of demonstrable anti-*Salmonella* antibody in the serum or absorbed on cells of the *Listeria*-infected mice (51). It has also been reported that in the infection of mice with *Salmonella enteritidis*, immunization with a live vaccine (294, 386, 487) or convalescent immunity (431) achieves high resistance against further infection with a virulent strain of the same bacteria. It was noted in this immunity that cultured macrophages derived from either the peritoneal cavity, the subcutaneous tissue or the liver of immunized mice, resisted the cell degeneration caused by *in vitro* infection with virulent bacteria regardless of the presence of antiserum in the culture medium. Furthermore, the serum obtained from immunized mice did not show any passive immunization against fatal infection and had no inhibitory effect on the intracellular growth of virulent bacteria in macrophages cultured *in vitro* (385, 387, 487, 492). Such resistance was referred to as cellular immunity and was also described in some other infections with cytophilic bacteria such as tuberculosis, brucellosis and listerosis (188, 322). In more recent studies (320), it has been shown that acquired resistance may depend upon the activation of host macrophages through a product resulting from the specific interaction between sensitized lymphoid cells and the pathogen or its antigenic products. This finding may help bring together the two interpretations of the term cellular immunity, in suggesting that the enhanced macrophage response is dependent upon the cellular lymphocytic response.

B. Cellular and humoral immune responses

15. Before embarking on a detailed analysis of the effects of radiation on the immune response, it is essential to stress that "the immune response" is a rather general term embracing several different types of immune reactions observed in animals and man. Any consideration of the effects of radiation must, therefore, be made separately for each type of response and in some cases for the separate components of a given type of immune response. This does not imply that the different clinical forms of immunity, hypersensitivity and allergy are all mediated by different mechanisms, but rather that there are a few basically distinct mechanisms of immunity within which there may be many slight variations expressed in different species or under different conditions.

16. The two basic types of immune responses are: (*a*) humoral-antibody formation, which involves the production of circulating antibody molecules found either in serum or in other body fluids; and (*b*) lymphocyte-induced cellular immunity, in which the actual site of the immune reaction contains both lymphoid cells and macrophages.

17. Although there are many results and experiments which support this basic dichotomy, the most striking demonstration that these are two distinctly separate forms of immunity comes from studies in experimental chickens (106, 603) in which the differentiation of the immunoglobulin-synthesizing plasma-cell system is under separate ontogenic control (the bursa of Fabricius) (206, 392) from that of the lymphocyte-mediated cellular immunity (26, 271, 611). Thus, by embryonic bursectomy, animals can be obtained which are totally agammaglobulinæmic and cannot form any antibody (612) but which have normal delayed hypersensitivity (609) and transplantation immunity. This experimental demonstration of two separate types of immune response is also clearly evident in several human clinical syndromes, in which either antibody formation or cellular immunity is selectively depressed (105, 141, 407, 450, 474, 496).

18. A schematic outline of the immune response is given in table 1. Three of the major distinguishing features of the dichotomy of immunity are listed. Antibodies found in serum and other body fluids are primarily synthesized and secreted by cells of the plasmacytic series (57, 133, 304) and also by lym-

phocytic cells (*B* lymphocytes) (119, 220), which ultrastructurally, have the endoplasmic reticulum characteristic of an active protein-secreting cell.

19. The differentiation of the plasmacytic cell line is controlled by the bursa of Fabricius in chickens (105, 603). Several candidates for a bursal equivalent in mammals have been proposed, including Peyer's patches (104), appendix (23), tonsil (451), diffuse intestinal epithelium (179), and even skin (180). There is, however, no universal acceptance of any of these as bursal equivalent sites. For example, the immunological role of the appendix of rabbits seems to be directed only towards the differentiation of IgM-synthesizing cells and not towards the differentiation of cells synthesizing other classes of immunoglobulins (104, 233, 298). In contrast, the bursa of Fabricius has an important role in the differentiation of cell systems involved in the synthesis of all classes of immunoglobulins. It has also been reported that Peyer's patches in the rat (101) and rabbit (249) are directly involved in the synthesis of IgM antibody when antigen is directly injected into the patch or when Peyer's-patch cells are treated with antigen *in vitro*.

20. In the earlier work by Cooper *et al.* (104), it was observed that combined removal of the appendix, the *sacculus rotundus* and all the Peyer's patches in rabbits followed one month later by whole-body exposure to 650 rads resulted in the partial, but not complete, depression of antibody-forming capacity when challenged 21 days after irradiation. Thus, in at least some animals, restoration of the antibody-forming capacity following near-lethal whole-body irradiation did occur in the absence of the postulated bursal equivalent. In a comprehensive examination of germinal centres in the rabbit appendix, it was concluded (409) that it is essentially the germinal-centre compartment which is responsible for the delivery of antibody-forming-cell precursors and that, contrary to the view of Good *et al.* (214), the germinal centres of the gut-associated lymphoid tissue represent plain germinal centres like those in the spleen and lymph nodes.

21. By contrast, cellular immunity is induced by lymphocytic cells which are also found in the immediate vicinity of the active immune lesion, together with macrophages, as for example, in the infiltrate underlying a rejecting skin homograft (49, 497) or in various organs in auto-immune diseases (323). The actual mechanisms of lymphocyte-mediated pathological changes will be considered in a later section in relation to radio-sensitivity. The differentiation of the lymphocyte-dependent line in cell-mediated immunity is thymus-dependent in most animal species studied, including man (213, 215, 373), although in sheep this could not be demonstrated.

22. Humoral immunity is manifested in a variety of different clinical and experimental forms which can broadly be considered as either the production of antibody, resulting in high serum titres of antibody and a state of elevated resistance to certain infections, or as the production of certain molecular classes of antibody which are capable of initiating immediate hypersensitivity reactions and some auto-immune disorders. Antibody molecules can be subdivided into different immunoglobulin classes (90, 171, 194, 306), for example, in man, IgM, IgA, IgG, IgD and IgE which have in common the basic molecular form of two light (*L*) and two heavy (*H*) polypeptide chains (158, 461) but which differ in that different structural genes code for the constant regions of the *H* chains of the various classes (195). Certain biological properties of antibody molecules are mediated through sites on the C terminal half of the heavy chain (433), and since the classes differ in their heavy chains, a given biological effect is usually mediated by only one or a limited number of immunoglobulin classes. These properties include the fixation of antibody molecules to mast cells, which is at the basis of the anaphylactic and reaginic hypersensitivities (433) and is associated with certain specific immunoglobulin classes (IgE-mediated reaginic hypersensitivity in man (268, 274), gamma-G1-mediated anaphylaxis in mice (34, 428, 435) and guinea-pigs (434), and another separate unidentified antibody-mediated reaginic hypersensitivity in mice (595)). Another form of hypersensitivity leading to tissue damage is the Arthus reaction (involved in serum sickness and some glomerulonephritis) mediated by those classes of immunoglobulins that are capable of forming a precipitating complex with antigen in tissue sites (for example blood-vessel walls), which then fix complement components (88).

23. The role of antibody in the rejection of antigenic tumours has not yet been fully elucidated. Cytotoxic antibodies are those antibodies which fix complement and cause lysis of tumour cells. These have been suspected to be active against dispersed leukæmic cell suspensions *in vivo* (16). On the other hand, it has also been shown by Hellstrom and Hellstrom (247) that some serum factors can protect tumours *in vitro* from lymphocyte-mediated tumour destruction. The nature of these serum factors and their role *in vivo* remains to be elucidated. It is by no means clear whether these blocking serum factors are the same as enhancing antibodies, which have been conventionally demonstrated by their ability to enhance tumour growth after prior injection into recipients which are then challenged with tumour cells (275). Although one study (598) suggested that enhancing antibodies were electrophoretically fast migrating (and possibly IgG1), two other studies (266, 543) implicated IgG2 molecules, which are also capable of fixing complement.

24. Cellular immunity is broadly recognized in two basic forms: (*a*) rejection of tissue allografts such as skin or kidney, or (*b*) delayed hypersensitivity reactions, best typified by the Mantoux reaction to old tuberculin or PPD in individuals sensitized to tubercle bacilli. As mentioned in the previous paragraph, there are reports suggesting that not all forms of transplantation immunity are mediated directly by lymphoid cells. Tumour allografts presented in the form of single-cell suspensions can be rejected by circulating cytotoxic antibody (16), and immunological damage to some organ transplants such as kidney has also been claimed to be antibody-mediated (299).

25. The morphological and hæmatological representation of this distinction of immunity into cellular and humoral is diagrammatically represented in figure I in which it is indicated that a multipotent hæmatopoietic stem cell has the potentiality to differentiate into any hæmatopoietic cell system. The true stem cell may possibly differentiate initially into two types of stem cell—a lymphoid stem cell (190) and a second type with potential to form other blood elements. On the other hand, Nowell *et al.* (427) reported evidence indicating the existence of multipotential lympho-hæmatopoietic stem cells in the adult rat. In this study, rats were given near-lethal x-ray doses to produce

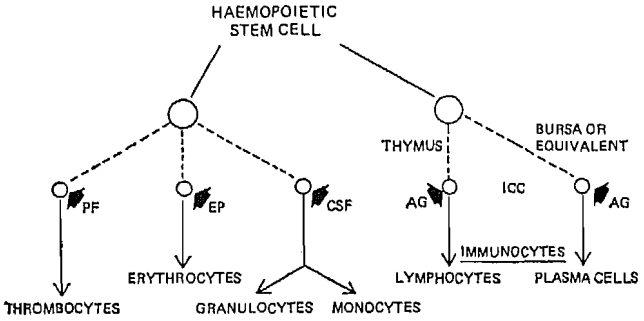

Figure I. Development of the hæmopoietic system from a common stem cell. The first division indicates a possible dichotomy involving a lymphoid stem cell. One type can then differentiate into thrombocytes, erythrocytes, or granulocytes and monocytes, under the induction of platelet factors (PF), erythropoietin (EP) or colony-stimulating factors (CSF), respectively. Immunocyte differentiation is from antigen (AG)-induced stimulation of immunocompetent precursor cells (ICC) which have differentiated under the control of thymus or bursa equivalent (bursa in birds only)

clones of hæmatopoietic cells marked by radiation-induced chromosome abnormalities. Subsequently, bone marrow from these rats was injected into lethally-irradiated mice to form erythropoietic spleen colonies, and peripheral blood lymphocytes from the same rats were stimulated to proliferate in a mixed lymphocyte interaction (MLI), an immunological response to histocompatibility isoantigens. Chromosome markers indicated that in several instances the cells of an erythroid spleen colony and a proportion of the lymphocytes reacting in the MLI were progeny of the same stem cell in the donor rat. In addition, lymphocytes of the same radiation-marked clone were shown to proliferate in response to several different histocompatibility isoantigens, suggesting that immunological specificity is determined during lymphoid differentiation, subsequent to the stem-cell stage.

26. Differentiation of the stem cell into lymphocytic elements is then directed by thymic induction, and differentiation into plasma cells by the bursa of Fabricius or its equivalent, although, as mentioned before, certain cells that are morphologically lymphocytes are also concerned with humoral immunity (*B* lymphocytes). Differentiation of stem cells into the erythroid series involves erythropoietin (182), whereas differentiation into granulocytes and monocytes involves a colony-stimulating-factor effect on a precursor cell (359, 524) and platelet factors are required for thrombocyte differentiation (442). The complete maturation into active immunocytes of lymphoid and plasmacytic immunocompetent cells then involves antigenic stimulation.

C. Stages within antibody formation

27. The injection of an antigen or vaccine into an animal is usually followed by a delay of a few days before detectable circulating antibody appears in the serum. During this period, several discrete steps leading to the production of antibody may be discerned. These can broadly be considered in three parts: (*a*) appropriate processing or handling of the injected antigen so that it effectively reaches the appropriate immunocompetent cell (the afferent limb); (*b*) the proliferation of certain immunocompetent cells and their interaction which, although involving specific antibody-like receptor sites on the surface of these cells, does not involve active antibody secretion (the inductive phase); and (*c*) the final process of differentiation of the plasma-cell line which progressively leads to a cell whose major function is the active synthesis and secretion of specific antibody (productive phase). These stages are schematically depicted in figure II.

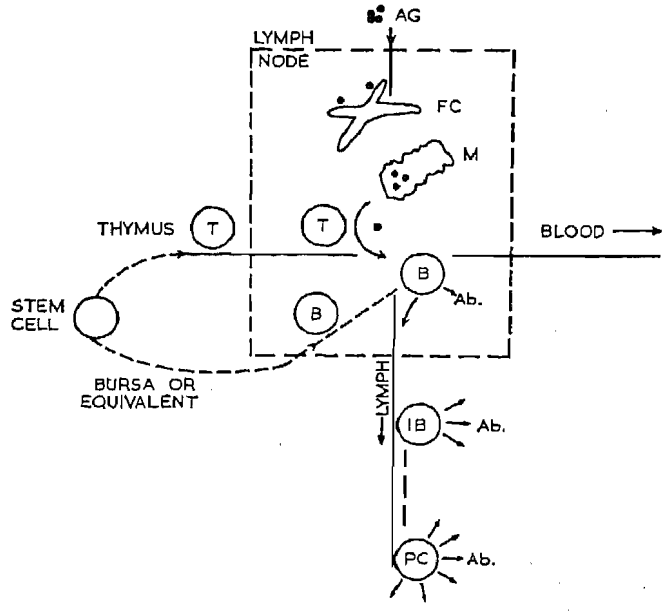

Figure II. Stages of antibody formation within a lymph node. The antigen (AG) often first requires "processing" by macrophages (M) or follicle cells (FC) (afferent stage) and then is transferred in a suitable form to the thymus-derived (T) and/or bursa (or bursal equivalent)-derived (B) lymphocytes for initiation of the immune response (induction). This stage may often require collaboration between the T and B cell types. The B lymphocyte then becomes the progenitor of the antibody (Ab) producing cell clone (efferent stage), first giving rise to immunoblasts (IB) or immature plasma cells, and thence to mature plasma cells (PC)

28. The relevance of this preliminary division of the immune response into separate stages to radiation susceptibility of immunity is that different cell types are involved in these steps and that these may show either over-all differences in sensitivity, or differences at critical stages of their function. The afferent limb involves granulocytic and macrophage cells which directly interact with antigens, and process or simply hold the antigen in a suitable manner for presentation to immunocompetent cells. The inductive phase then involves the presentation of this antigen or of some cellular product specific for the antigen to lymphocytic cells of thymic origin which then proliferate and may interact with another cell type (bursal-derived) which differentiates into the antibody-secreting cell line.

II. Effects of radiation on susceptibility to infections

29. Over the past eight years a vast body of literature has been assembled which repeatedly demonstrates one basic observation. Namely that, if an animal is given a moderate to high dose of radiation and is then challenged with an infectious agent, it will show increased sensitivity to the infectious agent. This observation has been made with virtually all experimental animals (and man), with most known infectious agents, including bacteria, viruses, protozoa, rickettsia and fungi, and with various sources of radiation and doses. Well over 1,000 such independent observations

have been reported and, as it would be extremely repetitious, these will not all be cited in this present document.

30. Of considerable relevance to this review is that increased susceptibility to infection is primarily caused by the decrease in immune responsiveness of the host. As several factors can influence the degree of increased susceptibility, this section will primarily concentrate on examining these variables with examples drawn from the abundant literature in this field. Probably one of the main things to stress is that exactly the same principles apply to radiation-induced immune depression, whether assessed by actual measurements of the immune response, or more indirectly by the death of the animal resulting from increased pathogen growth. In many instances, this latter estimation may be complicated by other factors and accordingly a direct relationship between the radiation parameter and true susceptibility is not observed.

31. Many reviews on the susceptibility of irradiated animals to infections are available (40, 55, 146, 150, 255, 365, 452, 453, 515, 534, 538, 551, 572, 648, 652, 659, 662, 678, 680). Approaches to this problem include assessment of the course of infection after irradiation following challenge with either (*a*) known pathogenic agents; (*b*) conditionally pathogenic agents (normal flora); and (*c*) no challenge but determination of the infection that spontaneously results. In considering the relevance of many of these data to man, it appears that the same principles found in animals also apply in man. For example, in one study (680) it was concluded from an examination of many species that radiation sickness in man closely resembled that observed in monkeys. In a study of patients with late-stage malignancy given whole-body irradiation, it was found (21) that the major cause of early death was infection, principally of gram-negative or fungal origin. Although there are a few other reports describing radiation infection in man, understanding of the basic principles have come from studies in mice, rats, rabbits, guinea-pigs, monkeys or dogs.

32. Antimicrobial immunity against infections in radiation sickness is so markedly impaired that susceptibility is increased not only towards pathogenic agents but also to bacteria which are part of the normal flora. These two aspects will now be considered, followed by examination of several variables such as timing of infectious challenge and radiation, and radiation parameters.

33. For more than 50 years (109) of experimentation in the field of immunology of infections associated with radiation sickness, investigators have determined the sensitivities of irradiated animals to various pathogens. For example, in a study by Yakovleva *et al.* (699) of 14 monkeys given approximately 4 10^{10} paratyphoid *B* organisms orally, only one died of paratyphoid. However, in monkeys also given 300 roentgens (in itself non-lethal in monkeys) four fifths died with paratyphoid five days later. In other studies of this type susceptibility to hæmolytic streptococci increased five times (689) and susceptibility to *S. enteritidis* increased hundreds of times (493) in mice exposed to 350 roentgens. A sharp drop in resistance to influenza virus has been demonstrated in experiments with irradiated mice and rats (682). Similar increases in sensitivity to gas gangrene organisms, to tetanus (673), to icterohæmorrhagic leptospirosis (673) and to tularemia (694) were observed in sublethally-irradiated mice. It is essential to note that any measure of increased sensitivity to an infection following irradiation will be accurate only for the given host, pathogen, and irradiation conditions. The over-all rule, however, is quite clear. The sensitivity of animals to microbes is markedly increased in radiation sickness.

34. In several studies with continuous exposure to low-dose-rate gamma radiation, increased susceptibility to chronic infections has also been observed. In studies in mice with *Listeria monocytogenes*, and using ^{60}Co gamma radiation at a dose rate of 1.0 to 1.5 rads per hour, it was found that the greater the total dose of radiation administered, the greater became the susceptibility (509). Mice receiving 500 rads were three times as susceptible as non-irradiated mice, while those exposed to 2,500 rads were approximately 30 times as susceptible. In an even more prolonged type of study (651), various animals were given continuous ^{60}Co gamma radiation at 1.2-4.3 roentgens per day, for 1.5-2 years. The cause of death of the irradiated animals was totally attributable to auto-infection with the development of septicæmia. Autopsy of these animals did not show the characteristic pattern of acute radiation sickness. The strongest disturbance of natural immunity occurred in young animals and particularly with radiation delivered during intra-uterine development.

35. In irradiated animals, the pathogenicity of conditionally pathogenic micro-organisms is often observed. For example, intravenous injection of doses of *B. proteus*, which are non-lethal in unirradiated mice, led to an increase in number of bacteria in the blood and to eventual death in mice given 400 roentgens three days previously (240). This phenomenon has also been demonstrated with colon and paracolon bacilli, *Pseudomonas aeruginosa*, type III pneumococci and many other bacteria which are non-pathogenic for normal animals.

36. In view of this striking increase in susceptibility of irradiated animals to both pathogenic and conditionally pathogenic organisms, it is reasonable to question whether irradiated animals might also become infected with an agent which characteristically does not infect normal animals of that species. In the main, the answer to this question is no. Species resistance to uncharacteristic infectious agents appears to persist (innate resistance). Thus Kolmer *et al.* (296) were unable to overcome the innate resistance of rabbits, guinea-pigs, rats and ferrets to poliomyelitis virus, despite the fact that the animals were twice irradiated. Many other examples of this type are documented in the review of Petrov (673), and include the agents for anthrax, tularemia, diphtheria, typhus, dysentery, typhoid and leptospirosis. The only exception that might be noted is that sensitivity to non-specific intoxication is increased after the injection of large quantities of microbial mass. It therefore appears that there is a high degree of stability of the animals' innate resistance to the effect of ionizing radiation in terms of certain infectious agents. In all probability, disease not characteristic of a given species does not occur even after irradiation. Irradiation is therefore incapable of abrogating the interrelationships which have been built up during the course of evolution between species of animals on the one hand and of micro-organisms on the other.

37. Although irradiated animals are severely compromised in their ability to undergo active immunization against bacteria and bacterial toxins, they can be satisfactorily protected by the use of passive immunization with antisera. This has been shown with diphtheria (659, 688), tetanus, and gas gangrene (653, 672). Although it has been claimed that irradiation does not change the rate of clearance of passively-transferred antibodies in syngeneic combinations, this has not been specifically evaluated with purified IgM and IgG antibody. In view of other observations on the loss of IgG and IgA through the irradiated gut wall (see paragraph 65), it might be expected that some loss of passive antibody would occur. Indeed, it has been shown that to obtain equal antitoxic effects in normal and irradiated recipients given passive antisera, three to five times more serum must be given to the irradiated recipients (659, 672). As was shown by Kaulen, an increased sensitivity to the complexes of toxin and antitoxin has also been observed in irradiated animals (689).

38. Increased susceptibility to virus infections following irradiation has also been observed frequently with many types of viruses including influenza, smallpox, ornithosis, mouse encephalomyelitis and mouse hepatitis. This is often seen as a shorter incubation period, more virus proliferation or more virus-induced pathogenic lesions, and is observed with sub-lethal doses of 200-500 rads. In several cases, however, the opposite result has been found, namely, a reduction in severity of the disease. On general grounds this might be expected on the premise that cell metabolism is markedly disturbed after irradiation and intracellular virus proliferation may be inhibited. Several examples from the earlier literature (211, 462) concern encephalitis in man, and show that alleviation of symptoms often resulted after radiation, possibly as a result of lymphocyte destruction. Similar results were also observed in studies of lymphocytic choriomeningitis in mice, a type of virus-induced auto-immune disease in mice whose pathogenetic basis is the induction of cell-mediated immunity. Mice exposed to 500 roentgens 24 hours prior to virus inoculation were protected for 48 days (256, 257), the depression of disease presumably being caused by inhibition of proliferation of the pathogenic lymphocytes.

39. Experiments for determining the time of increase in sensitivity to infection after irradiation can be divided into two groups: those showing an immediate increase in sensitivity, and those showing an increased sensitivity only after several days—usually about three days. In the first group, increased sensitivity to infection when given simultaneously with radiation has been shown for trypanosomes, plasmodia, influenza, yellow fever and tuberculosis (673). On the other hand, in a number of cases in which increased sensitivity to infection could be clearly demonstrated if the infectious challenge was given several days after radiation, no increased susceptibility occurred with simultaneous challenge. This includes studies with hæmolytic streptococci, pneumococci, staphylococci and colon bacilli. Irradiation after the infectious challenge leads to results similar to those in the first group (simultaneous administration). Thus, irradiation of mice three days after an inhalation of whooping cough bacilli led to a more serious infection than in control mice (684).

40. What is the reason for the existence of these two distinct timing relationships? The unifying concept is that these different results are related to the duration of the infectious process. Thus, those instances in which simultaneous challenge leads to increased sensitivity all involve chronic infections, whereas acute infections fall into the second group. In confirmation of this interpretation, it has also been found that irradiation after infection will aggravate a chronic infection, and that the difference in the two groups can be brought about with the same pathogen, if it is administered in ways which lead to either an acute or a lingering process.

41. In conclusion of this section, several points might be stressed which are derived from large numbers of individual reports: (*a*) radiation leads to increased susceptibility not only to pathogenic organisms (bacteria, rickettsia, parasites), but also to conditionally pathogenic ones (bacteria); (*b*) species resistance to infections that are not characteristic of that species is usually maintained in irradiated animals; (*c*) increased susceptibility to virus infections also results from radiation exposure, except in those cases where the cellular immune process is actually a part of the pathogenesis; (*d*) increased sensitivity to acute infections is only manifest if challenge is made at least several days after radiation, whereas simultaneous irradiation or irradiation after challenge is also effective with chronic infections; and (*e*) the majority of these consequences are mediated through the effect of radiation on the immune response. Accordingly, the duration of the period of reduced resistance to pathogens follows the period of immune depression and, as discussed in more detail in relation to the immune response itself, depends on many factors, such as the dose of radiation, the dose rate, and the animal species and its individual sensitivity to the particular infection.

42. The delayed consequences of radiation in man with respect to infection are not clearly defined at present. Considerable effort in this regard has been expended at ABCC and to date, with one exception, no relationship between a variety of infectious diseases and radiation has been documented. An analysis of mortality data among members of the Life Span Study Sample in both cities during the period 1950-1960 showed elevated ratios for all causes of death, all natural causes, leukæmia and other malignant neoplasms for persons located 0-1,399 metres from the hypocentre (269). Hiroshima males so located demonstrated a significant excess of deaths due to tuberculosis while Hiroshima females showed an increased frequency of deaths attributable to infectious or parasitic disease other than tuberculosis. These discrepancies were particularly marked during 1951-1952 and seemed to disappear thereafter. Periodic evaluations of the ABCC-JNIH Adult Health Study Sample have shown no clinical, radiographic or laboratory evidence of radiation-related infectious disease. Komatsu *et al.* (297) found no relation between absence from work and exposure dose in a group of male shipyard workers. A review of the ABCC autopsy experience also failed to document a consistent relationship between exposure status and inflammatory processes or infectious disease (22).

43. Finally it must also be stressed that immune depression is not the sole mediator of radiation-induced increased susceptibility to infection. It is almost certainly the major factor, but other components also play a role. Increased permeability of biological barriers has been demonstrated for the skin, the intestines and the blood-tissue barrier. Shortly after irradiation, even before

the development of an acute radiation syndrome, there is a depression of the bactericidal properties of the skin with respect to intestinal bacilli and other microbes applied to it (659). There is a decrease in the complement (655) and properdin levels (679) of the blood. These non-specific aspects have been discussed more fully elsewhere (673).

III. Effects of radiation on antibody formation

A. The afferent limb of antibody formation

44. The afferent limb of the immune response involves the handling of injected antigen in an appropriate fashion to ensure that some of it effectively contacts the immunocompetent cells. It is clear that the first cells to capture antigen are not the ones that synthesize antibody, although some of these cells—particularly monocytes and macrophages—do carry surface immunoglobulins adsorbed cytophilically from the serum (43, 59, 259). The amount of injected antigen is usually many orders of magnitude greater than the amount which ultimately reaches the appropriate lymphoid organ (416), and which then survives the initial degradation within macrophages (4).

45. The initial phase after antigen injection involves a diffuse distribution throughout the tissues without any special associations with the reticulo-endothelial system (4). The duration of this phase depends on the nature of the antigen, as some relatively poor immunogenic materials such as heterologous serum proteins may remain in a diffuse form for days, whereas bacterial products are usually rapidly cleared from the circulation. With particulate material, clearance is extremely rapid. Following its diffuse spread, the antigen is taken up by phagocytic cells, of which there are three main types: polymorphonuclear leucocytes, macrophages, and follicular reticular cells. As these three cells belong to slightly different, though interrelated, cell lines, we will consider their radiation sensitivity separately.

1. *Polymorphonuclear leucocytes*

46. Direct irradiation of polymorphs *in vitro* (498) or irradiation of whole animals appears to have no effect on the ability of polymorphs to phagocytose bacteria (517). However, if phagocytosis is permitted to occur and simultaneously the system is irradiated, increased bactericidal activity of the cell is observed (393). This enhanced killing has been shown to be due to an intracellular effect of irradiation, as irradiation of the cells after phagocytosis of the bacteria is also accompanied by an increased bactericidal activity (395). Furthermore, when active bactericidal fractions of polymorph homogenates are concurrently irradiated, the bactericidal activity is again increased (394).

47. Although the phagocytic capacity of polymorphs from *in vivo* irradiated animals is unaltered, they are not as efficient in killing ingested bacteria as are control leucocytes (647). The total H_2O_2 levels of polymorphs from these irradiated animals are higher, however, than those from normal guinea-pigs and, from the interpretation given above, they might be expected to be more bactericidal, not less. On a more detailed examination (440), it was found that polymorphs isolated from guinea-pigs three to five days after whole-body irradiation (100 R) showed decreased bactericidal activity, and that addition of foreign particles did not increase H_2O_2 production over resting cells as it did with non-irradiated cells. Although the total H_2O_2 content is elevated, the particle-associated (? lysosome) metabolic H_2O_2 is specifically decreased, possibly as a result of radiation-induced depression in production of H_2O_2 through the hexosemonophosphate shunt. Metabolic H_2O_2 thus seems to be more specifically related to bactericidal activity.

48. These results suggest that direct intracellular effects of radiation on the bactericidal properties of polymorphs can occur, being either suppressive or enhancing, depending on whether phagocytosis takes place at the time of, or later than, irradiation. This may therefore be one of the factors leading to increased susceptibility to infection after irradiation, even at exposures of the order of 100 roentgens. However, there is little evidence to suggest that polymorphs play any decisive role in the induction of antibody formation, although some claims have been made in this regard (521).

49. Irradiation also causes a profound depression of the production of polymorphs in the bone marrow by virtue of the destruction of the hæmatopoietic stem cells which are extremely radio-sensitive. This is clearly seen in an analysis of the *in vitro* colony-forming cells which are the precursors of macrophage and granulocytic progeny and which show a D_{37} survival dose of approximately 85 rads (79, 473). Within 6-8 hours after irradiation, a temporary rise in blood polymorph levels was observed, the mechanism involved being unknown (229). Regeneration of normal levels of *in vitro* colony-forming cells in the bone marrow takes about 16 days after 250 rads (229).

50. Studies in experimental animals have demonstrated that the hæmatopoietic stem cell is the essential precursor cell of the entire hæmatopoietic system, and if all cells of this type were completely inactivated by irradiation, then all activities of the immune system which are dependent on a continual input of differentiating stem cells would eventually fail. However, the reserve of stem cells in the body appears to be such as to outweigh any possibility of its complete eradication with moderate doses of irradiation. Following a dose of 150 rads all parameters of hæmatopoiesis had recovered to at least normal values by 7-8 days (150). On a daily schedule of 50 rads following an initial 150 rads, it required at least a further 250 rads to reduce stem-cell repopulating activity to 5 per cent of control values, which still represents a massive reserve of potential hæmatopoiesis.

2. *Follicular localization of antigen*

51. Primary lymphoid follicles in both spleen and lymph nodes represent rounded densely-packed collections of small lymphocytes in close relationship to a "web" of cytoplasm derived from specialized dendritic reticular cells. The web contains fine cytoplasmic strands with small spaces between them and no definite association with reticulin fibers (377). These cytoplasmic processes set up a very complicated three-dimensional network in the interstices of which many blast lymphocytes are found. The dendritic cells have few free ribosomes and an almost complete lack of lysosomes and of phagocytic inclusions (364) and the very thin cytoplasmic processes can be seen to form closely connected interdigitations with thin processes from primitive lymphocytes. After deposition of antigen in this webbed distribution, a germinal centre may form in the follicle with the original rounded web

being compressed into a crescent cap as the rapidly-dividing lymphoid cells proliferate.

52. These follicular antigen-capturing cells differ markedly from macrophages in their handling of injected antigen. With ^{125}I-labelled flagellar antigens and using electron microscopic autoradiography, it was shown (380) that a substantial proportion of the antigen localized in lymphoid follicles is not actually phagocytosed. These reticular cells retain antigen on the surface of their long dendritic processes where intimate contact is made with lymphoid cells. A similar finding has also been reported for germinal centres in lymph nodes of guinea-pigs injected with ferritin (340).

53. As will be discussed later, medullary macrophage phagocytosis of antigen is virtually unaffected by radiation. However, the retention of antigen in follicles can be profoundly affected by sublethal whole-body x-irradiation. The cytoplasmic fibril web is itself extremely radio-resistant since little direct damage could be observed with doses less than 1,250 rads, and it took 8,000 rads to destroy the structure completely. However, the process of follicular localization and retention of antigen was affected with exposures of 450 roentgens (272). Spleen autoradiographs and whole-organ counts showed that the follicle web was abnormally small, perhaps as a result of its collapse with the radiation-destruction of the lymphoid cells, and that a continuous cortical rim of antigen persisted in the lymph node, possibly indicating a radio-sensitive active process which is normally involved in the movement of antigen from the subsinus region into the follicle. Total retention of antigen in lymph nodes was not reduced, but was severely impaired in the spleen. This latter observation is perhaps more relevant, as initially all splenic antigen localization is in the follicles (420), whereas medullary macrophages are also very prominent in lymph-node antigen localization.

54. Localization of antigen in lymph-node follicles was further studied in rats exposed to whole-body x-irradiation (800 R) (624). This exposure markedly reduced the ability of the lymphoid follicles to retain antigen but did not affect the antigen uptake by the whole lymph node or the uptake by phagocytic cells of the medullary sinuses. It was then found that administration of specific antiserum to the antigen used, or even of larger doses of normal isologous serum, would result in significantly-improved follicular-antigen uptake when assayed 10 days after irradiation. Shielding of the popliteal nodes at the time of irradiation also improved follicular antigen uptake. It was suggested that the follicular antigen-trapping mechanism is extremely sensitive to changes in the level of serum opsonins and that substances present in normal serum act as follicular opsonins. Accordingly, the decreased follicular localization of antigen after radiation may be due to a decline in these opsonic materials, which must therefore be secreted by radio-sensitive cells (? lymphoid cells) in the lymph nodes. This point will be considered in more detail in a following section.

55. What role this impaired antigen trapping plays in the primary immune deficiency of irradiated animals is not clear, particularly as at least some antibody responses can be initiated in the total absence of follicular antigen localization (301). However, follicular trapping may be of considerable importance in the development of immunological memory (564) or continuance of the immune response, and thus its decline could play a major role in antibody depression. A specific study of this possibility has been made (403) in mice subjected to a whole-body exposure of 600 roentgens. The antigen-capture and retention capacity of lymphoid tissue, in particular of the germinal centre stroma, was found to be radio-sensitive, with maximum damage being evident about two weeks after 600 roentgens. Recovery was slow, taking several weeks to be complete. Preliminary electron-miscoscope evidence seems to indicate that the defect in antigen trapping may be attributed to direct damage of the antigen-capturing reticular cells, whereas a role of opsonic factors was not suggested in this study.

3. *Macrophages and the reticulo-endothelial system*

56. Mononuclear cells, of which the macrophage is the common free form and the Kupffer cell is typical of the fixed form, constitute the third and, in terms of antibody formation, the most important group of phagocytic cells. Macrophages take up particulate and soluble antigens within minutes of injection (71). As shown by electron-microscope studies (619), this involves the phagocytic and pinocytic vacuoles becoming surrounded by Golgi vesicles and lysosomes, with fusion to form a complex phagolysosome. Progressive digestion occurs in these vacuoles, but remnants of antigen persist for months. In quantitative studies (585) it has been shown that, although at least 90 per cent of the antigen is actually lost from the macrophage, it still retains its normal immunogenicity.

57. In general, x-irradiation in the LD_{50} range has not been found to affect phagocytosis or antigen degradation of a variety of substances in several species examined (33, 186, 196, 402, 628). Furthermore, macrophages in lymphoid tissue have been noted to be very active in phagocytosing the debris of cells damaged by x-irradiation (62, 514). In one study (445), x-ray exposures of up to 50,000 roentgens caused only a 15 per cent reduction of the engulfing capacity of isolated peritoneal macrophages. The migratory activity of macrophages is also quite radio-resistant (397). The capacity of phagocytes to replicate is, however, as radio-sensitive as that of any other cell population, and although only 1-5 per cent of a phagocyte population appears to be undergoing cell division, this could lead to a decrease in phagocytosis as a function of time following high doses of x rays.

58. In contrast, several reports have indicated that the phagocytic activity of animals can be reduced by whole-body irradiation. Several of these reports show impaired intravascular clearance of bacteria (82) or colloidal material (553) after whole-body irradiation, an impairment that can be considerably reduced by hepatic and splenic shielding during irradiation. Radiation-induced depression of phagocytic activity has also been demonstrated for macrophages from lung (646), intestinal wall (Fridenstein quoted in reference 689) and *in vitro* culture (689). Several reports (142, 196) indicate that a different tissue distribution of injected material may occur following radiation, without affecting the over-all phagocytic removal or rate of clearance. In a recent detailed study (482) the phagocytic activity of rats was significantly impaired after whole-body x-irradiation (800 R). The degree of depression was related to the post-irradiation time interval and was associated with a highly significant decrease in hepatic and splenic phagocytosis. In contrast, the lungs of the irradiated rats showed a significantly greater accumulation of the injected colloid.

59. Several early reports suggested that although no effects on actual phagocytosis or uptake of antigen by cells were induced by radiation, other subtle changes in the irradiated macrophages might occur. Donaldson *et al.* (147) and Kakurin (647) found that macrophages from irradiated animals had a depressed ability to digest intracellular material, and Gordon *et al.* (216) observed that reappearance of live organisms in the blood of irradiated rabbits occurred after a period of normal clearance, although Benacerraf *et al.* (41) found a normal breakdown of a denatured protein in the Kupffer cells of irradiated mice.

60. These results variously suggest that actual phagocytosis may in some instances be affected by irradiation (possibly mainly in liver and spleen) whereas in other cases, although the engulfment of material is normal after irradiation, changes in the normal intracellular digestion of the ingested material may occur as a result of radiation-induced enzymatic changes to the cell. These two stages will now be considered separately, in terms of the radio-sensitivity of phagocytosis as associated with opsonin changes, whereas changes in the actual fate of the ingested antigen will be considered in the light of the subsequent ability of macrophage-processed antigen to trigger the antibody response.

4. *Opsonins and immunoglobulins*

61. It is well established that serum or plasma factors, called opsonins, can augment the phagocytosis of both soluble and particulate material (479, 481). Accordingly, it is possible that depression of phagocytosis by radiation could be mediated through depression of opsonic activity or concentration. In some reports, sublethal irradiation has been shown to depress natural antibody formation with a rapid rate of decline of serum levels (550). This short half-life suggests that the globulins may have been IgM macroglobulins, which in some cases have been formally shown to be responsible for opsonic activity (469). Furthermore, there is mounting evidence that lymphocytic cells may synthesize small amounts of IgM molecules (56, 590, 604, 606, 607) which may be responsible for some or all of the serum opsonin. As these cells are relatively radio-sensitive, opsonic concentration in serum might thus be expected to decrease following radiation. The capacity of spleen cells for the total synthesis of IgG and IgM immunoglobulin was studied quantitatively with cells cultured *in vitro* (668). Mice received a dose of 500 rads and their spleens were extracted for culturing 1 to 12 days later. The rate of immunoglobulin synthesis was reduced by 80 per cent for a period of one to six days, but by the ninth day had over-compensated to a value of 70 per cent in excess of the control.

62. Decreased opsonin activity was proposed to be the most plausible mechanism for radiation-induced changes in follicular antigen uptake (624). Normal serum was shown significantly to improve follicular antigen uptake in irradiated animals as was specific antibody to the antigen. Shielding of the lymph node could lead to protection by either preventing direct damage of the cells, or by preserving some lymphoid cells which could continue to release opsonin. Furthermore, follicular localization of antigens is greatly accelerated by the passive transfer of specific antibody (417). The finding (250) that autologous immunoglobulins themselves tend to localize in follicles suggested that the opsonin coating might be a critical factor for follicular trapping. These results indicate that a serum factor, presumably specific antibody, whether "natural" or immune, is very important for follicular antigen localization.

63. In certain cases serum obtained from irradiated animals was slightly more effective in augmenting phagocytosis than normal serum (483, 490), and in another study radiation of young chickens failed to inhibit the production of natural opsonins (519). These apparent contradictions with other reports might indicate that generalizations are not valid in this context, and it might be proposed that, for some antigens and some species, the opsonic factor is produced by a long-lived, more radio-resistant cell, perhaps a mature non-dividing plasma cell.

64. In a detailed study (482) of rats in which the over-all clearance of a gelatinized ^{131}I-labelled triolein was shown to be reduced after irradiation and to be specifically associated with decreased splenic and hepatic phagocytosis, analysis of serum opsonins has been performed. Opsonization of the test material prior to its injection into irradiated rats significantly enhanced its clearance and therefore appeared to suggest a recovery of phagocytic activity. However, the enhanced clearance was found to be due to greater localization of the colloid in the bone marrow and no changes were found in liver or spleen. This emphasizes that even in the presence of elevated opsonic serum activity, x-irradiated animals can still manifest reticulo-endothelial depression in liver and spleen. This suggests that radiation-induced depression of phagocytosis in liver and spleen may be due to a direct effect on the macrophages in these sites. It also implies that different phagocytic cells may manifest variable radiosensitivities and therefore that their tissue distribution is required to analyse a given situation.

65. If all opsonin activity was associated with IgM macroglobulin, it might be expected in at least some cases to find lower IgM levels in irradiated animals. However, whereas IgG and IgA are depressed in mice exposed to 700 roentgens, IgM levels are virtually unaltered (38). Similar effects have been found when only the gut of mice was irradiated, whereas whole-body irradiation with the gut shielded caused only a slight change in serum immunoglobulins (37). Hence the characteristic early responses of the total serum-immunoglobulin levels to irradiation appear to result from x-ray damage to the intestinal epithelium and the consequent increased immunoglobulin secretion into the gut.

5. *Macrophage-antigen transfer studies*

66. The various reports described above have dealt primarily with specific studies of irradiated macrophages and their handling of antigens. In this section we will consider studies in which the process has been extended further in relation to immunity, and ask: in systems where macrophages are important for the elicitation of antibody formation, will irradiation of the macrophages interfere with the inductive phase of the immune response? This question can best be approached through the use of cell-transfer studies. Using radiation doses which will significantly suppress antibody formation in the whole animal, several groups have investigated whether normal macrophages will restore antibody-forming capacity to these animals.

Since cell suspensions are rarely completely homogeneous for a given cell type, it is important to control for possible contamination of macrophage preparations by immunocompetent lymphocytes.

67. Studies by Gallily and Feldmann (177) have indicated that the essential function of the macrophages in the induction of humoral-antibody formation can be destroyed with a sublethal exposure of 550 roentgens. Normal C57BL mice were given whole-body irradiation (550 R) and were found to be virtually incapable of making antibody to *Shigella* antigen. However, when macrophage preparations which had been pre-incubated *in vitro* with *Shigella* were given to irradiated mice, considerable antibody production then occurred. This activity was not due to contaminating lymphocytes since transfer of pure macrophage preparations also gave similar results and, if mice exposed to 900 roentgens were used as recipients, no restoration took place. In this latter case, if lymph-node cells were combined with the *Shigella*-treated macrophages and transferred to the irradiated recipient, antibody production could then occur.

68. A critical experiment was then performed with donor macrophages which were themselves derived from irradiated mice (table 2) (177). These were incubated *in vitro* with *Shigella* and transferred to recipients that had been exposed to 550 roentgens. Almost complete depression of the ability to transfer a capacity for antibody production occurred with irradiated (450 R) donors, and a significant depression occurred even with donors exposed to 150 roentgens. Macrophages irradiated *in vitro* and then incubated with *Shigella* also had lost the ability to aid in the induction of antibody formation. These results strongly indicate that with *Shigella* antigen, sublethal doses of irradiation will markedly interfere with induction of humoral-antibody formation as a result of a direct effect on an intracellular process (rather than inhibition of phagocytosis) of the macrophages.

69. A similar conclusion was reached by Pribnow and Silverman (465) who showed that both BCG-sensitized macrophages and normal lymph-node cells were required to restore antibody-forming capacity to rabbits exposed to 450 roentgens, whereas neither cell population alone would do so. It appears that in these rabbits 450 roentgens affected the lymphoid cells as well as the macrophages, whereas in Gallily and Feldmann's experiments with mice 550 roentgens did not sufficiently deplete the lymphoid component (compartment) but markedly affected the macrophages.

70. In some other studies, no radiation damage could be shown to the macrophages required for the induction of an antibody response. The critical difference may be solely in that a different antigen has been used. Ellis *et al.* (163) investigated the restoration of antibody response to sheep red blood cells in rats given different doses of x rays. Their results showed that even with lethal doses of radiation (1,000 rads), syngeneic lymphocytes were able to restore an impressive hæmolysin response in the irradiated animals, thus indicating radio-resistance of the host macrophage, as other studies have clearly indicated that macrophage processing or treatment of antigen is essential for the antibody response to sheep red cells (507). Gershon and Feldmann (201) investigated the response to sheep red cells in mice and could find no reconstitution of sublethally-irradiated mice with macrophage-ingested sheep red cells, again suggesting that another non-macrophage cell type had been acutely depressed by irradiation even with sublethal doses.

71. Mitchison (382, 383) has shown that a suspension of bovine serum albumin (BSA) containing macrophages is extremely efficient in priming mice for an antibody response to BSA, much more so than the free BSA. It was reported that the ability of this macrophage-bound BSA to prime mice was relatively radio-resistant. Spitznagel and Allison (523) also showed that macrophage-phagocytosed BSA (MBSA) is far more immunogenic for mice than comparable doses of free BSA. When the MBSA was given to mice exposed to 600 roentgens 24 hours previously, no antibody response occurred. If the recipients were also given 20 million normal lymph-node cells, good anti-BSA responses developed, suggesting that either MBSA can substitute for macrophage-processed antigen, or that only lymphoid depletion had occurred in the irradiated recipients and that macrophage activity is radio-resistant.

72. Although it is now quite clear that macrophages do play an important role in the induction of immune responses to many antigens, particularly in those cases involving large particulate antigens, the mechanism whereby they act is by no means elucidated. In view of the controversy in the literature on their radio-sensitivity, which in essence seems to say that for some antigens macrophages are very radio-sensitive and for others are very resistant, it is difficult to pinpoint a specific radio-sensitive stage in macrophage-antigen handling in general.

73. Various studies have recently indicated that RNA fractions from macrophages that have ingested antigens will transfer the ability to make antibody to normal lymphoid cells (183, 184, 185). In many cases this may be due to the presence of an antigen-RNA complex (25) containing minute amounts of antigen, which alone would not be immunogenic. The alternative possibility is that a true messenger RNA fraction coding for the specific antibody can be obtained from the macrophage preparation and transferred to normal lymphoid cells. Similar results have been obtained with mRNA fractions derived from macrophage-free lymphocyte preparations (12) and this raises the possibility that the mRNA fractions obtained from macrophages may in fact have been derived from a small contaminating population of lymphocytes. Such a possibility has been borne out in at least two reports, both involving allotype markers as evidence of transfer donor immunoglobulin to messenger (7, 39). Recently, Yamaguchi *et al.* (635) reported that a minimum dose of immunogenic RNA, which was derived from spleens of mice immunized with *Salmonella* flagellar antigens and was capable of transferring the immunity against the test antigen to normal mice, did not reveal an evidence of antigen contamination. In this study, it was shown that this immunogenic RNA fraction failed to initiate a secondary response to the test antigen when injected into animals that had been primed with immunogenic RNA or *Salmonella* flagella, while normal mice treated with immunogenic RNA were able to initiate a secondary response upon challenging injection of the test antigen. Regardless of the nature of the material presented by the macrophage to the lymphoid cell (free antigen, an antigenic fragment, an RNA-antigen complex or antigen-free RNA), there still remains the problem of how the material reaches the reactive lymphoid cell. In one study with hæmocyanin

(585) in which the material bound to macrophages was extremely immunogenic, it was proposed that the superior activity of the macrophage-bound antigen might be associated with a membrane-bound fraction which would have a far greater probability of contact with lymphoid cells, in much the same way as dendritic follicular cells are thought to interact with lymphoid cells. However, in experiments with larger hæmocyanin molecules, macrophage-associated antigen was less immunogenic than free antigen (443).

74. At the present time, the possible role of macrophage depression in reduction of the inductive phase of the immune response with relatively low doses of radiation appears to be uncertain. Since its importance has been strikingly demonstrated in at least one system (177), which is perhaps the most closely related to human resistance to infection of all the experimental systems studied, further studies with many different antigens, particularly bacterial antigens or organisms, rather than "laboratory antigens" such as heterologous serum proteins and erythrocytes, should be made in order to determine whether antigen processing by macrophages is a radio-sensitive phase which might account for radiation-induced depression of the immune response to many antigens.

B. The inductive phase of the antibody response

75. The antibody-forming plasma cell is a highly differentiated cell with the major function of secreting antibody and having virtually no prospect for further division. As will be discussed later, this cell is relatively radio-resistant. However, it is clear from a large body of data on the suppressive effect of radiation on antibody formation that there are earlier stages before the formation of the actual antibody-forming cell which are acutely radio-sensitive. In this section we will consider the origin of the antibody-forming cells and the radio-sensitivity of these precursor cells at stages before and after antigen-induced differentiation. Recent evidence of a collaboration between two cell types in the induction of many humoral-antibody responses has been obtained (84, 85, 122, 371, 384), and it is therefore most important to consider separately the radio-sensitivity of these two components. However, as most of the literature on radiation sensitivity of primary antibody formation was produced prior to the formulation of this recent collaboration concept, only a general consideration of this separation will be possible. Before discussing the actual radio-sensitivity of the early antibody response, it is relevant to consider briefly the origin of the immunocompetent cells.

1. *Radiation and the genesis of the immunocompetent cells*

76. In following the complete lineage of the antibody-forming cell, there is a striking demarcation into two stages. These are illustrated in figure II and are functionally distinguished as pre- and post-antigenic stimulation. In this section we are concerned with the radio-sensitivity of the precursor cells which have not yet been confronted with antigen. The true self-perpetuating cells, the hæmatopoietic stem cells, reside principally in the fœtal liver and in the bone marrow in adult animals and to a lesser extent in the spleen. Cells then travel via the circulation and may enter the thymus (390). In a manner which is as yet not entirely elucidated, these stem cells are induced to differentiate along the lymphoidal line and are thus rendered immunocompetent. A similar process (103, 389, 604) occurs with stem cells which enter the bursa of Fabricius in chickens, and the as yet unidentified bursal equivalent in man and other mammals. However, in this latter instance, differentiation into an immunocompetent cell involves the synthesis and expression of IgM molecules on the cell membrane (103, 566, 606). This IgM molecule may act as the recognition unit for antigen (45, 100, 468, 535, 539, 606). Particularly during early life (414), but also to a lesser extent throughout later life (311, 618), the potentially-immunocompetent cells then leave the thymus or bursa (or its equivalent) and form the recirculating pool of lymphoid cells (219) that move from peripheral lymphatic tissue via the lymph into the circulation and back into the lymphoid tissue. It is at this latter level that antigenic stimulation occurs and induces the formation of the true immunocytes, the effector lymphocyte of cellular immunity, and the antibody-producing cell. Since proliferating cells are the most susceptible to radiation destruction, three levels of acute radiation-sensitivity are suggested in this differentiation scheme.

77. The first level is represented by the hæmatopoietic stem cell which is capable of repopulating the bone marrow, the thymus and ultimately the peripheral lymphoid tissue of irradiated animals (363). This cell type is self-perpetuating, as has been shown by the *in vivo* colony-forming assay of Till and McCulloch (569), and is extremely radio-sensitive, with a D_{37} of around 95 rads (351, 511). Lethally-irradiated mice can be restored by injections of hæmatopoietic cells derived from *in vivo* hæmatopoietic colonies (571) and these recipient animals will eventually regain the capacity for humoral-antibody formation. However, as implied in figure III, recovery of immunocompe-

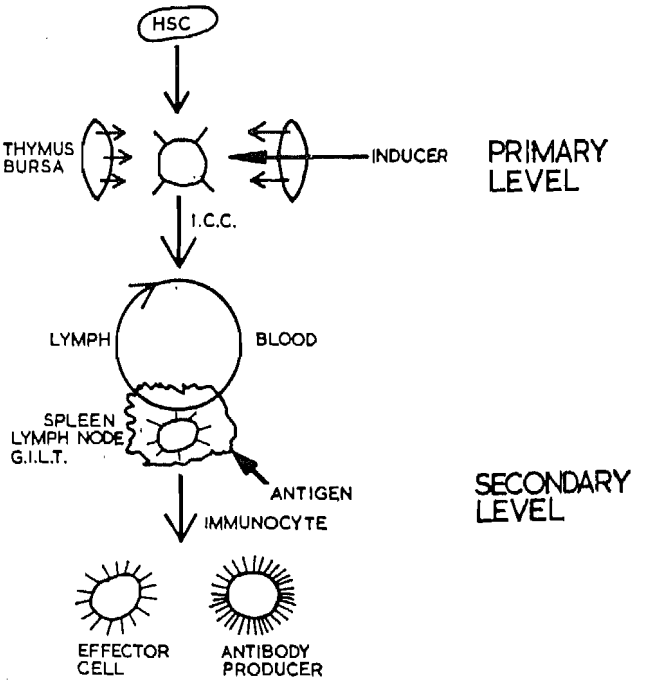

Figure III. Genesis of immunocytes. Hæmopoietic stem cells (HSC) are first directed toward immune pathways by inducing agents in primary lymphoid organs. They then emerge as immunocompetent cells (ICC) which enter the lymphocyte recirculation route passing through the lymphoid organs (including gastro-intestinal lymphoid tissue G.I.L.T.) from blood to lymph and back. It is in these peripheral organs that the second antigen-directed level of differentiation takes place, leading to the development of the antibody-producer or the effector lymphocyte of cellular immunity

tence after irradiation and injection of hæmatopoietic stem cells will only occur if the host animal has an intact thymus gland or a source of thymic inducer (114, 207).

78. The second stage of proliferation involves the lymphoid cells within the thymus (358) and the bursa of Fabricius which have been derived from the proliferating stem cell. X-irradiation causes a rapid involution of the thymus, and was in fact once used as a treatment for the spurious "*status thymolymphaticus*", a condition observed when a large thymus shadow was seen in the chest x-ray of a child (58). This procedure is not only of no benefit, since it is now well realized that the thymus is normally at its largest size in early life (358), but is actually dangerous as some irradiation of the adjacent tissue may lead to development of malignancy (see annex H). X-irradiation of the human thymus causes a rapid thymic involution and shrinkage, with regrowth occurring within a week (70). This effect not only involves a depression of the relatively-high proportion of dividing cells in the thymus, but also a direct lymphocytolysis of thymic lymphocytes (148). The stress of x-irradiation, resulting in an increased cortisol secretion (439), which is known to rapidly lead to thymic-lymphocyte destruction, makes a small contribution to this process.

79. This indirect action of x-irradiation on the adrenal gland leading to some steroid release might possibly affect the immune response more directly (apart from thymic cell destruction). Several studies (36) have demonstrated the sensitivity of antibody production to corticosteroids, and this has also been demonstrated recently *in vitro* (238), with the thymus-derived lymphocytes possibly representing the target for this effect (see also paragraph 298).

80. The third stage of active cell proliferation comes after presentation of the antigen to the immunocompetent cell. This rapidly leads to cell proliferation and accordingly to radio-sensitivity of this phase. This aspect will be considered in two sections: (*a*) the radiation sensitivity data, in which the immune response as a whole is discussed, and (*b*) the limited data available on the recently-demarcated two-component cell collaboration in antibody formation.

81. Radiation can therefore affect the differentiation sequence at three main points of cell proliferation: the hæmatopoietic stem cells; the early-differentiated cells in the thymus; and the antigen-stimulated immunocompetent cells. As all of these cell types may look morphologically like small lymphocytes, examination of the radio-sensitivity of lymphocytes as a distinct morphologically-defined population does not permit a clear demarcation of possible differential radio-sensitivities in these three compartments.

2. *Radio-sensitivity of the early primary immune response*

82. One of the most radio-sensitive phases of the immune response appears to be associated with the process of early induction (415, 546). Many authors (75, 145, 332, 333, 516, 532, 544, 673, 688) have reported on measurements of the radiation sensitivity of the antibody response, and, although somewhat different systems were studied in each case, their radiation sensitivities were similar and clearly indicated that cell proliferation must be an essential feature of the early immune response (330, 333). As it would be redundant to consider all the reports on radio-sensitivity of the early antibody formation (reviews in references 332, 531, 534, 540, 550, 551, 673, 688), we will consider in some detail only a few cases which clearly demonstrate the magnitude of the radio-sensitivity of the early phase.

83. The existence of an early radio-sensitive phase which rapidly moves into a radio-resistant phase was clearly shown by Dixon *et al.* (145) who irradiated rabbits two days prior to the injection of ^{131}I-BGG. A slight inhibition of the antibody response was observed with exposures of 75 roentgens or less, whereas 125 roentgens resulted in a considerable depression and 200-300 roentgens prevented the formation of all but traces of detectable antibody.

84. Makinodan *et al.* (333) tested the ability of spleen cells transplanted into lethally-irradiated mice (800-900 R) to produce hæmagglutinin against sheep erythrocytes when the donor spleen cells were derived from mice which themselves had been subjected to varying doses of radiation three hours before preparing the cell suspensions. The results showed that 37 per cent of the original antibody-forming activity remained after 130 roentgens. Based on the straight-line portion of the inactivation curve, the D_0 value was calculated to be 70 rads. Using a somewhat similar system, Celada and Carter (75) obtained a value of approximately 47-57 rads for this parameter. The immunization of mice with sheep erythrocytes one day after irradiation with an $LD_{50/30}$ reduces to 1 per cent of normal the number of antibody-producing cells accumulated in the spleen (662a, 676). Determination of the dose-effect relationship for spleen cells irradiated *in vitro* and subsequently placed *in vivo* together with sheep erythrocytes yielded a value of D_0=125 rads for the case n=1 (692). The radiation inactivation of immunity as shown by Makinodan *et al.* (333) and Simic *et al.* (510) is graphically represented in figure IV. As

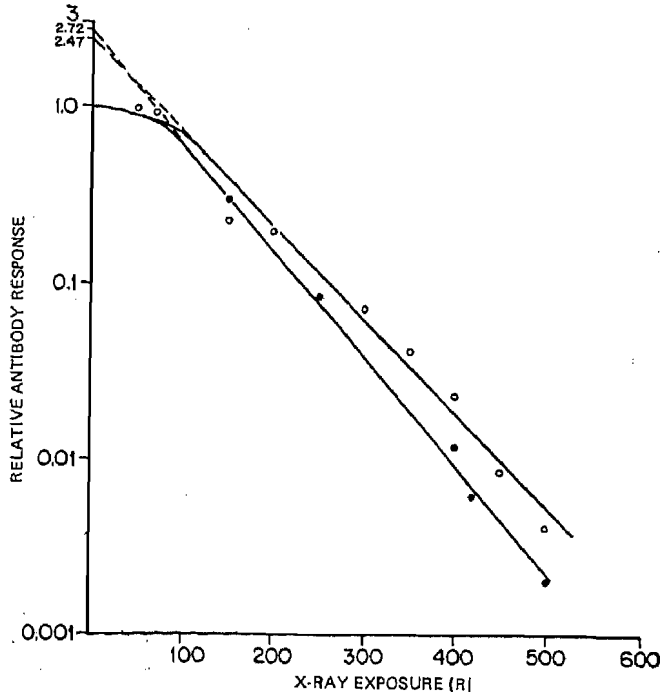

Figure IV. Inactivation of the immune response in rats (510) (open circles) and mice (333) (solid circles). Spleen-cell suspensions from donor mice given the indicated x-ray exposures were transferred to lethally-irradiated syngeneic recipients together with antigen. The resulting immune response is plotted relative to that given by control unirradiated donors

emphasized above, these data strongly indicate that the most radio-sensitive cellular event in the initiation of an antibody response is cell proliferation.

85. In several other biological systems (28, 46, 112, 459, 525, 652), it has been claimed that resistance to radiation is increased in animals repeatedly exposed to ionizing radiation. The population of immunocompetent cells in the body is one that is in a state of flux, showing a continual exponential increase until young adulthood, followed by a decrease with advancing age (335). If the concept of increased radio-resistance after pre-irradiation were to apply to this cell population, it would imply either that cells in a state of flux are more radio-resistant or that their capacity to repair radiation-induced damage is more efficient.

86. In an analysis of this possibility, Petrov and Cheredeev (454) studied the radio-sensitivity of splenic lymphoid cells derived either from normal spleens or from mice which had been given a whole-body dose of 500 rads 14 days previously. In each case, samples of the lymphoid cells were irradiated *in vitro* and transferred to irradiated recipients together with antigen. The immunocompetence of the population was then assessed by the resulting numbers of plaque-forming cells six days later. The dose-effect curve for the population of spleen cells taken from the pre-irradiated mice is characterized by D_0=220 rad (D_{37}=325 rad) and n=10.2. For the normal spleen cells, D_0=188.3 rad (D_{37}=125 rad) and n=0.8. This study therefore appears to indicate the induction of radio-resistance in lymphoid cells by pre-irradiation. A subsequent study (466), however, did not confirm this observation, although in this study whole-body irradiation of only 250 rads was used. Price and Makinodan (466) suggested that the results obtained in the former study might be related to other observations (662a) which show that the recovery of a normal splenic lymphoid population is a slow process, only partly completed in 30 days.

87. In more recent studies (455), the basic observation of Petrov and Cheredeev (454) has been confirmed, but it has also been found that it can be abolished by the prior addition of normal lymph-node cells to the pre-irradiated spleen cells. This suggests that the pre-irradiated spleen contains limiting numbers of radio-resistant lymphocytes which must then collaborate with the actual antibody-forming-cell precursors or with the progeny of hæmatopoietic stem cells. As the latter are in great excess in the spleen 14 days after receiving 500 rads (but not nearly as much in spleens after 250 rads), considerable reduction in their number by the second radiation treatment can occur without reducing the actual level of immunocompetence, which is dictated by the limiting number of lymphoid cells, possibly of the thymic-derived type. The important conclusion is that these experiments still do not prove that pre-irradiation induces radio-resistance in the cell lineage of the antibody precursor.

88. In most of the studies on radio-sensitivity of the humoral-antibody response, the methods used involved estimation of the amount of specific antibody present in the serum of animals following exposure to known doses of radiation. However, it is by no means certain that an assay for a serum antibody will give a value that is directly proportional to the number of surviving cells producing this antibody, unless it is first demonstrated that the doses of radiation employed have an all-or-none effect on the rate of production of antibody by individual cells, and that irradiation affects neither the rate of removal of antibody from the circulation nor the concentration of various serum factors which might alter the sensitivity of the assay. These objections apply mainly to whole-body irradiation studies rather than to the cell-transfer model of Makinodan. Recently, several techniques (120, 265, 273) have been developed which circumvent these problems by readily permitting the enumeration of antibody-releasing cells in a cell suspension.

89. This approach was used by Kennedy *et al.* (286) in a study of the radiation sensitivity of the ability of normal mice to respond to sheep erythrocytes. A typical result is shown in figure V, in which

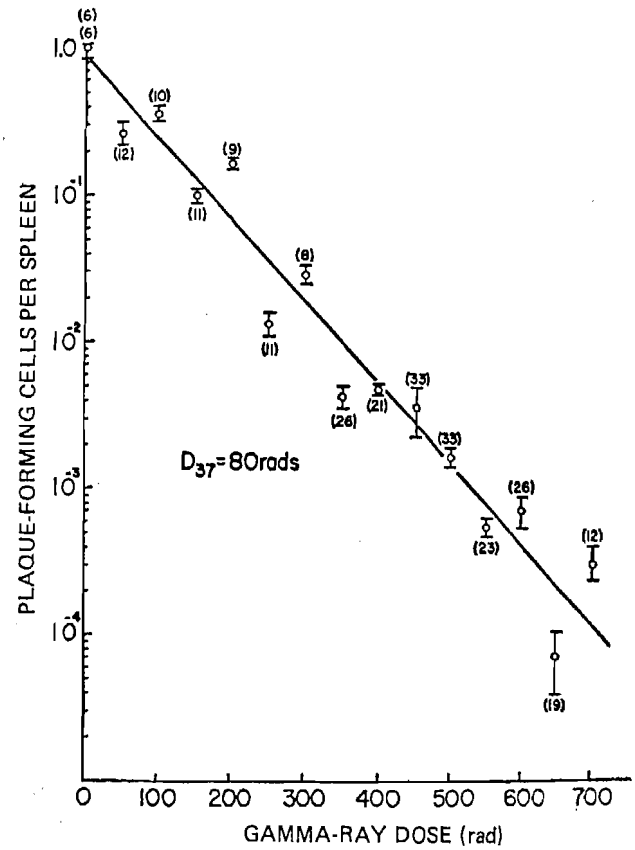

Figure V. Plaque-forming ability of the mouse as a function of radiation dose (286) when antigen (4 10^8 sheep erythrocytes) was given 10 days after irradiation and assays for plaque-forming spleen cells were made 4 days later. The results are plotted relative to the plaque-forming response of control unirradiated mice (control value 2.4 10^4 plaque-forming cells per spleen)

the plaque-forming ability of the mouse is shown as a function of the radiation dose given two hours before the injection of antigen. A D_{37} value of approximately 80 rads has been found in this experiment. The parameters of this curve were found to be unchanged for two different doses of antigen and for two different time intervals between antigen injection and assay for plaque-forming cells. These results therefore indicated that no significant repair of radiation damage to the plaque-forming cell system had occurred during the 2-hour to 10-day interval following irradiation. In a few experiments in which antigen was given 17 or 24 days after irradiation, some recovery of immune capacity was observed.

90. The D_{37} values reported by these various studies are all in the vicinity of 50-100 rads. This degree of radio-sensitivity suggests that it depends on continued cell proliferation because no cellular process other than proliferation is known which shows a radio-sensitivity of this order although interphase death of lymphocytes may also be of some importance (see paragraph 152). This interpretation is based on studies of the type reported by Puck and Marcus (467) and by others (251, 568). The effects of x-irradiation were quantitatively studied by Puck and Marcus with single cells of a human cervical carcinoma (*HeLa*) grown under conditions in which 100 per cent of the unirradiated cells reproduced in isolation to form macroscopic colonies. Survival of single cells (defined by the ability to form a macroscopic colony within 15 days) yielded a typical two-hit curve when plotted against x-ray dose. The exposure needed to reduce survivors to 37 per cent was 96 roentgens. This radiation sensitivity is tens to hundreds of times greater than that of any micro-organism studied in similar manner.

91. In considering the high radio-sensitivity of the early response, Kennedy *et al.* (286) suggested that a relatively small number of cells normally present in the mouse give rise by proliferation and differentiation to the large number of plaque-forming cells at the height of the immune response. The survival curves indicated that for values of fractional survival of 0.001 or more there was still enough residual immune capability for the system to react to an injection of antigen with the formation of antibody. In other words, the immune system could suffer at least a thousand-fold depletion of the proliferative capacity of its cells without completely losing its capacity to respond to antigen by the production of plaque-forming cells. This may not be true for all antigens, as it will depend on the number of precursor cells for the appropriate antigen.

92. Various investigations have compared the radio-sensitivity of the IgM *versus* the IgG humoral response. In general terms, as the IgG response usually appears later in time than the IgM, it might be expected to be more radio-resistant (as radio-resistance of the immune response as a whole appears to increase with time). Alternatively though, if the earlier IgM response were essential for the development of IgG response, the IgG response might indirectly appear more radio-sensitive than the IgM. In an examination of this problem, all combinations have in fact been found experimentally and will be briefly considered.

93. Several groups (406, 472, 541) have reported that the IgM response is more radio-resistant than the IgG response. Whole-body x-irradiation was administered to rabbits 20 hours prior to antigenic stimulation with polio virus. Antibody formation in rabbits exposed to 600-650 roentgens showed (541) a delay in IgM-antibody formation with a lower but more persistent titre than controls, and virtually-complete inhibition of IgG-antibody synthesis over a period of 2½ months after antigen.

94. The effect of x-irradiation on the sequential formation of immune globulins was studied (472) using flagellar antigens in rabbits given increasing whole-body doses. A delay in the appearance of IgG antibody was observed, whereas only a slight diminution in the timing or amount of IgM antibody was noted. In another study (406) on the regenerative potential of the immune response after irradiation, a preferential suppression of the IgG antibody was observed in mice exposed to 200, 400 or 600 roentgens. That is, the capacity to form 7S antibody was more heavily suppressed than the capacity to form 19S antibody, and this preferential suppression persisted throughout the recovery phase after irradiation. This was even more striking in thymectomized mice given 850 roentgens and isologous bone marrow, in which recovery of the 7S-antibody response was virtually abolished whereas the 19S response recovered substantially. X-irradiation may impair conditions necessary for the differentiation of 7S-producing cells, possibly by damaging the mechanism of antigen retention in lymphatic-tissue germinal centres, as discussed previously. These centres do in fact seem to be more closely related to the production of 7S antibody in the primary response (234). Alternatively, the IgM and IgG response may involve totally separate progenitor cells, and the IgM progenitors might either be more numerous or more radio-resistant than those of the IgG progenitors. Indeed, evidence consistent with this interpretation has been reported by Shearer *et al.* (501). These investigators considered that precursors of IgM and IgG plaque-forming cells are distinct populations and that the frequency of the former population in the normal spleen of a mouse is seven times higher than that of the latter. However, several studies (308, 424) have suggested that the IgM-IgG line is a single differentiating line of cells.

95. In a study with *Salmonella* antigen in rats (272), both the IgM and the IgG phases of the primary response were markedly inhibited by 450 roentgens given a day before antigen, and both phases could be restored by an injection of 200 microgrammes of Colchicine given at the same time as the antigen. The final possible combination of radio-sensitivity was reported by Berlin (44) who irradiated mice one to four days before immunization with influenza vaccine and observed markedly low IgM-antibody titres and a high degree of sensitivity of IgM antibody, as indicated by a D_{37} of 74 rads.

96. This latter value indicates that the radio-sensitivity of IgM cells is of the same order as that of the over-all immune response previously mentioned. Since all the data mentioned on greater sensitivity of the IgG response have been concerned with measuring IgG titres in sera obtained from irradiated animals, it should be noted from earlier discussion that, following irradiation, the IgG globulin is more selectively lost from serum than the IgM antibody. Accordingly, a greater radio-sensitivity of the IgG response might be falsely deduced from measurements of serum titres. A complete solution of this problem will again require the use of a direct cell-plaque estimation technique which is capable of determining both IgM and IgG plaque-forming cells following varying doses of radiation.

97. The effect of internal irradiation delivered from intravenously administered radio-active (^{32}P) colloidal chromic phosphate on the primary immune response of rabbits to sheep erythrocytes and typhoid antigen has been studied (636). When 14 days of internal irradiation from 520, 624 and 780 microcuries preceded a single immunizing injection, antibody responses to both antigens were significantly depressed, as shown by delayed appearance of antibody, decreased antibody synthesis rates, and lowered maximum antibody titres.

Splenic participation in the immune response of rabbits given 488 microcuries or more was judged non-operative. The spleens of these rabbits were estimated to have absorbed 7,000 to 14,000 rads during the 14 days preceding immunization. This result therefore parallels the marked suppression of primary antibody response with a single antigen injection after splenectomy in rabbits (545). In both cases, the impairment of antibody production could be corrected by the use of multiple antigen injections, which would induce the participation of non-splenic sites in antibody production. This might well indicate that intravenously administered radio-active colloid primarily affects the lymphoid tissue of the spleen, and has less effect on the circulating lymphocyte pool.

3. *Cell collaboration in the humoral immune response*

98. The irradiation studies described above indicate that cell proliferation is an early event following antigenic stimulation. The simplest view would be that the immunocompetent cell is stimulated by antigen (perhaps *via* macrophage) and directly proliferates and differentiates to become the antibody-forming cell (328, 423). Clonal expansion has been directly demonstrated in studies by Playfair *et al.* (458) and Kennedy *et al.* (287). These workers devised a method for the enumeration and characterization of cells which, on appropriate antigen stimulation, could produce a clone of antibody-forming cells. These they termed the "antigen-sensitive cells". They are detected by the injection of normal lymphoid cells into a lethally-irradiated animal. A proportion of the injected cells reach the spleen and settle there. A stimulus of antigen (sheep erythrocytes) then triggers a certain specific proportion of the injected cells to proliferate and differentiate into antibody-forming cells. Provided a small enough inoculum is given, these form discrete areas in the recipient spleen, which can be detected by laying thin slices of the spleen on agar containing the antigenic red cells. After allowing for diffusion of antibody and attachment to the red cells, hæmolysis is induced by the addition of complement. This system therefore appears to show a direct proliferation of cells following antigenic stimulation, because most studies are compatible with the concept that a single cell is the progenitor of the clone and ultimately produces many antibody-forming cells. It does not, however, establish that the cells that initially react with antigen are the ones that give rise to the clones observed. In fact, the "simplest" view described above has been controverted by later work demonstrating cell interaction in immune responses, interactions that produce specific second-order effects on the cells whose progeny eventually produce the antibody.

99. Collaboration between thymus or thymus-derived lymphocytes present in thoracic-duct lymph, and non-thymus-derived precursors of antibody-forming cells has been implicated in the immune response of mice to sheep erythrocytes (84, 85, 122, 371, 372). Neonatal thymectomy impairs the response of mice to sheep erythrocytes. This can be reversed by inoculating thymus or thoracic-duct lymphocytes simultaneously with the red cells (370). In this system, the identity of the antibody-forming cells was determined by using anti-H-2 sera in allogeneically-reconstituted hosts and chromosome-marker analysis in a syngeneic system (421). These techniques demonstrated that the antibody-forming cells were in general derived *not* from the inoculated lymphocytes, but from cells already present in the thymectomized hosts. In an attempt to identify the origin of the true precursor of the antibody-forming cells, a synergistic effect between thymus and bone-marrow cells on transfer into lethally-irradiated mice was demonstrated (85, 378). By means of a chromosome marker it was again shown (421) that all the antibody-forming cells produced were derived from the bone marrow. These series of experiments therefore indicated that, at least with some antigens, collaboration of thymus-derived cells with bone-marrow-derived cells is required to initiate the antibody response.

100. Although more recent studies (366) have further extended the list of antigens for which cell collaboration seems to be essential for antibody production, it is doubtful that this is an obligatory phenomenon for the initiation of all antibody responses. For example, current data would perhaps suggest that, although cell collaboration is important for heterologous antigens such as gamma globulin, albumins, erythrocytes and some haptens, it may not be involved in responses to many bacterial antigens. Recent studies with a congenitally-athymic strain of mouse have clearly shown that whereas this strain is quite incapable of making antibodies to heterologous erythrocytes, normal IgM-antibody production to several bacterial antigens occurs, although IgG-antibody responses are considerably depressed (110).

101. Various studies have demonstrated that although the thymus-derived cells do not become the actual antibody-producing cells, they do directly proliferate in response to antigenic stimulation. By the use of chromosomally-marked cells, Davies *et al.* (123) have demonstrated that thymus-derived cells will directly proliferate in response to either sheep red cells or an allogeneic skin graft, and will not become the antibody-forming cells (124). This has been extended in another study (500) showing by means of a limiting dilution assay that proliferation of the thymus-derived cell produces more cells which can collaborate with bone-marrow-derived cells and induce the latter into antibody formation. Similarly, using tritiated-uridine or thymidine markers, a proportion of injected thymus cells was observed (371) to transform directly under antigenic stimulation into blast-like pyronophilic cells which then divided into smaller lymphocyte-like cells. To confirm this interpretation, Koller *et al.* (295) assessed whether any significant frequency of mitosis would follow the antigenic stimulation of immunologically-incompetent (thymectomized) mice. Although the results are rather sparse, they do indicate that nearly all the mitoses seen in lymphoid sites after antigenic stimulation of thymus-grafted mice were indeed dependent upon the immunocompetence of the injected animal. This might be interpreted as indicating that the bone-marrow-derived cell does not proliferate unless it is somehow "stimulated" by the antigenically-stimulated thymus-derived cell. This interpretation would imply that a thymus-derived cell is the only cell type directly stimulated to proliferate by antigenic challenge. If so, this cell would represent a major radio-sensitive cell type involved in the radiation suppression of the inductive phase of the antibody response.

102. Studies on cell-to-cell interaction in the initiation of humoral-antibody responses in rabbits have given somewhat different results. In this species, antibody response to sheep erythrocytes of animals treated

with 800 rads can be restored by injection of allogeneic bone marrow from normal rabbits (2). Furthermore, with the use of allotypic markers of immunoglobulins, it was shown that the antibody-forming cells are derived from the irradiated host and not from the donor marrow (470). These results suggested that antibody-forming precursor cells are relatively radioresistant, while antigen-reactive cells which, in the rabbit, are found in bone marrow (1) are more radiosensitive. Several other alternative explanations might be given, however. As allogeneic marrow was used, it is possible that augmentation might result from a graft-*versus*-host reaction as has been proposed recently (283). It is also possible that the peripheral tissues of rabbits contain different proportions of thymic-derived lymphocytes than mice, and therefore that restoration is made by *T* lymphocytes in the bone marrow.

103. Several experiments have indicated that the lymphoid cells that reside in the thymus are very radiosensitive whereas the supporting thymic epithelial cells are not. The recovery of thymic epithelium after irradiation is the major thymic factor in effecting the full recovery of immunocompetence in the animal. As mentioned previously, virtually all thymocytes are destroyed by an x-ray exposure of 500 roentgens (573), leaving a residual stroma of reticular epithelial cells. Morphological observations indicate that these latter cells are resistant to exposures as high as 5,000 roentgens. Although several studies show that the irradiated thymus is capable of some lymphoid regeneration (within 3-4 days after 400 roentgens (166), within 1-2 weeks after 500 roentgens (367) and within 2 weeks after 850 roentgens (114)), normal regeneration is grossly impaired after exposures of 2,000 roentgens. Although lymphoid regeneration in thymus grafts exposed to 2,000 roentgens *in vitro* was observed after 11 days (154), two other studies (50, 125) showed that, despite almost normal lymphoid repopulation, the functional activity inducing immunological competence had not returned after three weeks.

104. A more direct evaluation of the radio-sensitivity of thymus-derived and non-thymus-derived cells would be to irradiate *in vitro* either suspension separately and then to attempt cell-collaboration experiments with the other cell type being unirradiated. Several recent reports on this type of experiment appear to give conflicting results. Claman and Chaperon (84) found that in thymus-marrow synergism in mice, both cell populations are sensitive to irradiation. Miller and Mitchell (370) also showed suppression of thymus-cell induction of antibody formation in bone-marrow-derived cells when the thymus cells were exposed *in vitro* to 1,000 roentgens. In contrast to this report, Goldie and Osoba (212) have reported synergism between heavily-irradiated (up to 2,500 rads) and non-irradiated normal spleen or lymph-node cells of the mouse in the development of plaque-forming cells to sheep erythrocytes *in vitro*.

105. It has been amply demonstrated that in the adoptive secondary response to hapten-protein conjugates in mice, co-operative interactions are mediated by hapten-specific and carrier-specific lymphoid cells (384). More recent observations (366) have confirmed that these correspond to bone-marrow and thymic-derived cells, respectively. Using this hapten-specific and carrier-specific cell-interaction system, Katz *et al.* (282) have studied the radio-sensitivity of the carrier-primed cells in guinea-pigs. The transfer of lymphoid cells, from strain-2 guinea-pigs immunized to bovine gamma globulin (carrier cells) into syngeneic recipients immunized with dinitrophenyl ovalbumin, was found to enhance markedly the recipient's secondary anti-dinitrophenyl response to challenge with dinitrophenyl bovine gamma globulin. This function of the carrier bovine gamma-globulin-specific cells was found to be resistant to 5,000 rads. However, the capacity to transfer immunological memory to bovine gamma globulin or to be stimulated by antigen to synthesize DNA *in vitro* was abolished by as little as 500 rads.

106. Similar results have also been obtained with an *in vitro* system (288). A primary immune response of normal spleen cells to trinitrophenylated sheep erythrocytes (TnpRBC) was studied *in vitro* and the number of anti-Tnp plaque-forming cells was determined. The number observed could be greatly enhanced by prior inmunization of the donor spleen *in vivo* with the carrier erythrocytes, or by using normal unprimed spleen cells in combination with spleen cells from mice that were immunized to the carrier erythrocytes. If these latter added carrier-primed cells were first treated *in vitro* with 1,000 or 4,000 rads before their addition to the normal spleen cells, they were still capable of enhancing the anti-Tnp response of the normal spleen cells. The immune response of the carrier cells themselves to erythrocytes was totally abolished by the irradiation. This observation also therefore demonstrates radio-resistance of thymus-derived helper cells.

107. These studies clearly indicate that in the transfer of immunological memory, where cell division is required, irradiation will abolish this function. However, in a primed system, reactive carrier cells are clearly able to co-operate with hapten-specific cells, without the need for division of the reactive carrier cells. This may therefore entail a presentation of the antigen by the carrier-primed cell to the hapten-specific cell, a task which can satisfactorily be performed by a lethally-irradiated cell. It is also possible that the reactive carrier cells may normally continue to divide, but that helper activity is needed only briefly at the initiation of the response. The experiments in which thymic cell function was destroyed by irradiation all involve primary immune responses. In this situation the virgin thymic cell on confrontation with antigen must proliferate in order to collaborate, and this is therefore a radio-sensitive step.

108. Although no direct data on radio-sensitivity in terms of collaboration potential have been obtained for the bone-marrow compartment, some data may be cited from avian studies. This is based on the view that the mammalian bone-marrow-derived cell is in effect "bursa-differentiated". The bursa is the primordial site of origin for cells that synthesize immunoglobulins in birds and the immunoglobulin specificity in the antibody-forming cell of mammals is of bone-marrow-type origin (270). Although embryonic bursectomy by hormones or surgery will totally prevent all potential antibody and immunoglobulin synthesis (102, 612), surgical bursectomy at hatch is not as effective in this respect. This is presumed to be due to the movement of bursal cells into peripheral tissues prior to hatching (103). If sublethal whole-body irradiation is given to newly hatched bursectomized chickens, much greater immunodepression is observed, even with doses of 250 rads (106). This suggests considerable radio-sensitivity in this cell line, although

the number of cells available in the periphery may only be very small at this stage, even in the normal animal. If the bursa of Fabricius is exposed *in vivo* to 1,000 roentgens at one and seven days of life (613) massive destruction of the bursal lymphoid follicles occurs without eventual normal regeneration. A diminished antibody response then results in most birds. Further studies are clearly needed to define the radiosensitivity of the bone-marrow component, and in chickens to confirm whether bursal cells play this role.

4. *Timing of irradiation and antigenic challenge*

109. The effect of irradiation on the immune response can be studied when the antigen is given before, at the time of, or after irradiation. In the latter case, where antigen follows irradiation, immunodepression is usually observed. The studies on radio-sensitivity of the inductive phase described above all deal with antigen given within a fairly short time after irradiation, namely, up to a few days later. When antigen is given many days or weeks after irradiation, this in essence is a study of the regeneration of the immunocompetent population and depends on many factors including stem-cell differentiation.

110. As discussed above, regeneration of the immune response appears to be thymus dependent, at least for certain antigens. Thymectomized, lethally-irradiated, bone-marrow-protected mice will respond only poorly to many antigens given even months after the irradiation (368, 406). The primary antibody-forming potential recovers very slowly from irradiation and this process does not require the presence of antigen, although impairment of the antigen-retention mechanism may be a factor in the delay of recovery of expression of immunity (403). In cell-transfer studies, the size of the immunocompetent pool was shown to be reduced for three to five months after sublethal x-ray exposure. It is not completely clear whether the renewal of the immunocompetent cell pool is partly due to a self-renewal system normally maintained in a steady state which slowly recovers after irradiation, or whether new competent cells are formed by differentiation from true progenitor cells. Some evidence (542) suggests that the resting antigen-reactive cell is not a rapidly dividing cell, as massive doses of the mitotic poison vinblastine yield only a slight reduction in the numbers of antigen-reactive cells. Thus most of these cells are in the G_0 state and are rapidly induced to divide by antigen. Although the relatively large amount of data on the importance of the thymus for the regeneration of immunocompetence tends to suggest a major role of differentiation of stem cells to immunocompetent precursors, the removal of the thymus does not totally deprive the animal of its capacity to regain immunocompetence even after severe suppression. Thus the recovery in thymectomized mice or rats given 400-600 roentgens was only moderately retarded in comparison with non-thymectomized irradiated animals (9, 153, 406). After higher exposures (850 or 500 + 500 R) the effect of thymectomy is much more marked, but nevertheless still not absolute, as particularly 19S responses still eventually recover (406). It therefore appears possible that in the absence of the thymus some regeneration of the immunocompetent cells (*x* cells or *PCl* cells) (444, 499) might occur from other surviving *x* cells, or from a more primitive precursor pool.

111. The classical studies of Taliaferro *et al.* (550) have clearly revealed that the timing of irradiation relative to the injection of antigen is of crucial importance in determining the amount of antibody eventually produced by an animal. When antigen was given prior to x-irradiation, an actual increase in the titre of antibody produced by the animal was noted. With an x-ray exposure of 500 roentgens given two days to two hours after the antigen, enhanced peak titres were the rule, though the latent period was lengthened. If, on the other hand, antigen was given one hour after irradiation, there was a slight inhibition, whereas the response to antigen was drastically inhibited if the injection took place 24 hours after irradiation. On the basis of these and other studies, Taliaferro proposed two types of radiation-induced enhancement. In the first type, seen with x-ray exposures between 25 and 300 roentgens given two days to two hours after injection, there is a heightened peak titre accompanied by a shortened latent period, and an abnormally high rate of antibody synthesis. In the second type, observed with exposures from 500 to 700 roentgens also after injection, there is an increased peak titre, but a lengthened latent period and a slower rise to peak titre.

112. Since the original observations of this phenomenon (338, 547, 549), many other workers have amply confirmed radiation-induced enhancement of antibody formation. Perhaps one of the most important of these studies was a detailed analysis by Dixon and McConahey (144). Before considering this in depth, mention of a few other confirmatory reports will be made.

113. A series of rabbits were given diphtheria toxoid, followed by x-irradiation (850 R) one, two or four days after injection (664). The synthesis of antibody took place in a large number of cells that were present for significantly longer periods of time than in unirradiated, immunized rabbits. In the early periods after irradiation, there was a tendency towards a reduction in the proportion of young forms of cells containing antibody. From the eighth to the fourteenth day after immunization, irradiated animals showed considerably greater numbers of young forms of antibody-containing cells than unirradiated animals in the same periods after immunization. After irradiation, the synthesis of antibody took place in the same types of cells as in unirradiated animals, although degenerative changes in the nucleus and protoplasm were seen in a large percentage of cells and the amount of antibody in the cell was altered. The percentage of lymphocyte-like cells in the antibody-forming population was considerably increased. These results are in contrast to a similar morphological study (665) made when antigen was given *after* irradiation, and somewhat opposite cellular changes were observed.

114. Antibody formation was analysed by the plaque method in mice treated with a dose of 660 rads one to two days after immunization with sheep erythrocytes. It was shown that exposure to radiation does not halt the increase in number of plaque-forming cells (677). The number of such cells accumulated in the spleen was only half that of controls. After irradiation, the number of antibody-producing cells continued to increase, becoming at least tenfold greater. This would suggest that after several mitotic cycles (before irradiation was given) the antigen-stimulated immunocompetent cells become capable of maturing (differentiation?) to antibody-producing cells without any, or only a limited number of, cell divisions. The general laws

governing suppression or stimulation of the immune response as a function of the relative timing of irradiation and immunization have also been discussed in detail by several authors (659, 675, 690).

115. In another study with rabbits exposed to 500 roentgens at various times before or after antigen, it was found (289) that essentially unimpaired responses occurred in animals irradiated immediately before, immediately after, or 12 hours after antigen. Marked depression was observed with irradiation given 12 or 24 hours prior to antigen. With irradiation 12 hours after antigen, there was usually a slight initial depression up to the sixth day after antigen, but the eventual peak titres rose to levels above those in the non-irradiated control. (This exactly agrees with the Taliaferros' observations on their second type of enhancement.) Radiation damage of the spleen was characterized by the complete degeneration of the lymphoid follicles, with survival of much of the peri-arteriolar lymphocyte sheaths. In the irradiated animals in which antibody responses were unimpaired (radiation after antigen), normal plasma-cell reactions localized in the surviving peri-arteriolar lymphocyte sheaths were observed two to three days after stimulation.

116. Irradiation after antigen is not always associated with antibody titres higher than in controls. In some instances (187), it is rather that the degree of immunodepression observed is not as great as when irradiation precedes antigen. Rats exposed to 500 roentgens at various times after antigen all showed some immunodepression which was greater when irradiation was given a few hours after antigen rather than four days after. Mice given a whole-body x-ray exposure of 710 roentgens immediately before or after antigen were severely immunodepressed (200), whereas radiation given five or more days after the antigen had only a slight enhancing effect on antibody formation.

117. Further studies (548) on the radiation enhancement of antibody formation with exposures from 25 to 100 roentgens have confirmed the original report that injection of antigen four hours to a week, or even one month, after irradiation in this dose range will lead to a prolonged production of hæmolysin and to transient high peak titres. It was suggested that after injury, the cells show an over-compensatory activity of a duration proportional to the x-ray dose. This stimulatory effect of small doses did not seem to be directed against the developmental and proliferative activities of immunocompetent cells or of memory cells, as in neither case was an effect observed during the latent period preceding antibody detection in serum.

118. These various reports taken together seem to indicate that radiation-induced enhancement of antibody formation is more marked in certain cases than in others. A detailed analysis of this problem by Dixon and McConahey (144) has revealed that many variables can indeed affect the degree of enhancement induced by radiation. They observed that (*a*) the degree of optimum stimulation varied from one antigen to another; (*b*) the time interval between antigen and irradiation differed in terms of optimum stimulation for different antigens; and (*c*) the optimal radiation dose also differed for the various antigens. In general, it appeared that the more rapid the antibody response the earlier x-irradiation may be given to enhance the response. For example, with soluble bovine gamma globulin (BGG) the interval between antigen stimulus and peak antibody response was from 10 to 11 days, and x-irradiation gave maximum enhancement when administered 2½ days after antigen. With heat-aggregated BGG as antigen, the interval between stimulus and maximum antibody was only seven to eight days and x-irradiation gave maximum enhancement when administered as early as two hours after antigen, although comparable enhancement could be elicited with irradiation one and two days after antigen injection.

119. It was proposed on the basis of these results that x-irradiation given early in the immune response destroys the majority of lymphoid cells, leaving behind depleted lymphoid tissues. Of the remaining lymphoid cells, many of which are primitive or immature, some are presumably responding to antigen and some are not. Those responding to antigen would be expected to proliferate more rapidly than those unaffected by the antigen. The cellular proliferation after antigenic stimulation and irradiation may then be more rapid and extensive than after either alone. During this exaggerated proliferation, the rapidly dividing antigen-stimulated cells can far outstrip their non-stimulated counterparts, resulting in the observed increased antibody formation and in large numbers of antibody-containing cells.

120. A second view has been proposed by Makinodan and Price (336) and is based on the concept of feed-back control mechanisms, that is, that the maximum immune expression of an individual, as measured by peak serum-antibody titre or number of antibody-synthesizing cells, need not necessarily reflect his full immunological potential. Several experiments clearly indicate that the immune system is capable of enhanced responses that are much greater than are achieved in conventional immunization schemes. Animals given 10,000 roentgens to exteriorized spleens with the rest of the animal shielded, will make a markedly-augmented antibody response to intravenously-injected particulate antigens (510). Cell-impermeable diffusion chambers containing antigen and spleen cells from pre-immunized mice, when implanted in irradiated recipients, can generate 10 times more antibody-producing cells per unit number of spleen cells than *in situ* immunization (331). In recipients made immunologically inert by the use of drugs, transfused histoincompatible spleen cells will generate more antibody than compatible spleen cells (489).

121. These and other studies therefore imply that the level of antibody response found in a conventionally-immunized animal reflects activation of only a fraction of its full immunological potential. Makinodan and Price (336) suggested that an immune response can be augmented most readily by radiation if it can cause a sufficient amount of cell destruction and thereby create a *milieu* for proliferation and differentiation of more immunocompetent cells than normally would participate in a response. However, it is essential that the dose of radiation be low enough so that the percentage of immunocompetent cells destroyed be less than the percentage normally expressed in the response.

122. Berenbaum (42) has determined the number of antibody-forming cells at various stages of the immune response when 450 roentgens were given between 1 day before and 20 days after antigen. At all times a rapid fall in the number of antibody-forming cells occurred, which was followed by a rise towards the levels found in unirradiated controls. Further stimulation of newly emerging antigen-reactive cells may have occurred from antigen lodged in depot sites in the

spleen. There was no evidence that radio-resistance rises after the antigen is given. As one of the first demonstrable effects of antigen injection is an increase in the number of antigen-reactive cells, an increased pool of antigen-reactive cells is exposed when irradiation follows antigen. Even if this post-antigen pool is reduced by radiation to the same extent as a pool that has not been exposed to antigen, the absolute number of surviving antigen-reactive cells may be considerably greater.

123. A third explanation for this apparent change in radio-sensitivity of the antigen-stimulated *versus* unstimulated immune response is to consider that there is a difference in the radio-sensitivity of the cells at these two stages, or that a better repair mechanism exists in the immunocompetent cell than in other proliferating cells. As previously discussed, there is little support for this view, although one recent report (638) has shown a basic change in the radiation-dose-response relationship when the immune system is irradiated before or after antigen injection.

124. Using a modification of the plaque technique (638a), Zaalberg and Van der Meul (638) found a significant difference in the effect of irradiation on plaque-forming capacity to sheep erythrocytes depending on whether the antigen was given 24 hours after or 1 hour before, or after, irradiation. The results given in table 3 show that when antigen is given after irradiation, a dose-dependent depression in immune response occurs. Shown graphically in figure VI, this

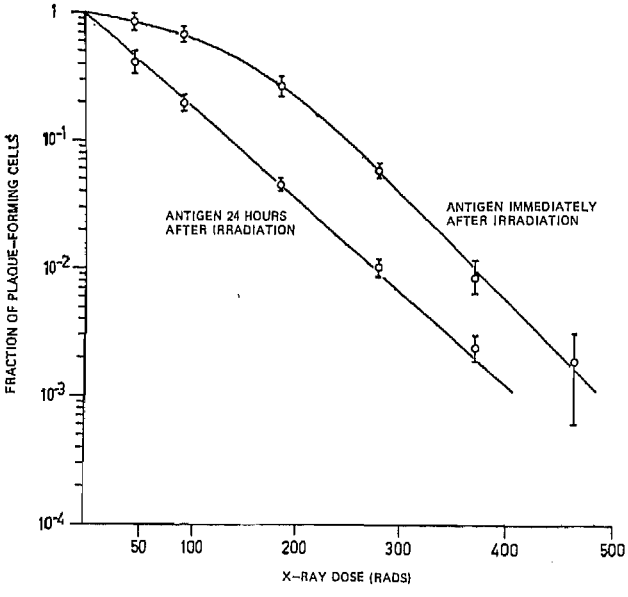

Figure VI. Radiation dose-response relation of IgM plaque-forming cells in mouse spleen (638). The points represent the number of plaque-forming cells determined four days after immunization, expressed relative to unirradiated mice. The bars represent 95 per cent confidence limits. The upper curve was obtained with mice injected with antigen within one hour after irradiation, and the lower curve with mice injected 24 hours after irradiation

is a linear depression. However, when antigen is given one hour before *or* after irradiation, an enhanced response is seen with 50 rads, and a totally-different dose-dependence is found (figure VI). As the radiation damage had already occurred in the animal given antigen one hour after irradiation, a repair mechanism might be postulated. It was suggested that the high level of radio-sensitivity of the non-stimulated small lymphocytes is connected with its relatively-inactive metabolic state. It is therefore not capable of repairing the radiation damage leading to interphase death. However, provided the antigen encounters the cell very soon after irradiation, it may stimulate the cell to repair the radiation damage, possibly by causing changes similar to those previously shown (622) to be capable of preventing interphase death.

125. From a practical point of view, the enhancement of antibody formation by x-irradiation has several interesting facets. It is a convenient laboratory tool for manipulating the immune response, in that antisera of very great potency can be obtained far more rapidly than in normal prolonged hyper-immunization schemes. For clinical medicine, these results serve as a caution against any attempts to inhibit the immune response of patients with x-irradiation if they have recently received the antigen. This may be of particular relevance to homograft situations such as kidney graft, where this type of model might imply that enhanced rejection rather than depression could result, if the antibody response is indeed participating in the graft destruction. Alternatively, if antibody production were acting to enhance graft survival (in the "blocking" sense referred to in paragraph 23), increased antibody production would not be disadvantageous. Also, in situations such as the response to a pathogen or a tumour antigen, an exposure to x rays after the administration of the antigen might greatly facilitate the immune process. As emphasized by Dixon and McConahey (144), the timing relationship of irradiation and antigen injection for either suppression or enhancement of the immune response depends on several factors, including the actual antigen used. Accordingly, this approach to achieve more efficient antibody responses to pathogens or tumours would be most dangerous to apply to man at the present time.

126. Although it might be expected that immunocompetence would be fully restored within months after irradiation, studies on antibody formation in survivors of atomic-bomb-irradiation have occasionally indicated persisting immunological changes. In several studies on blood-group antibody (254), bactericidal activity (253), or serum agglutinin to TAB vaccine (506), no appreciable difference in serum antibody titres of the survivors and controls was found. However, as these studies were performed at least 10 years after the atomic bombing, a more sensitive retrospective indicator for effects of irradiation on antibody formation was sought. Studies of Davenport *et al.* (121) have suggested that serum-antibody levels which appear in response to influenza-virus infection in a specific age group are highest against the strain of virus of the initial infection. Thus, following inoculation with influenza-virus vaccine antigenically related to the virus of primary infection, lower levels of serum antibody against the primary virus should develop in the heavily exposed subjects in comparison to the non-exposed controls.

127. The effect of atomic-bomb radiation on antibody production was studied (276) among persons living in 1961 who were exposed while *in utero* to the atomic bomb in either Hiroshima or Nagasaki. Patterns of the antibody levels in the group beyond three kilometres from the hypocentres suggested that the primary infection in these individuals was from a virus of type A1. Significantly reduced A1-type serum-antibody

levels were noted in pre- and post-vaccination sera of subjects within two kilometres from the hypocentre in Nagasaki. Depression in the pre-immunization sera was not, however, observed in the Hiroshima subjects. In both series, the heterotypic antibody response to Asian-influenza vaccination among those within two kilometres clearly demonstrated a considerably-poorer response to FM1. In subjects within 1.6 kilometres, antibody responses to type-A1 viruses were almost completely suppressed. In Hiroshima, subjects within two kilometres also failed to increase their serum-antibody response to Gotoh virus which is a variant strain of type-A1 virus. In the case of serum response, as tested with other type-A viruses, the results were somewhat more varied. All subjects showed a strong response to PR8 virus, although the response to the Weiss type was poor. The over-all development of serum antibody to the Asian virus following vaccination showed the opposite result, a somewhat better response being observed in subjects within two kilometres than in those beyond three kilometres. While this result might indicate involvement of the overcompensation mechanism discussed above, this is unlikely to have persisted for such a long period of time.

128. A further factor affecting the radiation-induced depression of the primary immune response is the exposure rate. A study was undertaken (74) to determine whether an exposure-rate-dependent suppression existed for antibody synthesis, and to establish the range of exposure and exposure rate over which an effect could be seen. Adult mice were exposed to ^{60}Co gamma radiation in doses of from 200 rads to 1,100 rads at exposure rates ranging from 4 R min^{-1} to 100 R min^{-1}. Irradiated mice were injected with rat- and sheep-erythrocyte antigen at various times before or after irradiation, and the titre of circulating antibody was determined. Greater suppression of antibody production occurred at the higher exposures rates, particularly when the total dose was in the sublethal range, 600 and 700 rads. Rate-dependent suppression of antibody production was dependent upon the type and dose of antigen, the route of antigen administration, and the time interval between antigen administration and radiation exposure. When antigen preceded irradiation by 12 hours and the dose was 700 rads, the suppression at 72 R min^{-1} was 64 times that at 8 R min^{-1}. The exposure-rate effect was demonstrated at the cellular level by culturing irradiated spleen cells in irradiated (950 rads) syngeneic recipients. In this experimental system both primary and secondary antibody formation were differentially sensitive to exposure rate. At the level of maximum exposure-rate sensitivity for formation of antibody against sheep erythrocytes (700 rads), responses were depressed with increasing exposure rates up to 40 R min^{-1}, whereas insignificant additional depression occurred after exposure to higher rates, up to 100 R min^{-1}. The exposure-rate dependence of radiation mortality was determined, and the responses of mortality and immune suppression were compared. No correlation was observed.

C. The productive phase of antibody formation

129. A great deal of data obtained through histological correlative studies (170), studies on antibody content of cells (305), and observations of direct *in vitro* antibody formation by single plasma cells in microdrops (413) has shown that plasma cells are important antibody producers. However, it has also been recognized that other cell types of different morphology can secrete antibody. Many of these include DNA-synthesizing cells (305, 413) and other smaller lymphocyte-like cells were also found to be active. The majority of these differ from the main bulk of small lymphocytes in having a distinct rim of cytoplasm rich in RNA and are now known to be non-thymic-derived *B* lymphocytes. More detailed electron-microscopic studies of antibody plaque-forming cells have appeared more recently (57, 119).

130. In studies combining detection of antibody formation at the cell level with ability to synthesize DNA (328, 423, 446), the conclusion was reached that every antibody-forming cell which arose during the primary or secondary response was the result of a recent mitotic division. It is quite clear that antigen-reactive cells usually enter a proliferative phase and divide, probably several times, to produce mature antibody-forming progeny. Multiplication and the expression of specialization (differentiation) occur over the same interval. Thus, some actual antibody-secreting cells (plasmablasts and immature plasma cells) still retain the capacity for division. However, after a sequence of about six to eight mitoses, division stops and fully specialized non-dividing end-cells dominate the scene.

131. In a detailed analysis (446) of the rate of cellular proliferation and recruitment in the spleens of mice undergoing a primary immune response, it was concluded that, although cellular proliferation during the lag phase is the dominant event, many recruitment events also occur with an exponential increase. It was found that (*a*) antigen-induced cellular proliferation begins about 12 hours after antigen injection; (*b*) plaque-forming cells begin to significantly appear after a lag of about 24 hours; (*c*) most, if not all, of the precursors of the plaque-forming cells during the lag phase are proliferating; (*d*) the number of these cells increases in a staircase fashion suggesting a considerable degree of synchronous growth; (*e*) a series of recruitment events occur in phase with division of plaque-forming cells (this possibly involving the cell-collaboration phenomenon); and (*f*) cells responsible for these recruitments are themselves proliferating before they transform into plaque-forming cells. Similar findings have been reported for both 19S and 7S plaque-forming cells from spleen-cell cultures undergoing secondary anti-sheep RBC response in millipore diffusion chambers (486).

132. In this section of the productive phase we are therefore concerned with cells which are actually synthesizing and releasing the antibody molecules. In terms of cell-collaboration concepts, this refers to the bone-marrow-derived (bursa-induced?) compartment in which the proliferative events referred to above may be induced either simultaneously with or, more probably, only after antigen-induced thymus-cell proliferation. The productive phase therefore is heterogeneous, in that it involves some immature blast cells, some of which are capable of several further division cycles and would be relatively radio-sensitive, fully differentiated plasma cells, the "background" antibody-forming cells present in animals not deliberately immunized, and finally, although not strictly an active secreting cell, the memory cells involved in the elicitation of the secondary response. Virtually no direct data are available on the immature plasma cell. Its contribution to the total serum antibody would be rather small

and direct single-cell experimentation would be required to assess its radio-sensitivity. We shall therefore concentrate on the major antibody-producing cells and the secondary response.

1. *Plasma cells and the active immune response*

133. Various early studies on whole-body x-irradiation of animals after antigen injection clearly indicate that a depression of antibody formation does not result (as discussed under enhancement). Thus, Dixon *et al.* (145) showed that 800 roentgens given three days after antigen had no suppressing effect on the antibody response. Mice given about 650 or 775 rads from a ^{60}Co source at the time of peak serum-antibody formation to tetanus toxoid showed (228) only slight depression or no change in their antibody level 5 to 10 days later. Rabbits immunized with bovine serum albumin were given 450 to 550 roentgens during the steady-state phase of antibody production after either primary or secondary antigen challenge (83). Irradiation during the primary-response steady state produced a continuous fall in antibody levels, but was without effect when given during the declining phase of the secondary response. This would indicate that, at least in some cases, the steady state of persisting serum antibody, particularly after a primary response, is maintained by a balance between proliferation of differentiating cells (probably involving many immature plasma cells) and the half-life of the antibody molecules. In another study, rats received bacterial antigens and gave a prolonged and sustained antibody response (302). Whole-body irradiation during this steady-state phase did not affect the antibody titre for at least a period of several weeks after irradiation. This, in comparison to the report previously mentioned, probably indicates that in other systems, that is, with different antigens, the steady state of persisting antibody production may involve only the mature non-dividing element, and does not require the continuing recruitment of other immunocompetent cells.

134. From these and many other similar studies it its clear that, as the phase of detectable serum antibody develops, the over-all immune response appears to become much more radio-resistant. However, this type of study could involve many factors. Particularly with the use of different antigens, there may well be marked differences in the proportion of mature non-dividing cells and newly-recruited dividing antibody-producing blast cells in the steady-state level. Radio-sensitivity of the antibody molecules, their loss through the gut, the actual radio-sensitivity of the antibody-forming cell or of the antigen depots, are all further factors affecting serum titres in an animal given whole-body x-irradiation.

135. Before considering the effect of irradiation on antibody-producing plasma cells, it is relevant to determine whether irradiation can directly affect the product of the plasma cell, namely, the antibody molecule. The effect of ionizing radiation on the hæmolytic activity of rabbit IgG and IgM hæmolytic antibodies was studied (477). Protein fractions of rabbit serum were irradiated in a beam of 2-MeV electrons generated in a Van de Graaff electrostatic accelerator or by a beam of 5-MeV protons generated in a linear accelerator. The antigen-binding capacity of hæmolysin, unirradiated and irradiated, was measured by determining the number of sheep erythrocytes required to neutralize (absorb) hæmolytic activity. Other investigators who have studied the inactivating effect of ionizing radiation on biologically active proteins of known molecular weight, have found a good correlation between the apparent target size of the active molecule and the size of the whole molecule. Inactivation curves with these other systems were linear. However, with the IgM system, non-linear radiation inactivation curves were obtained. For IgM, 10 per cent of hæmolytic activity was retained with a dose of 14 10^6 rads and for IgG 30 per cent remained after 18 10^6 rads (2-MeV electrons). Some structural characteristics of IgG and IgM hæmolytic antibodies were then deduced by target theory analysis of the relation between the dose of radiation and inactivation of the molecule. Destruction of a single target with a molecular weight of 52,000 in the IgG molecule was sufficient to destroy hæmolytic activity. These data are consistent with a model of the IgM molecule containing more than three sub-units, each of molecular weight from 1.6 to 1.8 10^5. In this model, each sub-unit was capable of combining with antigen, and two adjacent sub-units were required for the fixation of complement (C') and for hæmolytic activity. In the context of the present discussion these results clearly indicate that no inhibitory effect on antibody molecules themselves is detected within the dose range used in experiments with antibody-forming cells.

136. In most cases mature plasma cells are relatively short-lived, surviving for two to four days only. However, a small but important minority, perhaps one in a thousand of all antibody-forming cells created during the proliferative phase, live for many months and maintain continued antibody production (375). These long-lived cells will therefore become of increasing importance in the maintenance of serum-antibody levels once the productive phase is reached. In assessing their possible radio-sensitivity, Miller and Cole (376) gave rats and mice a secondary stimulus of TAB vaccine, followed by injections of tritiated thymidine twice a day for four days. Thirty days later two groups of mice were given 850 rads and 500 rads, respectively, and one was left unirradiated; the rats received 850 rads, controls being unirradiated. Large numbers of persisting labelled plasma cells were found in lymph nodes after irradiation. No difference could be found in the numbers or distribution of labelled plasma cells in lymph nodes from irradiated animals compared to lymph nodes from those non-irradiated. This ability of plasma cells to survive irradiation may partly explain the radioresistance of established antibody production.

137. It has been shown (334) that exposure of spleen cells in millipore diffusion chambers to 10,000 roentgens during the plateau phase of secondary antibody formation results in a decrease in the total number of cells and in a relative increase in the proportion of plasma cells. Based on the incorporation of amino-acids into specific antibody, Vann (591) showed that spleen cultures exposed to 10,000 roentgens continue to synthesize antibodies at a normal rate. This indicates that the antibody-synthesizing polyribosomal units, which contain the messenger RNA for specific antibody-peptide synthesis, as well as enzymes required for protein synthesis, are not only stable but remarkably radioresistant. In a further analysis of the ultrastructural changes in irradiated antibody-forming cells exposed in chambers to 10,000 roentgens, Sado (484) showed by plaque assay that 3 out of 10 nucleated cells were specific-antibody formers four days after the irradia-

tion (10 per cent in the unirradiated control). The half-life of irradiated antibody-producing cells was not different from that of unirradiated cells. Electron-microscope studies showed pronounced nuclear damage, but fully developed endoplasmic reticulum rich in ribosomes. A low but significant number of blast and mature plasma cells were still capable of incorporating tritiated thymidine several days after exposure to 10,000 roentgens. Based on several studies, it was suggested that these cells represent those which were in the *S* phase at the time of irradiation and were incapable of generating further progeny. This study indicates an extremely high radio-resistance of plasma cells according to all parameters.

138. The direct effect of x-irradiation on antibody-plaque-forming cells *in vitro* has been studied by Kennedy *et al.* (286). Four days after the injection of antigen (sheep red blood cells), spleen cells from mice were taken and irradiated *in vitro*. They were then plated for content of antibody-forming cells as assayed by the Jerne plaque technique. As shown in figures VII-IX, doses of less than 2,000 rads had no effect

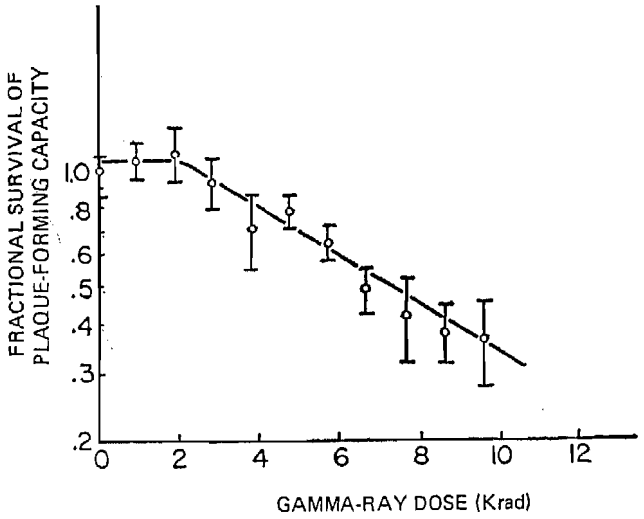

Figure VII. Plaque-forming ability of spleen cells from immunized mice as a function of radiation dose *in vitro*. Four days after the injection of antigen, the spleen cells from 12 mice were pooled, irradiated and assayed, 10 aliquots being used for each point. The 95 per cent confidence limits shown were calculated from variation in the number of plaques per plate (286)

on the capacity of plaque-forming cells to form plaques and doses in excess of 2,000 rads, up to 10,000 rads, had only a moderate effect. Although an accurate D_{37} could not be obtained, approximately 9,000 rads were required to reduce plaque-forming capacity to 37 per cent of its initial value.

139. In a more recent study, Sado *et al.* (485) determined the characteristics of the survival curves of 19S- and 7S-antibody-producing cells irradiated *in vivo*. In this study, the antibody-producing cells were derived from spleen-cell cultures undergoing secondary anti-sheep erythrocyte responses in cell-impermeable diffusion chambers and their numbers were assessed three days after irradiation by a modification of Jerne's hæmolytic plaque procedure. The results indicated that the number of 19S-antibody-producing cells decreased exponentially with increasing doses, giving a survival curve with a D_0 of 6,200 rads and an n_0 of 1.0. On the other hand, the survival curve for 7S-antibody-producing cells gave a shoulder portion at exposures below 15,000 roentgens which was followed by an exponential decrease with increasing doses, indicating that there exists a threshold for inactivation of this type of antibody-producing cell. This survival curve gave a D_q dose of 8,000 rads, a D_0 of 4,250 rads, and an n_0 of 1.62.

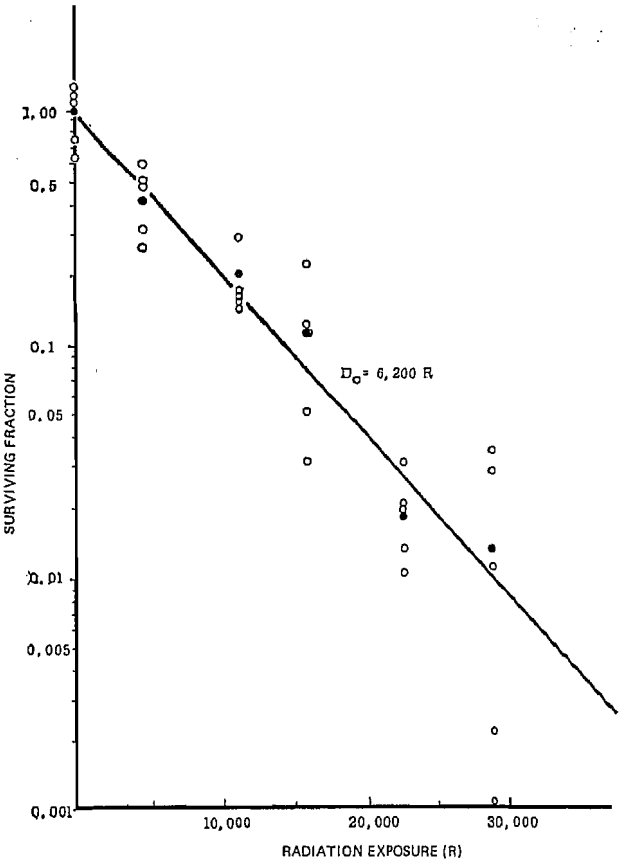

Figure VIII. Survival curves for direct plaque-forming cells. Each circle represents a value obtained from individual chambers and the solid circles represent the mean. Mice bearing diffusion chambers containing immunized spleen cells were irradiated with the indicated doses and the chambers then transferred to new hosts. Plaque-forming cells in the chambers were then assayed three days later (485)

140. In contrast to these situations involving direct irradiation of plasma-cell populations, some results with cell-transfer situations have indicated a depression following radiation. For a quantitative estimate of the radio-resistance of the productive phase of the immune response, mice of the C57BL strain were immunized with non-pathogenic leptospiræ (667). After 14 days of antibody production the spleens were removed, and a cell suspension was prepared and then irradiated *in vitro* with doses ranging from 100 to 20,000 rads. After this the cells were placed in culture *in vivo* (i.e. injected into irradiated recipients), and after six days the antibody titres in the blood of the syngeneic recipients were measured. In this case the dose-effect curve consists of two parts. The first part—in the 100-800-rad dose range—has characteristic values of $D_0 = 260$ rads and $n_0 = 1.3$. The second part of the curve is less steep: an increase in the dose from 1,000 to 20,000 rads produces no substantial additional depression of antibody formation in the cell suspension. Irradiation with a dose of 800 rads depressed the antibody formation by 81 per cent, and 20,000 rads by 93 per cent. The conclusion drawn from this was that in the pro-

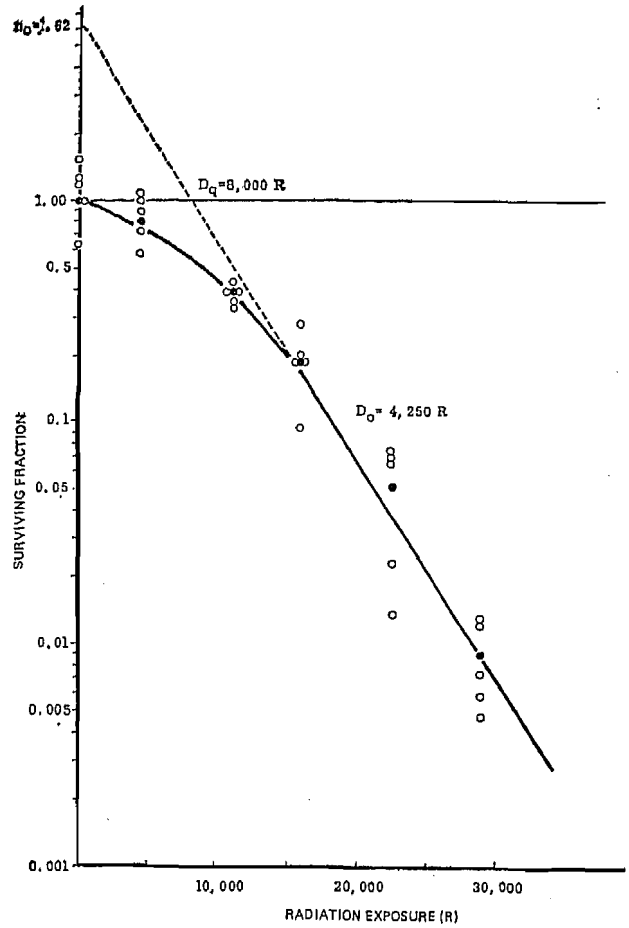

Figure IX. Survival curves for indirect plaque-forming cells. Other details as for Figure VIII

ductive phase the population of producing cells is heterogeneous: some are in the blast stage and require cell division in order to develop, while others are mature non-multiplying plasma-cell elements. The results were analogous when the synthesis of antibodies in an *in vitro* culture after the inclusion of tagged amino-acids was considered (668).

141. In normal spleens of unimmunized animals, there are varying numbers of cells which will form antibody plaques against several red-cell antigens. These are referred to as background plaques and may represent persisting plasma cells from previous immunizations (spontaneous or induced) to cross-reacting antigens. The numbers of these background plaque-forming cells are unaltered (241) when measured two and seven days after x-ray doses of up to 200 rads. Doses of 500 rads and 900 rads caused some slight decrease in background plaques (approximately 20 per cent at two days and 30 per cent at seven days after 900 rads). The lack of sensitivity to radiation at whole-body doses of 50 to 100 rads indicates that maintenance of normal levels of background plaque-forming cells is not dependent on rapid proliferation, and that the average lifetime of these cells is greater than seven days. This result is also consistent with the relative radio-resistance of the mature plasma cell.

2. *The secondary antibody response*

142. The secondary antibody response is elicited in an animal after the second injection of antigen. This may be given at a time well after the first injection when the primary response has completely disappeared, or earlier, when persisting antibody is still present. The three main hallmarks of a secondary, memory or anamnestic response, are a shortened latent period (time between antigen injection and appearance of serum antibody), a higher peak titre, and a greater and earlier contribution of IgG rather than IgM to the antibody population. All three of these criteria are not always manifest in a secondary response, and generalizations are not very relevant in this regard as differences occur with different species, antigens, doses, timing, etc.

143. In general, when the secondary response is considered in its entirety, without attempting to separate the true secondary from a decaying primary, or without giving attention to the quality (avidity) of antibody, the secondary response is quantitatively more radio-resistant (figure X). Thus Dixon *et al.* (145) found

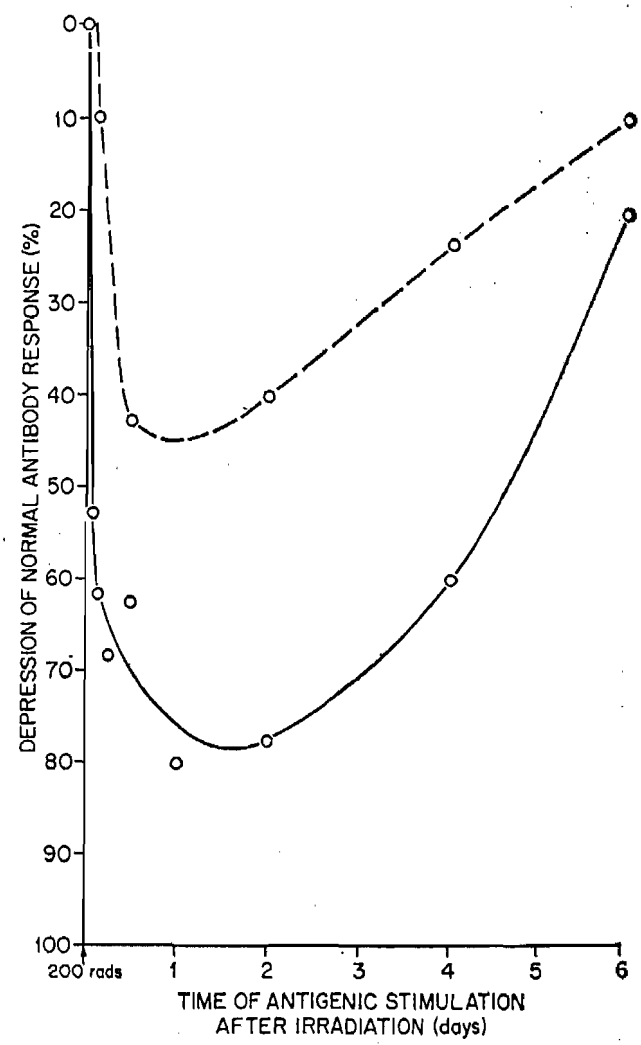

Figure X. Relative radio-sensitivity of the primary (solid line) *versus* secondary (broken line) response (532). In these studies the effect is estimated in the whole animal by measuring over-all antibody response (for both primary and secondary, the results are plotted relative to control unirradiated animals given primary or secondary immunization, respectively)

little effect of 400 roentgens two days before the secondary antigen injection; but some delay occurred with 800 roentgens (400 R was fully effective in the primary). Similarly, Silverman and Chin (508) found

no effect on the anamnestic response in rabbits given 400 roentgens 24 hours before second injections of egg albumin. However, in the earlier studies of Taliaferro *et al.* (549), it was reported that the specific anamnestic response to Forssman antigens was as susceptible to x-ray damage as the initial response. Crosland-Taylor (113) also found that the secondary response of rabbits to tetanus toxoid was radio-sensitive but differed from the primary response in that an exposure of 400 roentgens had to be given two or more days before the antigen to reduce the peak titre. Porter (460) found that 550 roentgens given to rabbits during the latent period between the first and the second antigen injection destroyed or markedly inhibited secondary response. Following this further, Thorbecke *et al.* (565) showed that whole-body irradiation (450-500 R) given to rabbits 21 days after a primary injection produced a permanent partial inhibition of the booster response, whereas irradiation eight days after the primary injection resulted in some inhibition followed by a rapid recovery. This recovery appeared to be correlated with the destruction and reappearance of secondary nodules in the white pulp of the spleen.

144. These earlier studies therefore seemed to indicate that the secondary response could be inhibited by pre-irradiation, but perhaps not to the same degree of sensitivity as the primary response. Detailed measurements of radio-sensitivity using the cell-transfer system were then made by Makinodan *et al.* (333). When the antibody-forming activity of spleen cells is assayed on a given day after an antigenic stimulus, the logarithmic relation between antibody titre and viable-cell number is linear up to a certain cell dose, and the slopes of these regression lines are not significantly different regardless of whether the response is primary or secondary. The slopes of these regression lines remained unaltered even after sublethal x-irradiation, and the magnitude of the decrease in the primary and secondary antibody-forming activities of spleen cells after a given dose of x rays was approximately the same. These findings therefore suggest that the apparent difference in radio-sensitivity between primary and secondary antibody responses among intact animals exposed to sublethal whole-body doses of radiation is mainly due to the difference in absolute number of competent cells surviving after radiation treatment. It follows then that radio-sensitivity of secondary antibody-forming capacity of intact animals can be best shown with those given a minimum pre-immunization treatment.

145. In further extending their original observations made in 1952, Taliaferro and Taliaferro (548) have shown that, in rabbits immunized with sheep erythrocytes, the anamnestic response to Forssman antigen was still depressed when sheep erythrocytes were injected two to six months after exposure to 500-700 roentgens. The results demonstrate that the IgM response is of equal radio-sensitivity in the primary and secondary response when maximally depressed by 500-700 roentgens, but that the anamnestic response is more radio-sensitive than the primary response during the phase of recovery from these high doses. These authors accordingly suggested that the memory cells themselves might be more sensitive to radiation than the initial immunocompetent cells.

146. It has been suggested (499) that the cell pool responding in the secondary response is a specific differentiation product of the memory-cell pool induced by antigenic stimulation. As the antigenic stimulation subsides, its expansion ceases and it does not regenerate after injury if the stimulus is lacking. Since its self-generating capacity may be limited, it can be permanently reduced in size or even abolished by irradiation. This general conclusion (406) is based on earlier studies of the regenerative potential of antibody formation after irradiation. It was shown (404) that the minimum antigen dose necessary to initiate a near-maximum antibody response is about 10^5 times greater for irradiated than for unirradiated spleen cells. Accordingly, various factors affect post-irradiation recovery of the ability to give a secondary response. These include the type and amount of priming antigen, the primary x-irradiation interval, and the x-ray dose (405). Recovery of the memory-cell pool after irradiation does occur provided the antigen persists until uncommitted progenitor cells again become available and are stimulated to form memory cells.

147. A recent report (552) has described the radiosensitivity of the *in vitro*-induced primary and secondary antibody responses to a bacteriophage antigen. In this culture system both types of responses could be compared in an identical environment. Radiation-induced depression of the secondary response initiated *in vitro* with lymph-node cultures from immunized rabbits was clearly demonstrated with 500 rads given 3 hours before or 24 hours after antigen. Peak antibody production was both delayed and reduced. The radiosensitivity of the secondary response was as great as, if not greater than, that of the primary response. This type of direct study, taken with the recent reports described above, clearly demonstrates the equal radiation sensitivity of the actual antigen-induced cells whether they be of virgin or memory type. The actual expression of the radio-sensitivity of the primary or secondary response, as measured by serum titres in the whole animal, involves other factors, which in turn are mainly related to the number of virgin or memory cells that are respectively irradiated.

148. In a previous discussion of the enhancing effect of radiation on antibody formation, Makinodan and Price (336) considered the phenomenon in terms of the actual immunological expression in relation to the full potential response that would be possible. They also discussed the apparent paradox of radio-resistance of the secondary response in these terms. Previous studies had clearly shown that although the difference in the magnitude of response between individuals undergoing primary and secondary responses might be only twofold, the secondarily-stimulated animal actually possesses up to 100 times more potentially responsive immunocompetent units (331). In other words, the ratio of immunological expression to potential is much larger in a primary than in a secondary response. This in turn implies that, for a given dose of radiation, even though both primary and secondary cells have equal radio-sensitivities, a much greater number of unused potentially reactive cells remain in the secondarily-challenged animal. A sample calculation of this nature has been made by Makinodan and Price (336) and is shown in table 4. In this example it is seen that although 300 roentgens reduced a primary response to 5 per cent of control, no effect was observed on the secondary response.

IV. Effects of radiation on cellular immune reactions

A. Cellular components involved in cellular immunity

149. As originally outlined in this review, immune responses are broadly divisible into those involving humoral antibody mediated by plasmacytic and some lymphocyte-like cells, and cellular immunity mediated by lymphoid cells. The small thymic-derived lymphocyte (*T* cell) is the cell involved in immune reactions such as delayed hypersensitivity and graft rejection. This cell may undergo various changes and appear as a pyrinophilic blast cell which then may give rise again to lymphocyte-like progeny (523a). Although this cell is known not to secrete appreciable amounts of immunoglobulin molecules, it is likely that the recognition unit on the cell surface, which is responsible for specific reaction to antigen, is an immunoglobulin molecule (30, 35, 225, 343, 425), or possibly only a free light chain or light-chain component. Many studies of this problem are currently in progress, and at least agree that the density of immunoglobulin molecules on the surface of the *T* cell, if present at all, is only of the order of 1 per cent of that on non-thymic-derived lymphocytes. The *T* lymphocyte is part of the recirculating pool and is markedly depleted by thymectomy, particularly neonatal thymectomy (374, 379). Those lymphocytes which are involved in cellular immunity are directly derived from the thymus and carry surface marker antigens such as theta, which distinguishes them from the non-thymic lymphocytes that are precursors of antibody-forming cells. The phenomenon of cell collaboration has been repeatedly stressed in discussions on antibody formation. Although cell collaboration may be equally relevant for cellular immunity, there is at present only slight direct evidence of such interactions (72), involving two thymic-derived cells. There is no evidence for collaboration between thymic-derived cells and bone-marrow (bursa equivalent?) derived cells in cell-mediated immunity (91, 536, 577). This section will first examine morphologically-defined lymphocytes, as a heterogeneous population, and then discuss in functional terms specific cellular immune responses.

B. Lymphocytes, lymphoid tissue and radiation

150. Organized lymphatic tissue and individual lymphocytes are extremely radio-sensitive. This fact was recognized within a few years of the discovery of x rays, and has been the subject of numerous detailed reviews over many years (54, 156, 165, 408, 495, 663, 670, 675). The striking effect of a single lethal whole-body dose of x rays on the mouse lymph node is indicated in figure XI. The effect of a large acute exposure to ionizing radiation is to destroy the cortical masses of tissue lymphocytes and the dividing cells in the germinal centres of the lymph node, leaving intact the stroma, blood vessels, mature plasma cells and reticulo-endothelial cells (95). A few lymphocytes usually remain and these will be considered later. This pattern is typical of all organized lymphatic tissue.

151. Regeneration of lymphatic tissue usually involves reappearance of parenchymal elements in the same order as in the original ontogenic development, that is, collections of cortical lymphocytes appear first, and are followed by germinal centre formation. As previously discussed in relation to immunological regeneration, the regeneration of lymphoid tissue and of functional activity depends on the presence of an intact thymus. Extramedullary myelopoiesis in lymphatic tissues preceding lymphoid regeneration has been observed (54) but is of unknown significance. Regeneration of lymph nodes after local, rather than whole-body, irradiation is extremely rapid, presumably because of the influx of normal cells from the unirradiated areas (98). If a high local dose (e.g. 3,000 rads) is given, an extreme secondary atrophy develops in subsequent weeks, apparently following vascular damage and destruction of the original stroma (165).

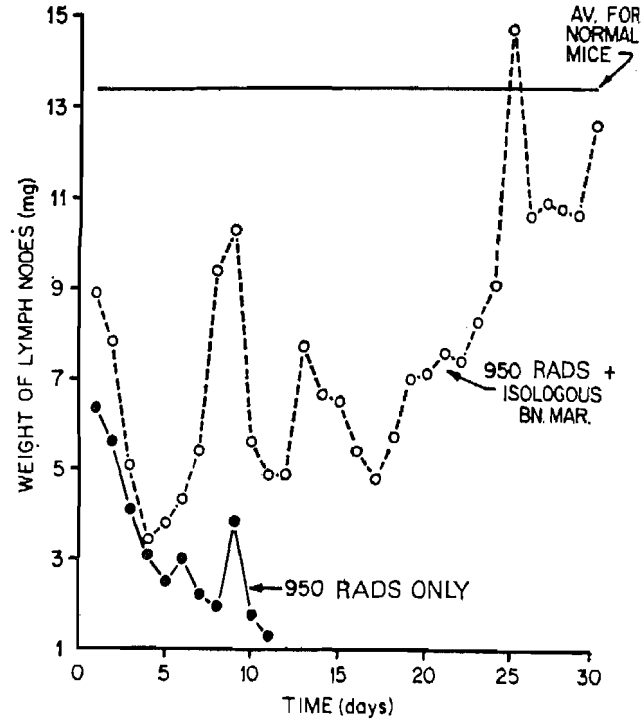

Figure XI. Weight changes in four peripheral lymph nodes in mice exposed to supralethal whole-body doses of x rays. Also shown are the weight changes in lymph nodes in mice similarly irradiated but given isologous bone marrow afterwards. The temporary weight increase between 5 and 10 days after the treatment was caused by extramedullary granulopoiesis. Each point represents five mice (95)

152. In considering the effect of radiation on lymphocytes, it is important to distinguish between two general mechanisms. Firstly, lymphocytes are virtually the only cell population of the body to show interphase death from radiation, and this may play an important role in radiation-induced depression of immunity. On the other hand, as has been previously discussed (paragraph 90), the doses of radiation that affect the primary antibody response suggest that a main effect of radiation is on cell division.

153. The biochemistry of necrosis has not been studied to the same extent as the morphology of necrosis and there is no firmly established biochemistry of radiation necrosis in lymphatic tissue. Bacq and Alexander (27) consider interruption of energy supply and enzyme release as two main theories on the nature of the early biochemical lesion in cells exposed to ionizing radiation. Major consequences of radiation exposure are the interference with the biosynthesis of nucleic acids and chromosome breakage. It is generally considered that the inhibition or delay in DNA synthesis is the single most important biochemical change

in lymphatic tissues caused by radiation and it may be presumed that ionizations are in some way affecting those portions of the cell genome that control DNA synthesis itself.

154. Normal human blood lymphocytes have been found (527) to show extreme sensitivity to x-irradiation *in vitro*. A statistically significant sensitivity to x-irradiation was shown with two and five roentgens, producing, respectively, 13 to 21 per cent and 35 per cent effect (scored by morphological and motility changes). In other studies *in vivo*, as little as four hours after receiving a radiation exposure of 100 roentgens, the peripheral lymphocyte count is 25 per cent of normal in four- to seven-month-old rats (495). In addition to a reduction in count, the lymphocyte shows direct changes with pyknotic nuclei beginning to appear in four to six hours in lymphocytes exposed *in vitro* to 100-400 roentgens. It should be noted that the extreme sensitivity of lymphocytes to two and five roentgens was only observed with cells irradiated *in vitro*. It is possible that this represents an artificial condition in so far as the cells are not in their normal environment, and that these results might therefore not be relevant to *in vivo* irradiation, whereas *in vivo* results may also depend upon abscopal effects (e.g. as discussed in paragraph 298).

155. Lymphocytes within the gut epithelium in mammals have been termed theliolymphocytes, and it was proposed that they constitute a specialized type in that the gut epithelium may function as the first-level lymphoid organ. It has been shown (178) that on the whole they are as radio-sensitive as blood lymphocytes, although the number of theliolymphocytes is restored to normal values much earlier than blood-lymphocyte levels after irradiation. This may indicate a selective radio-resistance of the unknown source of the theliolymphocytes, or a preferential localization of the regenerating precursor cells.

156. Some persisting lymphocytes are still seen in lymph nodes of animals given whole-body irradiation in the lethal dose range. These cells may represent either a random fraction surviving the particular dose of radiation, or a specific population of more radio-resistant lymphocytes. Some *in-vitro*-culture studies with phytohæmagglutinin-stimulated lymphocytes have pointed to the existence of a separate resistant population (93). It has been shown that some small lymphocytes can persist for at least a year (375) and some of these may be responsible for immunological memory (222, 426). Several studies have accordingly been carried out to determine whether there is a difference in the radio-resistance of the long-lived and short-lived lymphocyte. In two reports (169, 621) no change in the proportion of long-lived to short-lived lymphocytes was found in blood lymphocytes or thoracic-duct lymphocytes after 215 or 300 rads. In another study (376) where doses of 500-850 rads were used, lymphocytes were examined in the local lymph nodes draining an antigen-injection site. Despite a marked generalized destruction of lymphocytes, the nodes examined contained significantly higher proportions of the long-lived lymphocytes (identified by tritiated thymidine introduced at time of antigen stimulation one month prior to irradiation). It was felt that these cells were probably not part of the circulating pool of small lymphocytes, and the results therefore do not necessarily contradict the other two reports which are concerned with the recirculating pool. It was therefore proposed that at least some types of long-lived lymphocytes are relatively resistant to quite high doses of x rays.

157. There is a clear-cut dose-response relation for lymphatic-tissue damage and repair when the dose is delivered over a short interval. However, the dose rate as well as the total dosage is important. In two studies (108, 198) on transplantation of foreign bone marrow, dose rates of 1-4 rads per minute were much less effective in immune depression than dose rates of 29-54 rads per minute, although the same cumulative dose was given. Dose rates in the range of 1.1 to 1.8 rads for eight hours per day had only a moderate effect on morphological changes in lymphatic tissue, as it often took several months to produce discernible changes.

158. A paradoxical finding on radiation exposure and thymic destruction has been reported by several authors (574, 575, 597). Whereas increasing exposure usually leads to enhanced lymphoid destruction, when it reaches the kiloroentgen range an opposite effect is observed. Thus rat thymus given between 10 and 30 kiloroentgens *in vivo* showed less damage (by morphology and weight) than in animals exposed below 10 kiloroentgens. With 30 kiloroentgens, virtually no thymus weight reduction was observed, whereas maximum depression in thymus weight occurred with approximately one kiloroentgen. A similar phenomenon has also been observed with thymus irradiation *in vitro* (574). Within the lymphatic-tissue system all sites appear to be equally radio-sensitive. The thymus, however, regenerates faster than other lymphatic tissues, presumably because it is the site of differentiation of new lymphocytes from immigrant stem cells.

159. X-ray exposures in the 10-200-roentgen range produce stimulation of adrenocortical secretion as judged by depletion of either adrenocortical sudanophilic material or total adrenocortical cholesterol (148). Accordingly, it is possible that x-irradiation may damage the lymphocyte through an indirect corticosteroid-mediated effect. In a study on atrophy of lymphoid organs in unoperated and adrenalectomized mice given different doses of radiation, it was found (149) that acute involution of lymphatic tissue (that is, steroid-independent lymphocyte destruction) occurred in both groups of animals with x-ray exposures from 25 to 200 roentgens, but that with 10 roentgens destruction of lymphoid tissue was more pronounced in intact mice than in adrenalectomized animals.

160. Thoracic-duct lymphocytes enter the splenic white pulp *via* the blood and, after traversing a pathway within the splenic pulp, subsequently re-enter blood (192). This suggests that local continuous irradiation of the spleen would lead to a marked fall in the recirculating lymphocyte pool and therefore of the primary immune status of the animal (221). This has been studied (191) by attaching a ^{32}P-impregnated polythene strip to the antihilar surface of the rat spleen. This resulted in a profound drop (to 15 per cent in four days) in the output of small lymphocytes from a thoracic-duct fistula. No other type of blood cell was affected. It appears that the lymphopenia was brought about by radiation death of small lymphocytes (possibly mainly interphase death) passing through the spleen from the blood. Other studies (230) on isolated lymph nodes had previously shown that large acute doses of radiation do not impair the organ structures essential for the recirculation of lymphocytes at least in the

immediate period, although later effects have been noted (see paragraph 289).

161. Lymphopenia has also been produced by chronic extracorporeal irradiation of the blood (111), by intra-atrial implantation of a beta-emitting source (31) and by intralymphatic infusions of radio-isotope-labelled agents (159, 567, 620). In this latter instance studies in man with intra-lymphatic infusion of ^{131}I-lipidiol have shown that even with a unilateral lower-limb infusion an appreciable volume of lymphoid tissue is irradiated, and histological examination of lymph nodes revealed widespread destruction. Many workers have proposed that depression of lymphopoiesis accounts for the lympho-cytopenic state. However, in the experiments with the ^{32}P-soaked strip (191), the lymphopenia occurred far too rapidly (50 per cent fall in one day) to be accounted for by depressed lymphopoiesis. A direct radiation death of the recirculating small lymphocytes seems far more likely. Leukæmic lymphocytes also appear to be markedly radio-sensitive and accordingly chronic extracorporeal blood irradiation may be of potential value in removing leukæmic cells. Several of the relevant findings from a recent international symposium on chronic extracorporeal blood irradiation are summarized below.

162. Reports at the experimental level clearly indicate the efficacy of chronic extracorporeal blood irradiation in producing lymphopenia. This may either be due to radiation destruction of the lymphocytes or to their inability to recirculate after irradiation. It was felt that there was still a bewildering amount of variability in technique for a relatively small amount of clinical information. In general, the experience with different clinical situations after chronic extracorporeal blood irradiation could be summarized as follows:

Acute myelocytic leukæmia: rare hæmatological remissions. Survival does not appear to be greatly changed;

Chronic myelocytic leukæmia: relatively few cases reported. No remission reported. White-cell count rises again rather rapidly;

Acute lymphocytic leukæmia: again relatively few cases reported and generally poor results;

Chronic lymphocytic leukæmia: best results with chronic extra-corporeal blood irradiation are in this disease. There have been clinical but no hæmatological remissions. Some decrease in spleen and lymph-node size has occurred.

C. Delayed hypersensitivity

163. Delayed hypersensitivity reactions can be readily induced in man and various laboratory animals. The guinea-pig is the classic species favoured for studies of this type of immune reaction. Studies on delayed hypersensitivity *in vivo* suffer from the disadvantage that the reaction can only be assessed semi-quantitatively at best, and that little information is available on the relation between the sensitivity of development of the skin lesion and the number of sensitized lymphocytes. Accordingly, the possibility of detecting accurately small radiation-induced changes is more limited than for antibody production, particularly when in the latter case actual numbers of antibody-forming cells are measured. At present, there is no universally accepted technique for enumerating sensitized cells involved in delayed hypersensitivity reactions comparable to the plaque type of assay. (Although one recent plaque type of assay has been reported (53) it is rather complex, and has yet to be fully confirmed.) This absence of a satisfactory plaque assay implies that a rather substantial reduction in the immune reaction probably has to be induced before it becomes observable by current methods such as measuring indurated skin lesions.

164. In several early studies, the induction of delayed hypersensitivity was not markedly inhibited by irradiation in doses which suppressed antibody formation. Thus 300 rads given 18 hours before sensitization with diphtheria toxoid resulted in a period of pure delayed hypersensitivity up to the twenty-first day post-sensitization without any antibody being detectable. When 300 rads were given 18 hours after sensitization, delayed hypersensitivity lasted for the usual period (488). This was confirmed (584) with another antigen, ovalbumin, which again showed retention of delayed hypersensitivity in the absence of antibody formation. However, when high radiation exposures (800 R) were given to rabbits before sensitization, complete suppression of delayed hypersensitivity was observed. A single exposure of 200-250 roentgens to guinea-pigs failed (494) to suppress the acquisition of allergic contact dermatitis to dinitrochlorobenzene, which is a manifestation of delayed hypersensitivity. Radiation will also depress the development of hypersensitivity to tularin and brucellin (661).

165. A febrile reaction is often associated with the state of delayed hypersensitivity. In guinea-pigs given 200-300 roentgens before sensitization, a febrile response occurred on systemic challenge with antigen in both irradiated and control groups (584). This reaction occurred in animals showing suppression of antibody response but not of delayed hypersensitivity.

166. Most types of experimental allergic autoimmune diseases such as experimental allergic encephalomyelitis appear to involve predominantly a cellular immune response (437). Administration of 150 roentgens 18 hours prior to antigen was reported (181) to result in an increased severity of experimental allergic encephalomyelitis in guinea-pigs, rather than a depression. There was no significant diminution of delayed sensitivity to the original brain material used for inoculation. However, in another study (438) of allergic encephalomyelitis induction, 400 roentgens (whole body) given to rats prior to sensitization with spinal cord and adjuvant suppressed the encephalomyelitis. This suppression was dose-dependent and was observed in two strains of rats sensitized by either of two routes. A reduced production of complement-fixing antibodies occurred, but there was little, if any, suppression of delayed immunologic reactivity as based on tuberculin skin testing. In two other reports, x-irradiation enhanced rather than depressed the development of experimental allergic encephalomyelitis in guinea-pigs (13) but suppressed it in rabbits (94). These apparent contradictions in radiation effect on the induction of experimental allergic encephalomyelitis may be due to differences in species, doses of x rays, etc. The studies of Paterson (437) would seem to indicate that a reduction of cytotoxic-antibody formation might explain the reduced clinical disease. It might be speculated that reduced antibody formation could also lead to the enhanced severity observed in guinea-pigs. Since certain types of antibodies (enhancing antibodies) may protect animals from the disease (437)

it is possible that these, rather than a cytotoxic antibody, are normally produced in the guinea-pig with the immunization scheme used. Accordingly, radiation-induced depression of this type of antibody formation would lead to an apparently more aggressive immune cellular response that would further the disease process.

167. Some contradictions also exist in the literature regarding the question of radiation sensitivity of the transfer of delayed hypersensitivity. Experiments with donor cells irradiated *in vivo* or *in vitro* and with normal recipient animals have been described. As regards irradiation of the donor *in vivo*, it was shown (118) that a whole-body x-ray exposure of 150 roentgens diminished the tuberculin reaction of sensitized donors when irradiation was given prior to antigen. Comparable x-irradiation of recipient animals four days before cell transfer from either irradiated or non-irradiated donors also produces a diminution in the tuberculin reactivity of the recipient animals. Irradiation of sensitized cells *in vitro* prior to transfer has also been reported (24) to reduce the resulting reaction, provided the exposure is above 1,500 roentgens. Exposure to 1,000 roentgens did not affect transfer. Since the small lymphocyte is very sensitive to radiation, it might be expected that reduction of transfer by irradiated donor cells would occur very readily.

168. Three possible explanations for this apparently high radio-resistance of sensitized cells might be considered: (*a*) that, as discussed by Makinodan *et al.* for antibody production, the immunized cell population is as radio-sensitive as unimmunized cells, but contains so many specifically sensitized cells that high doses of radiation are needed to eradicate enough of the component donor cells; (*b*) that the sensitized memory cell responsible for the transfer of delayed hypersensitivity may belong to a different category of lymphocytes (possibly of the type described by Miller and Cole (376)) and be inherently radio-resistant. This might imply that its radio-resistance has in fact been induced by the antigenic stimulation, as suggested by Stefani (526); and (*c*) by analogy with the experiments of Katz *et al.* (282) on the radio-resistance of carrier-primed cells, it may be that the donor-sensitized cells in transfer of delayed sensitivity also collaborate with host cells, and that this collaboration involves very little, if any, donor-cell proliferation, and is therefore relatively radio-resistant.

169. X-irradiation (550 R) of recipient rats (89) before transfer of sensitized cells totally prevented the expected delayed reaction, provided skin-test challenge was given soon after the cell transfer and irradiation. The passive transfer by sensitized cells of experimental allergic encephalomyelitis into recipients was also inhibited (309) by x-irradiation of the recipients 24 hours before cell transfer. Complete inhibition occurred with 700 or 1,000 roentgens, partial inhibition with 400 roentgens and no inhibition with 100 roentgens. These two experiments strongly suggest that a host component involving cell proliferation is essential for successful passive transfer of delayed sensitivity of experimental allergic encephalomyelitis. This is consistent with various studies (review by Bloom and Chase (52)) which clearly indicate that the majority of infiltrating cells in the delayed-hypersensitivity lesion are host-derived cells.

170. One of the more classical hallmarks of delayed-hypersensitivity reactions is that they can be passively transferred by cells but not by serum (52). A recent report (157), however, has described the passive transfer of delayed hypersensitivity to PPD by plasma from BCG-immunized x-irradiated (800-1,000 R) donors. Neither plasma from non-irradiated BCG-sensitized donors, nor plasma from x-irradiated non-immunized donors, could transfer PPD sensitivity to normal recipients. Passive transfer of PPD sensitivity was also achieved by normal spleen cells which had been incubated *in vitro* with plasma from immune x-irradiated donors. Repeated washing of these cells failed to remove their ability to passively transfer PPD sensitivity. It was suggested that some factor of unknown nature which is normally bound to cells was released into the plasma by irradiation and could then bind to host cells *in vivo* or *in vitro* and "confer reactivity". Such a factor could theoretically be an immunoglobulin-type molecule with appropriate specificity, a nucleic acid coding for a polypeptide with the specificity, a transfer factor of one of the types recently reviewed by Lawrence (303), or a very immunogenic antigen. This is a complex problem as a failure to detect migration-inhibition factors in supernatants of sensitized lymphocytes irradiated *in vitro* was also recently reported (19).

D. Transplantation immunity

1. *Experimental allograft rejection*

171. The feasibility of pretreating prospective recipients with ionizing radiation to promote survival of foreign grafts was clearly demonstrated by Murphy in 1914. This work appears to have been forgotten until the early 1950s when, following on the pioneer studies of Medawar (355) on the immunological basis of transplantation rejection, Dempster *et al.* (131) showed suppression of skin homograft rejection by pretreatment of the recipients with x-irradiation. An exposure of 250 roentgens given to rabbits before the application of skin grafts from another rabbit markedly prolonged the survival of the grafts. The second-set response, however, was unaffected by this dose of radiation.

172. Prolonged survival of skin grafts with only minor genetic differences can be induced by pretreating recipients with ionizing radiation in non-lethal doses. A moderate delay in primary homograft rejection was observed (362) in mice given 400 rads, although the depression of antibody formation was far greater. Prolonged rejection of male-skin grafts on female syngeneic mice has also been induced by exposing recipients to 300 roentgens (285). Exposures of 1,000 roentgens were far more immunosuppressive on both primary and secondary graft rejections (63).

173. The effect on graft survival of extracorporeal gamma irradiation (ECI) of the circulating blood of calves before and after skin homografting has been described in 13 calves (77). In all the ECI-treated calves, the normal acute and violent skin-homograft-rejection process occurring at 9 to 10 days was modified to a slow and mild process with an increase in rejection time by 1 to 11 days. In one calf where thoracic duct lymph was drained for eight days and cell-free lymph was returned to the animal followed by four days of ECI to the lymph, the skin-graft-rejection time was 40 days.

174. These results clearly indicated that the homograft-rejecting capacity could be depressed by prior irradiation, although the relative radio-sensitivity of

primary *versus* secondary graft rejection was not clear. Tyan and Cole analysed this problem in a series of papers in which different variables were considered, such as radiation dose, method of presensitization, comparison of hæmagglutinin production *versus* graft rejection, and comparison of xenogeneic (heterograft) with allogeneic (homograft) grafts. It was found (580) that the second-set response of mice presensitized by means of allogeneic or xenogeneic skin grafts was more resistant to a lethal dose (850 rads) of x rays than the first-set response. This was also shown (578) with mice given sublethal irradiation (670 rads). Differential radio-sensitivity of the xenogeneic and allogeneic reactions was also observed but in opposite directions in primary *versus* second-set rejections (579). The method used for presensitization can also affect the apparent radio-sensitivity of the second-set-rejection mechanism (576). Thus, if spleen cells in Freund's adjuvant are used for presensitization, the resulting homograft response is as radio-sensitive as that produced by application of skin grafts. However, if the spleen cells are anatomically separated from the Freund's adjuvant in the recipient, a more radio-sensitive response develops, this difference possibly being due to a differential proportion of proliferating and mature cells induced by the two régimes. Consideration of hæmagglutinin production and skin-graft rejection by irradiated mice also tends to suggest that these two immune responses are mediated by separate cell lines (581), as has been discussed previously.

175. Accurate measurements of radio-sensitivity of the homograft immune mechanisms are again difficult unless a quantitative cell assay can be used. Two approaches to this problem have been reported. In one case (76), for estimating second-set rejection, recipient mice are primed with donor homologous (transplantation) antigens and then given irradiation and an injection of spleen cells of donor type which have been previously sensitized to sheep red cells. The ability of the transferred cells to form anti-sheep-red-cell antibody in the recipient is then dependent upon radio-sensitivity of the cellular immune response of the recipient. When recipients were given 500 roentgens, only a few animals responded, indicating an almost complete failure to take on the part of the infused homologous cells, that is, evidence of a still functioning host immune response. With 700-850 roentgens the antibody responses by the donor cells were intermediate and, with 900 roentgens, titres comparable to isologous controls were observed (complete homograft suppression).

176. Further quantitative evidence of the radio-sensitivity of homograft immunity came from a second assay method (75) in which the killing effect of parental (P1 or P2) cells was studied in irradiated, immunologically inert (P1 × P2) F1 recipient mice, by determining the decrease of anti-rat agglutinins synthesized by P2 cells. The data showed that the homograft-rejecting capacity is more radio-resistant than the agglutinin-forming capacity. Slight strain differences were also observed. The LD_{37} values for agglutinin formation by C3H and C57 cells were 58 and 47 rads, respectively. The corresponding value for homograft-rejecting capacity (C3H cells) was calculated to be 78 rads, which is in the range of radio-sensitivity calculated for cells in the inductive phase of the humoral antibody response. This suggests that cell proliferation is also the major radio-sensitive step in the development of a homograft response.

177. One problem with this interpretation is that the particular assay system used has not been proved to represent graft rejection by a direct *T* lymphocyte cellular process, and that cytotoxic or protective antibody formation might also be involved. In fact, in a further extension of this assay method (73), evidence was presented that the reaction could proceed through a porous membrane. Critical studies of the radio-sensitivity of the actual effector cells that mediate cellular immunity are still needed, and several suitable methods for this have recently become available. These involve *in vitro* assays directly measuring cytotoxic effects of sensitized lymphocytes on target cells (64, 65, 205, 447). Recent data suggest that there are two categories of specific cytotoxic lymphocytes, one of which retains cytotoxicity after doses of 2,000 rads, whereas the other is much more radio-sensitive, being markedly inhibited after doses of around 500 rads. There is also some evidence to suggest that stimulation by antigen renders the cytotoxic lymphocytes more radio-resistant (224).

178. Inactivation of stem cells has also been used as a target assay for homo-transplantation activity of lymphoid cells (681). When both lymphoid and hæmopoietic cells are grafted from CBA and C57Bl mice to lethally-irradiated F1 hybrids, the lymphocytes of CBA genotype inactivate 90-100 per cent of the colony-forming elements of C57Bl type, which is detected by the reduction in spleen colony formation (677). CBA donors were irradiated with $LD_{50/30}$ doses of gamma rays, after which the ability of their spleen cells to inactivate the stem cells of C57Bl mice was investigated. One hour, one day, seven days and fourteen days after irradiation the index of inactivation was 0 to 10 per cent. A partial re-establishment of lymphocyte homograft activity was observed after 30 days. Normal values were not obtained until 60 days after irradiation.

2. *Hæmopoietic grafts*

179. Bone-marrow transplantation to a lethally-irradiated recipient within a syngeneic system will produce complete restoration of the hæmopoietic system and thus, in situations in which the radiation damage causes lethality due to hæmatopoietic damage, the survival of the animal. This effect was well studied in laboratory animals for many years and is known to be due to the repopulation of hæmatopoietic tissues in the depleted host by the injected cells and their descendants (189, 310, 314, 315, 587, 644).

180. When marrow transplantation is performed in allogeneic situations, two problems are encountered. Firstly, if the bone marrow is foreign to the host, then the immune competence of the host must be sufficiently depressed by irradiation or by other means to permit the survival of the injected cells. It was estimated (570) that, when a major histocompatibility difference is involved, the minimum dose of radiation (followed by homologous marrow) necessary to permit survival of the injected cells, and therefore tolerance to the donor, lies between LD_{18} and LD_{90}. With minor histocompatibility differences, lower radiation doses are effective (126). In studies in mice, insufficient radiation leads to marrow-graft rejection and an early (within 5-21 days) mortality (570). This occurs even in the high sublethal range, presumably because the graft-rejection mechanism appears to be more radio-resistant than the animal's own hæmopoietic stem

cells. Thus, although the number of cells that persist is sufficient to reject allografts, the animal's own hæmopoiesis is suppressed below limits required for its survival.

181. The second problem with allogeneic grafts occurs when the host carries a major transplantation antigen not present in the donor's genotype. This results in a late mortality (21-60 days) when bone-marrow cells are injected into allogeneic lethally-irradiated recipients (e.g. parental strain into F1 hybrids). This type of mortality is attributed to an immunological reaction against the foreign host antigens by the homologous lymphoid elements (or their progeny) that have been introduced with the grafted bone marrow (92, 99, 136). The immunological nature of both of these problems is now well documented (162, 512) and will not be extensively reviewed. Instead, a brief consideration of hæmopoietic transplantation in larger animals and man will be undertaken, particularly in terms of the radiation conditions and doses required for adequate immunosuppression of the recipients. Several other factors concerned in this problem, such as dose rate, will also be discussed in relation to the animal experiments.

182. In a study of the survival of irradiated rats injected with allogeneic bone marrow, Courtenay (108) found a relation between survival and the x-ray dose rate. The study suggested that the lower rates of 0.28 and 1-4 R min^{-1} were less effective in depressing the host's immune response than the higher rate of 29 R min^{-1}. This was confirmed by Gengozian (198) who irradiated mice at several different exposure rates so that they received a total of 900 roentgens over the whole body. Within two hours they were injected with rat bone marrow. The higher the exposure rate, the greater was the success of the grafts. The results strikingly indicated that in mice given 900 roentgens at a rate of 3.75 R min^{-1}, virtually no take of donor cells occurred. This phenomenon was further studied by Gengozian *et al.* (199) who gave mice lethal whole-body exposures of 900 and 1,200 roentgens at different exposure rates followed by allogeneic or xenogeneic bone marrow transfusion. With both grafts and both total doses, mice exposed at 3.75 or 19.8 R min^{-1} did not show permanent survival. In fact, with 900 roentgens at 3.75 R min^{-1} no increased survival could be shown. Mice given 1,200 roentgens at high rate (39.7 R min^{-1} or 53.4 R min^{-1}) had a permanent take of grafted marrow.

183. It is to be emphasized that in these experiments the difference between dose rates is not large. Previous work (547) has stressed the importance of high dose rates for immunosuppression in comparing chronic (days, weeks) with acute (minutes, hours) irradiation. The distinction drawn in the experiments with bone-marrow transplantation is between a time of delivery of only 22 minutes (1,200 R at 53.4 R min^{-1}) and one of about 5½ hours (1,200 R at 3.75 R min^{-1}). These findings may have great relevance to clinical attempts at allogeneic marrow transplantation after whole-body irradiation, because many of the irradiators used on humans operate at low exposure rates. A survey of various clinical studies (20, 346, 352, 555) reveals that radiation is often delivered at exposure rates ranging from 0.5 to around 5 R min^{-1}, so that even though a total dose of 800 to 1,800 rads may be given, it is delivered at a very low rate. As will be discussed in the following paragraphs, most attempts at allogeneic bone-marrow transplantation in man have been relatively unsuccessful. Similarly, the difficulties in obtaining successful foreign-marrow grafts in large animals are well documented and again may be related to the low dose rates used, usually of the order of 4-20 R min^{-1}.

184. As a result of the geometry of large animals and of man relative to the radiation sources used, the absorbed tissue dose and the tissue-dose rate may be even lower. Exposure rates greater than those found (199) satisfactory for transplantation of bone marrow in the mouse may therefore be necessary for success in large animal studies. These considerations clearly indicate the need for careful evaluation of this basis for transplantation failure, as opposed to the more conventionally accepted graft-*versus*-host reaction.

185. Grafts of bone marrow from donors which differ at major histocompatibility loci can survive for about a month or two if the prospective recipients are pretreated with sub- and mid-lethal doses of radiation (197). Rejection of the foreign graft, which is related to the recovery of the host's immune system, can occur with a severe reaction leading to the death of the recipient. This effect is often referred to as the "mid-lethal killing effect" and is observed in these situations in mice where a mid-lethal dose is used together with strongly-antigenic donor bone marrow (587). The effect may be analogous to the enhancing effect of irradiation on the antibody response, and accordingly may have an important bearing on clinical attempts with allogeneic marrow transplantation after total body irradiation in so far as, with the doses and dose rates of irradiation used, this mid-lethal killing effect may be involved in apparent failure of takes of allogeneic donor marrow. A similar problem may also occur with situations of minimal donor-host genetic differences, since Barnes and Mole (32) showed that the injection of a minimal number of lymph-node cells from H-2 compatible mice into sublethally-irradiated recipients (CBA) caused a significant fraction to die 2-18 months later of a lymphoid deficiency (? graft *versus* host) syndrome.

186. The lethal effects of whole-body irradiation (1,800 R) of dogs can be overcome by administration of the dog's own marrow taken before irradiation (227). However, when allogeneic bone marrow is used, permanent takes are extremely rare. When methotrexate is given early in transplantation, controlled studies (558) show that an increased number of long-term survivors results. Survivors for five months to four years have been observed after whole-body exposures of 1,200-1,800 roentgens and injection of marrow with methotrexate (561). In some animals, mild secondary disease developed and then subsided. In studies with dogs given 1,200 roentgens and cross-circulated (168) with normal dogs of opposite sex or injected with marrow of opposite sex (167) donor-type mature granulocytes were readily evident in the irradiated partner. In this study also, methotrexate was of some value in diminishing the secondary disease (168). In view of the previous discussion of dose rate, it is to be noted that in these studies with dogs dose rates of less than 10 rad min^{-1} were used and the effect of methotrexate may have been to aid the immunodepression of the host. However, the clinical symptoms are claimed (562) to be different in dogs dying of graft rejection than in those dying of secondary disease, and care should be taken to clarify this in all cases.

187. In contrast to much of the experience in dogs and man, bone-marrow takes appear to be relatively successful in primates. In recipients given whole-body doses in the range of 550-930 rads and 4.2 10^8 allogeneic bone-marrow cells, takes have occurred in at least 95 per cent of the cases (116). However, at this stage the major problems with primate-bone-marrow transfusions are encountered. In mice and rats, although takes of bone marrow require suppression by reasonably-high radiation doses, permanent chimeras are then relatively frequently established. In primates, on the other hand, secondary disease is a far more common problem (135). This difference may be partly due to the numbers of cells required to protect the lethally-irradiated recipient. Estimates (588) range in the order of 5 10^6 cells per kilogramme for mice, 40 10^6 cells per kilogramme for monkeys and of the order of 100 10^6 cells per kilogramme for man. If it is assumed that comparable proportions of immunocompetent cells exist in the bone marrow in the different species, and that a similar absolute number of immunocompetent cells will initiate the graft-*versus*-host process, then it is possible that the excessive severity of secondary disease in primates is mainly due to the larger absolute numbers of immunocompetent cells. On the other hand, by varying the number of allogeneic bone-marrow cells used in transfusion, Vos (599) has shown that mouse bone marrow indeed contains less immunologically active cells than monkey bone marrow. Dicke (137) has also shown that mouse bone marrow contains far fewer phytohæmogglutinin-sensitive cells than monkey bone marrow.

188. Attempts at bone-marrow transplantation after whole-body irradiation in *Rhesus* monkeys which have received multiple transfusions only rarely leads to acceptance of the graft, in distinction to the almost invariable takes in non-immunized monkeys (593). This was also demonstrated with prior transfusions of blood from third-party donors. Decreased takes may well be due to the existence of a heightened state of the immune response in the recipients prior to irradiation, which, by increasing the number of immunocompetent cells, would accordingly lead to an increased number of reactive or potentially-reactive immunocompetent cells surviving after irradiation. The time interval between transfusion and irradiation (minimum 30 days) is probably too long to account for the decreased takes being caused by increased levels of antibody resulting from irradiation-induced antibody enhancement. In presensitized recipients, this problem might be overcome by giving the recipients higher doses of radiation with a view to a more complete eradication of the population of immunocompetent cells. However, if some of this population should involve the more long-lived radio-resistant subpopulation, irradiation at a sufficiently high total dose would not be feasible. Before considering the various approaches to amelioration of the secondary disease problem, a brief report on human bone-marrow transplantation is relevant.

189. Bone-marrow grafts were first introduced in man in patients with leukæmia (344) and in the victims of the radiation accident in Vinca, Yugoslavia (348). Although it has been clearly indicated that marrow grafts can take initially, the secondary disease problem in man is very severe, as its onset is generally very early, when the aplasia from total irradiation at the high dose of 800 rads is still uncorrected. Several groups have studied the effect of marrow infusion in patients with leukæmia. Using dose rates of up to 2 rad min^{-1}, total exposures of 1,200 to 2,000 roentgens do not appear to induce early gastro-intestinal complications. However, even in this exposure range it was found (556) that, although initial takes of allogeneic marrow (usually from related donors) occurred, survival of the patient was only of 2-4 weeks duration. Death was either from infection or, occasionally, from recurrent leukæmia. It appears that extremely high doses of radiation would be needed to completely eradicate the leukæmic cells.

190. With the increasing frequency of marrow transplantation in man, the syndrome of graft-*versus*-host disease as seen in man is becoming well defined. In a study by Meuwissen *et al.* (361), the following clinical findings were noted in 7 of 13 patients treated by marrow transplantation. The classical syndrome of fever, rash, liver disease and diarrhea was present in most instances although exceptions were noted. Skin showed destruction of the basal cell layer, a predominantly mononuclear cell infiltration, acanthosis, and dysparahyperkeratosis. A most striking finding was the frequency of localized or generalized ulceration of gastro-intestinal mucosa. Bone-marrow abnormalities occurred less regularly, and included hypoplasia and infiltration with plasma cells and histiocytes. Although elevation of liver enzymes occurred regularly during the graft-*versus*-host reaction, serious liver lesions were rare at *post-mortem*. It is noted that some patients showing these changes included those in whom an HL-A-matched marrow transplant had been given.

191. Although occasional remission of leukæmia has been observed (557, 560) with whole-body irradiation in a sublethal exposure range of 325 or 880 roentgens, most studies have involved higher exposures combined with marrow transfusion. In several studies with identical twins, the leukæmic individual was given 800-1,600 roentgens and marrow from the normal twin. In each instance (557, 559), a remission was achieved but the improvement was followed by a discouraging early return of the leukæmia (554). These studies confirm that the lethal effect of high doses of radiation in man can be counteracted by marrow transfusion, but again indicate that the same doses are not sufficient to eliminate all the leukæmic cells. A series of reports on allogeneic-marrow grafting in irradiated leukæmic patients was reviewed in 1965 (554). It was stressed that large numbers of cells must be given and that addition of immunosuppressive therapy to the radiation treatment is advantageous. Over-all success, however, is rare.

192. The seven years since that review have not seen any major improvement. A summary by Mathé (347) showed that 17 out of 24 marrow grafts had taken in the recipients. However, of the 17, 10 died with acute secondary disease, 3 with a later subacute or chronic secondary disease, and 4 with recurrent leukæmia. Several workers, including Mathé (345), have considered the possibility that presensitization of the recipient by repeated blood transfusions might occur and some evidence for this is suggested. It has been indicated (345) that administration of Imuran during the period of the transfusions reduces or annuls this immunization.

193. To stress the point again, it is the eradication of secondary disease which appears to be the major

problem for human-bone-marrow transplantation. Thus, although low dose rates are usually used, it appears that a sufficient depression of host immunity has been achieved. It is possible, however, that the low dose rate may partially account for the incomplete eradication of the host leukæmic cells, particularly as recurrences occur even after very high total radiation doses. Approaches to prevention of secondary disease consist mainly of (*a*) pre- or post-treatment of the host and (*b*) direct efforts to reduce or remove immunocompetent cells from the donor inoculum.

194. Treatment of recipients includes the addition of other immunosuppressive agents in the few days after marrow grafting, in an attempt to suppress the proliferation of the injected immunocompetent cells. Some success in this regard has been achieved with cyclophosphamide, amethopterin or antilymphocyte serum (396, 589), although it now appears that cyclophosphamide only postpones the onset of secondary disease and does not suppress it sufficiently to allow long-term survival.

195. Suppression of the induction of secondary disease by manipulation of the donor cells can be approached in various ways. The essential aim is to transplant an inoculum which contains sufficient numbers of hæmopoietic stem cells without containing any immunocompetent cells with specificity against the host. The ideal source of cells is a bone-marrow donor of identical histocompatibility type, at least for "major" antigens, and considerable effort is currently being invested for the complete histocompatibility typing of man. However, this can only be a complete solution provided there is ready availability of all types of donor marrows, which involves problems of procurement and storage.

196. The other approaches are all concerned with using allogeneic bone marrow lacking immunocompetent cells. Again, a possibility for the use of an unmanipulated cell suspension exists. In the adult, hæmopoietic stem cells are found predominantly in the bone marrow. However, in fœtal life the liver is the major site of hæmopoiesis and, if taken at an early stage, liver does not contain any immunocompetent cells, as the thymus induction of immune differentiation has only barely commenced. Studies in mice (582, 583, 586) clearly show that fœtal-liver-cell suspensions will not induce secondary disease in primary irradiated hosts. However, they become differentiated to immunocompetent cells provided the host has an intact thymus, and at the same time become tolerant to the host's histocompatibility antigens. The use of fœtal-liver-cell suspensions for the treatment of irradiated *Rhesus* monkeys has been studied by Van Putten *et al.* (594) who found that more than one complete fœtal liver was required per recipient. With the use of pooled fœtal-liver-cell suspensions, 4 10^8 to 11 10^8 cells per kilogramme gave repopulation in one fifth of the monkeys after 800 roentgens and in three fifths after 900 roentgens. It was concluded that fœtal liver is relatively less effective than bone marrow, possibly because the initial immune attack of the bone-marrow competent cells on the recipient aids in depressing the host's immunity. However, studies in mice (388) clearly show that fœtal liver is extremely rich in hæmopoietic stem cells provided it is taken at an appropriate fœtal age, and it is possible that the optimal age of monkey fœtal liver was not used. Perhaps the main drawback of fœtal liver cells as potential donor cells for use in clinical medicine is the problem of availability. If large numbers of cells are needed, this approach could only be realistically achieved through the use of pooled donors with a successful storage method. It was concluded (594) that the pooled frozen liver cells of roughly 50 human fœtuses of 20-26 weeks of age would be required for one adult. As such a large amount of material cannot be transfused safely, the use of fœtal liver cells was thought to be clinically unrealistic. On the other hand, it is possible that a more judicious choice of fœtal age for the liver source, and possibly fractionation of the cell populations giving more effective cells might permit the use of this approach.

197. If allogeneic bone marrow is to be the source of donor cells, the next general approach is either to purify the stem cells, or to selectively kill or remove the immunocompetent cells. Various physical cell-separation methods (for example, gradient centrifugation) with mouse bone marrow have shown (138, 388) that fractions with enriched hæmopoietic stem-cell activity can be obtained, although as yet not in pure form. In one study (139) it was also shown that certain fractions could be obtained which contained up to 50 per cent of the original stem-cell activity, but produced no secondary disease even with large numbers of cells. This approach is therefore promising and should be attempted with primate cells, provided suitable *in vitro* assays for measuring separately the activity of stem cells and immunocompetence can be developed.

198. By the use of the *in vitro*-colony-forming method (61) as a measure of stem-cell activity, recent data with monkey bone marrow strongly suggest that this approach may be very successful (391). Separation of *Rhesus* monkey bone marrow by buoyant density gradient has demonstrated a reproducible and homogeneous light density distribution profile of cells capable of forming hæmopoietic colonies in agar culture. An average hundred-fold enrichment of these cells was obtained, with the most enriched fractions containing the majority of these cells in the original marrow inoculum. Although assays for immunocompetent cells have not been performed on these inocula, it is most probable that the content of these latter cells would be considerably reduced, particularly in those fractions where up to 33 per cent of the cells are *in vitro*-colony-forming cells.

199. Recent studies (343) of graft-*versus*-host reactions in mice have indicated that the receptor site on the mouse immunocompetent cell that is responsible for recognition of the foreign histocompatibility antigens is an immunoglobulin molecule, possibly either a free L chain or a new type of immunoglobulin. Pretreatment of adult mouse spleen cells with rabbit antisera against mouse-immunoglobulin light chains completely prevented the cell suspension from inducing a graft-*versus*-host reaction. Viability of the cell suspension after this treatment was indicated by dye-exclusion tests with trypan blue. However, of even greater relevance was the observation that hæmopoietic stem-cell activity, as measured by the mouse spleen-colony assay, was unaffected by the anti-light chain serum treatment (605). This general approach should be extensively applicable to clinical work if it proves reliable in experimental studies. The treatment involves a short (approximately

1-2 hours) incubation of the cells with an appropriate concentration of the antiserum, followed by cell washing and then transfusion.

200. Specific removal of immunocompetent cells reactive to host-histocompatibility antigens could be achieved by treating them with the specific antigens and in some manner then effecting their removal. This might be approached by determining whether the specific immunocompetent cells formed rosettes or aggregates on mixing with allogeneic cells. If this occurred, the aggregates could be removed by some method involving particle size. Another potential method might be based on the studies of Ada and Byrt (3) which showed that the potential for the formation of specific antibody to a bacterial antigen could be removed by pre-incubating a normal cell suspension with a very heavily ^{125}I-labelled preparation of the antigen, thus inducing radiation killing in those cells which specifically bound the antigen. The potential to produce antibodies to other non-cross-reacting antigens was not destroyed, indicating that the radiation effect was only induced at very close range, presumably through the labelled antigen held on the cell surface (337). For this approach to be applicable to man, it would be necessary to purify specific histocompatibility antigen which can be successfully labelled with ^{125}I, and to apply a concomitant histocompatibility-matching scheme, so that the appropriate cells are removed prior to their transfusion into the recipient. This latter necessity, however, could be avoided, at least theoretically, if a radio-labelled antigen preparation were to be prepared from each recipient and then applied to the donor cells before transfusion. This method offers the potential advantage of involving only a relatively short pretreatment of the donor cells before transfusion.

201. Other measures that have been attempted to eliminate the graft-*versus*-host reaction include (*a*) an *in vitro* exposure of donor cells to antigens of the prospective recipients before use in transfusion (107), (*b*) sublethal irradiation of donors before collection of their bone marrow (117), a procedure which, however, would also considerably reduce the hæmatopoietic stem-cell activity of the marrow, and (*c*) control of the radiation dose rate (199, 562).

202. At the present time, none of the above methods has yet been shown to work completely satisfactorily, which probably indicates that several variables are involved. This is substantiated by the studies of Congdon *et al.* (96, 97) who undertook a comprehensive "4-factorial" study in mice, assessing the effects of variation in the interval between whole-body irradiation and injection of allogeneic bone marrow, the number of bone-marrow cells, the age of bone-marrow donor and sex. On the basis of these experiments the 90-day mortality could be reduced tenfold by controlling these factors. These results indicate that, combined with other approaches mentioned above, complete elimination of the secondary-disease mortality is a very realistic possibility.

203. At present, there is very little information bearing directly on the question of possible cell collaboration in cellular immunity as related to radiation sensitivity. Several aspects of the induction of a graft-*versus*-host reaction by bone-marrow cells are at present confusing. The basic immunological question is whether the bone-marrow population contains immunocompetent *T* lymphocytes which will immediately initiate the graft-*versus*-host reaction on injection into recipients, or whether maturation of the potentially-immunocompetent cells in marrow (stem cells) is required. If the latter is true, or if a cell-collaboration step is involved, the process may take place in the host and the radiation dose rate used in man and other primates may be insufficient to prevent a rapid expression of secondary disease by the donor cells. This possibility is based on the following experiments.

204. Adult thymectomized irradiated mice given syngeneic bone marrow are immunologically unreactive. When an allogeneic thymus graft was also placed in the recipient, the mice recovered their immune capabilities and rejected the thymus graft itself (369). At no time was there any evidence of repopulation of the allogeneic graft. This indicates that the injected syngeneic bone-marrow cells already carried the potential to react against the histocompatibility antigens of the allogeneic graft but first required something from the graft, probably humoral in nature (432), to express this activity. Thymus grafts irradiated *in vitro* (2,000 R) failed to restore neonatally-thymectomized mice to full immunological capacity (367), thus suggesting the existence of a radio-sensitive stage in the synthesis, release or activity of the thymic factor. These experiments therefore suggest that bone marrow contains an immunocompetent cell capable of reacting against histocompatibility antigen, provided a thymic factor is available.

205. If this is also applicable to the injection of bone-marrow cells into allogeneic recipients, it implies that the host animal must provide a thymic factor for the injected cells to be able to induce the graft-*versus*-host reaction. As this effect of the host thymus may be radio-sensitive, it is quite possible that the relatively-late onset of secondary disease in lethally-irradiated mice is due to radiation damage to the host's thymic epithelium, and that this must first recover before the injected cells can attack. As the dose rates used in man and primates are of a low order (<5 R min^{-1}), it is quite possible that this radio-sensitive phase of the thymic effect has not been sufficiently destroyed, thus allowing for immediate maturation of injected stem cells to immunocompetence. This concept would suggest that higher dose rates might also be advantageous in delaying thymic epithelial restoration and therefore development of immune competence. It is possible that local thymic irradiation might even produce a sufficient delay to permit the injected cell population to become tolerant to host antigens.

206. Experimental verification of this concept could come from a direct demonstration that removal of the host thymus prevented the induction of secondary disease in irradiated mice given allogeneic cells. Such an experiment has been reported by three groups, but the interpretation of the results is difficult, because adult thymectomized lethally-irradiated mice given syngeneic bone marrow also develop a wasting disease as a result of lymphoid aplasia. Even if the secondary disease were prevented in thymectomized allogeneic recipients, the mice could still die of wasting through lymphoid aplasia. In one study (592) with heterologous combinations, a marked reduction in the incidence of secondary disease was observed in thymectomized mice, and in two other studies a marginal prolongation of life was observed (209, 537). A critical test of this hypothesis would require the use of germ-free irradiated thymectomized recipients, to avoid wasting disease from lymphoid atrophy.

3. *Organ grafts*

207. Human renal allograft transplantation has become a major accepted form of clinical therapy for certain kidney diseases. Inherent in any successful organ grafting is the prevention of a host immune response from rejecting the graft. This can basically be approached in two ways: (*a*) by avoiding presenting the recipient with an effective foreign antigen, and (*b*) by suppressing the host's immune response. Irradiation has been a valuable tool for immunosuppression over the past 10-15 years, but it is certainly not the ultimate ideal approach. The majority of current approaches to suppression do not involve irradiation, and accordingly the field of organ transplantation is currently of less direct relevance to the topic of radiation and immunity. The main current efforts are aimed at (*a*) developing histocompatibility typing in order to select the most closely matched graft possible and therefore to limit the degree of foreignness in the donor graft, and (*b*) achieving immunosuppression by means of drug therapy, anti-lymphocyte serum, or immunological tolerance. However, as radiation has been used very frequently in the past, certain aspects which involve different uses or types of radiation will be discussed here. In terms of the two approaches to suppression of homograft immunity mentioned above, radiation has been aimed at either (*a*) the graft itself, or (*b*) the immune system of the host. These will be considered separately.

208. An impressive body of data clearly indicates that local irradiation of the kidney soon after transplantation is of definite value in delaying acute rejection (261). An example drawn from the kidney-transplant registry is shown in figure XII. The influence of local

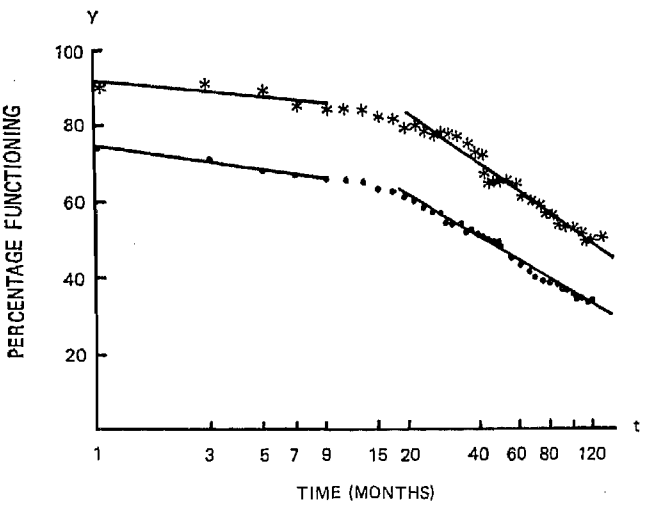

Figure XII. Effect of local kidney-graft irradiation on survival of the graft. The figures are drawn from the kidney-transplant registry (8), and plot the percentage of functioning grafts against time for both irradiated (stars) and non-irradiated (closed circles) grafts

irradiation can be seen as early as one day after grafting. Local graft irradiation is usually performed by fractionation of some substantial dose of radiation, e.g. 1,000 rads, into smaller doses of not less than 150 rads, which are given at some appropriate interval. Before considering the possible mechanism involved, it is of interest to mention a few of the direct experiments on local irradiation. Some complications of local irradiation will be discussed in the section on delayed effects (VII.C).

209. In 1953 Dempster (130) claimed that the pyrinophilic reaction in the transplanted kidney could be reduced by irradiating it prior to transplantation. Approximately 250 rads were given to the kidneys while still in the donor. However, in other early reports or experimental studies, high doses given to the donor did not influence the characteristic reaction (193, 260). In a further study in dogs, local irradiation was given as six fractions of 150 roentgens every two days (631) to the graft *in situ*. The mean kidney survival in the irradiated group was 23.4 days compared to 9.9 days in the controls. This prolongation was confirmed clinically (261) in a review of many patients, and again experimentally in a further trial in dogs (631). With experimental heart transplants in rats a similar schedule of repeated local irradiation (6 × 150 R) of the graft starting immediately after transplantation also produced a slowed interstitial infiltration of the graft by lymphocytes, and its longer survival (430).

210. The critical question in relation to the radiation effect is whether radiation is destroying the actual immunogenicity of the donor graft or is suppressing the early phase of the host response. There is some experimental evidence for both points of view. The former interpretation is based on the notion that the circulating lymphoidal cells of the graft constitute the major immunogenic stimulus. It has been shown that the mixed lymphocyte reaction can be inhibited when one of the component cell populations is exposed to 1,000 roentgens and it was suggested that this acts by destroying the capacity to stimulate the allogeneic lymphocytes (300). However, this was not consistent with another study using 2,500 roentgens in which no loss of activity was observed (326). In transplantations of allogeneic tissues together with adult leucocytes onto the chick chorioallantoic membrane, rejection only occurred if the transplant contained a relatively large component of reticular tissue. Treatments such as gamma-irradiation, which reduce the amount of reticular tissue in the graft, protected it from transplantation damage (300).

211. In another study (161) it was shown that in a direct graft-*versus*-host reaction induced by parental strain cells injected in the kidney of an F1 rat, several factors were operative. Since Gowans has reported (218) that sensitization of lymphocytes is a consequence of their perfusion through an isolated allogeneic kidney, and since rat kidney cells have been used as target tissues for *in vitro* cytotoxic immune reactions (625), it is likely that kidney parenchymal cells may offer an immunogenic stimulus to specific cells. However, by themselves, the donor parental strain cells cannot do much damage to the host kidney or even generate more than a very sparse local infiltrate. It therefore appears that a host component is necessary for the full development of interstitial infiltration and parenchymal destruction. This was deduced from experiments which showed that whole-body irradiation of the hosts 24 hours before the injection of allogeneic parental cells resulted in an inhibition of the subsequent reaction, to a degree commensurate with the radiation damage sustained by the lymphoid system of the host.

212. These results in general suggest that local irradiation of the kidney after transplantation may be beneficial and that several factors are possibly involved,

including both destruction of any donor lymphoid cells which may act as a strong immunogen, and destruction of early-infiltrating host cells, many of which may be acting in a non-specific destructive manner, perhaps in a fashion analogous to the recruitment of normal host lymphoid cells in delayed-hypersensitivity reactions (52).

213. Even apart from other more serious considerations of the disadvantages of using radiation, in immunological terms whole-body irradiation for suppression of organ-graft rejection is by no means an ideal approach. If radiation were to be the sole agent for immunosuppression, the accompanying problems of bone-marrow transplantation would also have to be solved as the dose required to create sufficient immunosuppression would lead to marrow aplasia. Accordingly, only sublethal exposures are practical, which, although aiding in immunodepression, may still be expected to provide significant radiation damage. Before the advent of the more recent methods of immunodepression, whole-body irradiation at doses of the order of 400 rads was used with some possible success (232, 357). Additional localized irradiation of the spleen and the right lower abdomen has also been given to depress immunity and to obliterate the lymphatic field draining the transplant (633).

214. Under certain experimental circumstances, whole-body irradiation has *facilitated* the destruction of renal grafts. Studies in inbred rats (174) with renal grafts placed into immunologically-tolerant hosts afforded a means of examining the rejection process under controlled conditions. Graft rejection could be induced by the injection of large numbers of competent syngeneic lymphoid cells. If whole-body irradiation (550 R) was also given, graft rejection was greatly facilitated, in that fewer injected cells were needed to induce rejection, and graft destruction was hastened. Total-body irradiation *per se* was occasionally followed by the destruction of skin homografts. This effect may have occurred through a variety of mechanisms, such as (*a*) depletion of lymphoid cells in host organs allowing better seeding of the injected cells; (*b*) enhanced cell growth and preferential mitosis in the presence of antigen; (*c*) alterations in the target cells rendering them more susceptible to rejection, or (*d*) reduction or suppression of the state of tolerance in the host. This latter mechanism will be discussed in more detail in a later section.

215. Various other means of achieving radiation-induced immunological depression have been reported (29). Although it is through the discovery of many immunosuppressive drugs that the results of organ transplantation have greatly improved, in certain instances it may not be advisable to use these agents, and resort to radiation-induced depression may still be required. For example, it has been reported that a severe toxic reaction to Imuran and prednisolone may occur in Japanese people, and alternative immunosuppression by intralymphatic administration of radio-active isotopes (^{198}Au) to destroy lymphoid tissues was attempted (505). This method resulted in a reduction of peripheral lymphocytes and a decrease in serum gamma globulin. A useful reduction in dosage of Imuran and prednisolone thus became possible, safeguarding against the development of post-operative complications in these patients. Several other studies with intralymphatic radio-active materials have been reported. Wheeler *et al.* (620) also used intralymphatic colloidal gold (^{198}Au) combined with splenectomy and direct injection of the isotope into the mesenteric lymph node of dogs. A marked selective lymphopenia was observed in the dogs for three to five weeks. The rejection of homologous renal transplants was delayed. Severe lymph-node destruction was produced and it was felt that, combined with Imuran, additive immunosuppression occurred.

216. Intralymphatic injection of ^{131}I-ethiodal into dogs was followed by a marked lymphopenia for four weeks, with progressive reduction in lymph-node size (567). Repeated doses were found to have a cumulative effect. Radio-active chromic phosphate (^{32}P) given by direct intralymphatic injection into dogs has also been used (81) and produced a severe destruction of a majority of lymph nodes with subsequent lymphopenia. However, although antibody production against human serum albumin was significantly inhibited in this series, the reactivity to the allografted heart was not altered. It was noted that intralymphatic injection of radio-active material leads to selective destruction or change only in lymphoid tissues. All other organ systems appeared quite normal following intralymphatic injection, in contrast with intravenous injection of ^{32}P which affects all systems. The intravascular implantation of a high-energy beta-emitting source (^{90}Y) into dogs was also shown (630) to produce a profound lymphocytopenia. Within 12 hours, levels fell to 0 to 10 per cent of pre-implantation values and remained low for three weeks. Ten animals given renal homografts (with the implant as the only source of immunosuppression) showed a mean functional survival of 16.9 days (controls 5.3). Biopsies showed minimal cellular infiltration.

217. These approaches suffer from the disadvantage that it is very difficult to control the radiation dose. Their use is still in the experimental stage and they are not at present recommended for human clinical use.

218. Another approach to the reduction of the circulating immuno-competent lymphocyte pool is through extracorporeal blood irradiation (ECIB). This approach was first developed by Heymans in 1921 (252) and subsequently refined extensively by Cronkite *et al.* (111) who described a method of producing a profound lymphopenia in calves by shunting the blood around a ^{137}Cs or ^{60}Co source. In dogs, a short course of ECIB produces a significant lymphocytopenia, and repeated daily doses give a prolonged lymphocyte suppression (324). Two patients also were so treated and in one case some reversal of the early acute rejection process was reported. However, no effect on the later rejection process was observed. In a study (476) of 11 patients waiting for renal transplantation, to whom approximately 9-37 kilorads were given by ECIB, lymphocytopenia was observed in only three. An impairment of renal-homograft rejection in dogs given continuous ECIB has also been observed by Wolf *et al.* (632). Several other reports (262, 356) have indicated that ECIB has been successfully applied in the treatment of rejection crises. In a recent detailed study (448) of 18 patients on ECIB before renal transplantation, followed by drug-mediated immunosuppression, a significantly smaller number of rejection crises occurred in the irradiated patients as compared to 60 controls given only the same drug treatments, and in general survival rate was higher.

219. Despite the encouraging results of currently-used schedules of ECIB in human renal transplanta-

tion, some recent data suggest that there may be a need to further evaluate different schedules (78). In this study, two alternative schemes of ECIB were used in goats and, following one of these, the mean goat renal allograft survival was doubled as compared to control goats. This success was achieved without the help of immunosuppressive therapy or the benefit of donor-host matching. The preliminary results with two goats indicate that a combination of pre- and post-transplant ECIB might be better than pre-transplant ECIB. This study also showed that, as with other techniques of immunosuppression, the degree of blood lymphocytopenia following ECIB does not correlate with the transplant survival.

220. The general lack of close correlation between lymphopenia and improved allograft survival following ECIB, raises the possibility that more subtle inactivation changes may have occurred in the remaining lymphocytes, or that there may be an alteration in the proportion of *T* and *B* lymphocyte types. This latter possibility was investigated (614) by means of lymphocyte-transformation tests with blood samples taken before and after ECIB. The response to phytohæmagglutinin was unchanged, while the response to purified tuberculin and allogeneic cells was reduced per unit number of lymphocytes after ECIB. These results were interpreted as indicating that the fraction of thymic derived cells left in the peripheral blood after ECIB was unchanged, but that the immunological functions of these cells was impaired. Similar results were observed after irradiation of the blood *in vitro* with single doses of from 100 to 500 rads.

221. In all these reports, it is fairly clear that ECIB, like intralymphatic irradiation, implant irradiation or even local lymphoid-organ irradiation, will reduce the level of the recirculating lymphocyte pool and reduce the incidence and severity of the early rejection crises. As a complete and permanent immunosuppressive régime it is clearly not enough, but may still be useful as an adjunct to immunosuppression by other means, perhaps particularly during rejection crisis. Again it should be stressed, however, that in view of the damaging effects of radiation, a goal of transplantation research should be to avoid and replace its use wherever possible.

V. Radiation and immunological tolerance

A. Two antigen dosage zones for tolerance induction

222. The concept of immunological tolerance as first proposed by Burnet and Fenner (69) was based on the discovery by Owen (436) that erythrocyte mosaicism existed in dizygotic twin cattle and persisted for a long period of time. This mosaicism results from an interchange of primordial hæmopoietic cells through vascular anastomoses between the co-twins. It was the persisting nature of this chimeric state that led Burnet and Fenner to the hypothesis that the immunological system of the organism becomes non-reactive to antigens with which it comes into contact in embryonic life and that the normal function of this mechanism is to ensure the non-antigenicity of self components. Further evidence of the anomalous situation in dizygotic twin cattle was found in the acceptance of skin homografts between the partners (17, 48) and the phenomenon was termed immunological tolerance. Experimental demonstration of the production of tolerance was then made by injection of embryos with homologous cells (47).

223. Since these earliest demonstrations of tolerance, a vast literature on the subject has developed and has been the subject of various reviews (152, 239, 307, 518). As much of this is not relevant to this present topic, we shall be concerned in this section only with the recent development of the concept of two zones of antigen dosage in which tolerance can be induced, as one of these zones may be involved in maintaining the normal homeostatic mechanism and thus preventing anti-self reactivity. Theoretically, a disturbance in this system, such as might be induced by radiation, could lead to breakdown of tolerance and the production of auto-immunity. This will be considered in section V (D).

224. In normal adult mice, the repeated administration of high doses of antigen paralyses the immune system and leads to a progressive decline in reactivity. Lower doses of antigen (for example, 0.1 to 1.0 mg of bovine serum albumin) lead to immunization and to stabilization of the serum antibody at a high level. The studies of Mitchison (381, 382), Dresser (151), Shellam and Nossal (504) and Ada and Parish (5) have now revealed that another antigen-dose zone for the induction of tolerance exists with amounts of antigen below the immunizing dose. The actual dose range involved appears to differ for different antigens used. With bovine serum albumin, repeated doses of 1-10 micrograms will induce a partial tolerance but, with *Salmonella* flagellin, the picogramme range is more effective.

225. Although the detailed mechanism of tolerance, particularly of low-zone tolerance, is not completely understood, the existence of such a phenomenon may be of some fundamental importance. The results suggest that tolerance might be the most likely result of an interaction between an antigen-sensitive cell and a molecule of antigen, although it must be noted that low-zone tolerance has not been demonstrated to occur after challenge with low doses of living infectious agents. Immunity appears to require the presence of more antigen, either because it has a higher threshold or because it requires antigen-processing by macrophages or localization of antigen on the dendritic processes of reticular cells. Some recent studies (244-247, 456) have suggested that true allogeneic tolerance of *T* lymphoid cells may not exist, and that the phenomenon may be explained by the presence of a blocking factor which prevents the cell-mediated attack by *T* lymphocytes. On the other hand, recent data by Rouse and Warner (478) demonstrates the induction of allogeneic tolerance in agammaglobulinæmic animals, which indicates that the formation of blocking antibodies cannot be the sole explanation for allogeneic tolerance.

B. Induction of tolerance

226. It appears likely that tolerance induction and immunity induction are alternative effects of the antigen on particular lymphoid cells. One injection of antigen may drive some of the cells in one direction and other cells in the opposite direction (381, 419). The great usefulness of x-irradiation in regard to tolerance induction relates to the use of adult animals, when moderate to high immunogenic materials are used. In the absence of x-irradiation, the antibody formation which results from antigen-reactive cells being driven

towards immunity masks or blankets any simultaneous tolerance induction in other individual cells. When sublethal irradiation precedes antigen injection, many of the antigen-reactive cells are killed in proportion to the dose of radiation. Recovery of the immune system then occurs mainly by recruitment of stem cells from the bone marrow which, under thymic influence, are induced to become antigen-reactive cells. This recovery phase in essence resembles the immunological maturation around the time of birth, and many studies have clearly indicated a greater ease of tolerance induction in new-born than in adult animals (152, 518), even with the low-zone tolerance model (6, 418). As the recruitment and differentiation of new antigen-reactive cells after irradiation is a progressive occurrence, paralyzing antigen concentrations must be maintained for some time in these tissues. Several studies have been performed on the detailed kinetics and exact requirements for tolerance induction after x-irradiation and these will be briefly reviewed.

227. In normal adult rabbits, repeated infusions of large amounts of heterologous plasma proteins can induce a state of specific immunological unresponsiveness. This normally lasts for about 3-4 months. However, if the rabbits had been given 400 roentgens two days before the start of the antigen infusions, the tolerant state persisted for at least 10-11 months (143). These studies were then extended by Nachtigal and Feldmann (399) who assessed the influence of two variables on tolerance induction, namely, (*a*) dose and timing of irradiation, and (*b*) dose of antigen. Evidence was presented that the degree of unresponsiveness was a function of the time interval between x-irradiation and the beginning of antigen administration. If antigen was given 24 hours or 16 days after irradiation, complete tolerance was produced, whereas 42 days later administration of the antigen led to only partial tolerance. In this system, doses of antigen that would be immunogenic in normal animals were found to bring about tolerance in the irradiated rabbits. In another study (471) adult rabbits were given either 10 or 100 milligrammes of bovine serum albumin 24 hours after irradiation. Antibody response to the lower dose was suppressed but not that to the higher dose. It was further shown that the 10 milligramme dose had in fact established a state of specific immunological unresponsiveness.

228. Kinetic studies (400) in rabbits given 550 roentgens and human serum albumin revealed that tolerance can be induced with small amounts of antigen which in non-irradiated animals would constitute small immunizing doses. This only occurs when the antigen is injected over a prolonged period. Thus 20 milligrammes given in a single injection applied shortly after x-ray treatment did not induce tolerance. This result is contrary to the overloading concept of tolerance induction, since cellular depletion is most severe immediately after irradiation and the overloading of cells with antigen would be most pronounced at that time. Tolerance was most effectively induced in the x-irradiated rabbits when administered in small doses spread over the post-irradiation period. Tolerance induction could occur even when the antigen treatments were started four weeks after irradiation. Moreover, it appeared that smaller amounts of antigen are required for tolerance induction in this period, which suggests that susceptibility to tolerance does not develop immediately following inactivation of immunocompetence by x rays and that it may perhaps be a transient phenomenon appearing closer to the immune recovery phase. In other words, this would indicate that tolerance induction is acting on a cell at a certain stage of differentiation which is present particularly in new-born animals and during the recovery phase after irradiation.

229. The effects of small amounts of proteins given over the course of ten weeks immediately following whole-body irradiation (600 R) has been examined in mice (383). Four different proteins acted in much the same way, all but one showing a similar threshold dose of antigen for tolerance induction. Doses of antigen given three times a week are more effective in paralyzing than doses more widely spaced or than a single injection. The only exception to this statement is that in rabbits a single injection of bovine serum albumin was shown to paralyze after irradiation (312), but this may be due to the relatively slow elimination of bovine serum albumin from the circulation of the rabbit.

230. The concept of radiation-enhanced immunological tolerance might be applicable to problems of graft rejection. A soluble histocompatibility antigen prepared either directly from the potential kidney donor or from another source of histocompatibility-matched (to the donor) material, might be used to induce tolerance in the recipient, at least for the initial period when graft rejection is most likely to occur. The graft itself might then act as a continuous source of transplantation antigen to permit the maintenance of the tolerant state. The critical problem then is to be assured of inducing tolerance rather than immunity in the recipient. Theoretically there are two approaches. As it is rather unlikely that enough material will be available to induce high-zone tolerance in adult, either low-zone tolerance or radiation-induced tolerance would be required. As the former is rarely an absolute and total tolerance, and to err on the side of too much antigen might easily provoke an immune response, the use of sublethal irradiation of the recipient two to three weeks before the transplant, combined with repeated injections of the soluble antigenic material, would be more likely to result in specific tolerance. Indeed, studies in mice (285) have clearly shown that non-lethal exposures (e.g., 150 R) can be used as an excellent facilitating agent in inducing skin-graft tolerance to weak histocompatibility antigens. Further doses of radiation would not be advisable, in part because radiation-induced breakage of tolerance might then occur (see next section).

C. Breakdown of tolerance by radiation

231. The state of immunological tolerance persists for only a certain finite period unless continuing, albeit low, levels of antigen are maintained. There are several factors involved in the loss of the tolerant state, the two major ones perhaps being the decrease in antigen concentration and the emergence of new immunocompetent cells *via* the differentiation pathway. Regardless of whether or not there is such an entity as a reversibly tolerant cell, new immunocompetent cells are constantly arising throughout life. Thus if tolerance is to be maintained, there must be sufficient antigen still present to make tolerant each new immunocompetent cell as it arises. Thus, measures that reduce the rate of appearance of new competent cells, such as thymectomy, prolong the state of tolerance (86, 87). On the other hand, if antigen were to be more rapidly eliminated

or an excessive production of immunocompetent cells were stimulated, then breakdown of tolerance would occur much faster.

232. If the continued presence of antigen is indeed required for the maintenance of the tolerant state, it was predicted by Denhardt and Owen (132) that x-irradiation of tolerant animals would result in a loss of the tolerant state. This would be expected either from possible radiation destruction of the cells storing antigen, or by the excessive proliferation of stem cells which occurs following irradiation. In the first experimental test of this idea (132) rabbits made tolerant to bovine serum albumin were given 300 roentgens and immunized with bovine serum albumin 16 days later. No evidence of a break in tolerance could be detected. In a similar experiment but using 450 or 1,000 roentgens, Weigle (616) also could not find any break in tolerance. However, this particular tolerance model represents one of the most stable tolerance situations known and may therefore be the most resistant to change.

233. In studies with rats, Nossal and Larkin (422) induced tolerance to mouse red blood cells by starting injections at birth, and then gave lethal irradiation when the animals were adult. The rats were then given bone marrow from a tolerant donor, and on immunization with mouse red cells were shown to be capable of antibody production. This was then extended (327) to a simpler system in which the tolerant rats were given sublethal irradiation. Tolerance breakdown was again observed with the formation of substantial amounts of antibody. Similar data were also obtained by Stone and Owen (530) using rats tolerant to sheep erythrocytes. These results also showed that the loss of tolerance could not be demonstrated unless the antigenic challenge was given at least 6-18 weeks after irradiation. The results of both groups indicate that the cells emerging by proliferation and differentiation after irradiation are less likely to be made tolerant by antigen and perhaps are more prone to stimulation towards antibody formation, thus aiding further in tolerance breakdown by immune elimination of any residual antigen. Breakdown of transplantation tolerance has also been demonstrated; partial tolerance across H-2 barriers was induced in mice at birth, and tolerance was completely abrogated by exposure to 350-450 roentgens (173).

234. Attempts to break tolerance induced with antigen doses in the low-zone range have recently been described (503). Tolerance to flagellin was induced in rats by repeated daily doses of 10 microgrammes for several weeks. These animals were then given normal thoracic-duct lymphocytes with or without added irradiation of the recipients prior to cell injection. Challenge with antigen was also made at the time of thoracic-duct cell injection. Irradiation alone did not produce any loss of tolerance in the three weeks following injection. In view of the preceding reports, this may well have been too short a time to allow for recovery. However, the injection of normal thoracic-duct cells combined with host irradiation led to a breakdown in tolerance, even when only the recipient's spleen was irradiated. In this instance, irradiation may have aided by creating some lymphoid atrophy in the lymphoid organs of the host, thereby permitting a more successful colonization of the injected normal mice by the transfused lymphoid cells. In general, it appears that, regardless of the exact detailed mechanism of tolerance breakdown, the effect is essentially an *acceleration* of the anticipated eventual breakdown. Thus tolerance situations that are inherently more stable and permanent may be relatively more difficult to break by radiation.

D. Implications for auto-immunity

235. It has been frequently pointed out that the immunocompetent cell population is often called on to produce antibodies or cellular immune reactions against materials which are of a nature very similar to that of the tissues of the animal itself. This includes recognition of histocompatibility antigens, allotypic forms of immunoglobulins including those derived by maternal-fœtal transmission (522, 608, 627), various tissue-specific iso-antigens (e.g. those within the thymus, TL, theta, etc.) (60) and various tumour-specific antigens (e.g. Prehn, (463)). The cell population of the body must therefore have some means of distinguishing these from self-antigens or of preventing the continual emergence and activation of potentially autoreactive cells (66). If the maintenance of the normal state of immunological homeostasis (non-reactivity to self) involves a tolerance type of mechanism which eliminates or inhibits anti-self reactions, then agents which break down induced tolerant states might behave similarly with potential anti-self reactions and play a role in the induction of auto-immune diseases. A precedent for this argument comes from studies that show that injection of related antigens can break down a state of immune tolerance. Weigle (615) showed that injection of either human serum albumin or chemically-modified bovine serum albumin into rabbits tolerant to bovine serum albumin will break the state of tolerance to a certain extent. He then (617) extended this observation in showing that auto-immune disease in rabbits could be induced by the injection of a similarly chemically-modified self protein. Hence it is reasonable to consider the possibility that, as radiation can break tolerant states, particularly weak states, it may also be capable of breaking self-tolerance, that is, of inducing an auto-immune disease.

236. Recent studies with an *in vitro* system of mouse spleen cells and a fragment of a bacterial flagellin, have shown that specific tolerance can be induced purely *in vitro* with either a high-zone (140) or a low-zone dose of antigen, provided that an optimal concentration of antibody is present in the latter case (176). It is the critical ratio of antigen to antibody that determines the capacity to induce tolerance in the antigen low-zone dose range. If this mechanism is also applicable *in vivo*, a source for this critical amount of antibody must be envisaged. Such a source could either be found in the so-called natural antibody, or be induced by the initial dose of antigen. Ada *et al.* (6) have in fact reported a concomitant antibody production to occur during induction of low-zone tolerance *in vitro*. It was therefore considered by Feldmann and Diener (176) that such a mechanism of low-zone tolerance may be operative in the maintenance of self-tolerance. Possibly the small amount of antibody synthesized by the antigen-reactive cell, and normally exposed on the cell surface, may serve this purpose. Regardless of the actual source of this antibody, it might be proposed that radiation-induced proliferation of the stem-cell system and differentiation towards potential antibody production might alter the balance between the normal homeostatic levels of self-antigens

and of their respective antibody. Such an event might then swing the system in either direction. Excess antibody would possibly not provoke any break in control of self-reactivity as it would perhaps continue to mediate feed-back inhibition at the central level (175). However, the alternate direction of increased antigen levels, perhaps as a result of radiation-induced release of antigen, might trigger an auto-immune process. Further experimental studies on the relevance of the different current mechanisms of tolerance to the normal homeostatic control are clearly warranted.

237. In the light of these general considerations on radiation and auto-immunity, it is of considerable importance to examine any available human data that may relate to this problem. The most suitable material would derive from an examination of the immunological consequences of exposure to the atomic bombs of Hiroshima and Nagasaki. Interest in this area at the Atomic Bomb Casualty Commission is of relatively recent origin, and much of the attendant data is as yet incomplete. However, various observations have been made and should be considered. Two studies have been carried out in an examination for effects on auto-antibodies. In connexion with a study for the presence of thyroid disease, the Hyland thyroglobulin autoprecipitation test and the Wellcome thyroglobulin hæmagglutination test were applied to approximately 1,100 sera. No relation between agglutination titres and radiation experience was observed. In a study of rheumatoid arthritis, examination of sera by the latex agglutination test for rheumatoid factor was made. Again, no relation between the findings of this test and exposure to radiation was apparent (281). A further index of auto-immunity that has been studied concerns the spleen weight. The ratio by weight of the spleen to the entire organism has been used to document experimentally-induced auto-immune disease, although other causes may lead to the same observation. One study of this parameter, made prior to the availability of the T65D dose estimates, showed no radiation-related effects with respect to spleen index (18).

238. On the whole, the available data on incidence of auto-immune findings in individuals exposed to radiation is sparse, but does not at present indicate any significant connexion. It should be strongly noted, however, that, in animal species, the maximum radio-sensitivity is in the early young adult period, and accordingly the incidence of auto-immune changes among highly-irradiated persons who were exposed at relatively young ages will be of particular interest. The available studies on the effect of spleen shielding (described elsewhere) certainly indicate that the maximum effect may be in persons exposed in the second and third decades of life. Thus, a future relationship with radiation of, for example, spleen index and collagen disease, may well become apparent, but probably only in a select age group. Detailed studies on cellular criteria of auto-immune immunological activity should also be sought for, as these may more directly relate to the actual disease process.

239. In considering the possible relationship between auto-immunity and radiation, it is also relevant to consider this association in terms of the various concepts relating to radiation and ageing. Much of the attention placed on studies of ageing has related to the use of parameters of ageing in non-dividing cell populations and static tissues on the *a priori* assumption that these are most intimately concerned with ageing. On the other hand, it is possible that a more indirect biological principle may be operative, which involves proliferating cells. Such a theory has been expounded by Walford (602) in propounding an immunologic theory of ageing. This theory basically considers that ageing is due to somatic-cell variation, particularly of those factors which determine self-recognition patterns among cells. In higher animals the cells of the reticulo-endothelial system are especially involved. Ageing in these species is brought about by the unleashing of self-destroying processes of the nature of auto-immunity or transplantation disease. The initial cause of the somatic-cell variation, whatever it may be, is extrinsic to this pathogenetic mechanism, although cell variation may be further stimulated by auto-catalytic immune processes. If irradiation increases the rate of somatic-cell variation, and therefore the potential development of an auto-immune state, and if at the same time is immunosuppressive, it will tend to inhibit the auto-immune tendencies of the somatically-variant cells. Thus irradiation may have two opposing effects on the onset of auto-immune disease, one accelerating and one retarding. The actual result might therefore depend on the balance of these two factors and in turn depend upon the type of radiation, total dosage, dose rates, age of animals at time of irradiation, species, nutrition, and many other factors. In particular, if age is a factor, it may well relate to the greater radio-sensitivity of the young animal. If ageing is an auto-immune process, then in adults the process may well be sufficiently underway to be autocatalytic, and irradiation at this time would not lead to any greater observable rate of change. This conclusion (from Walford, (602)) is indeed similar to that reached by Anderson (18) in considering the preliminary data available on the immunological effects of radiation on atomic bomb survivors.

240. Another connexion of auto-immunity with irradiation lies in the possibility that radiation-induced somatic mutations in lymphoid cells might enable these to directly react with self-components (14). Spleens from inbred mice were taken seven days after lethal whole-body irradiation. Cell suspensions were injected intracutaneously into the skin of normal syngeneic mice. A marked reaction was observed which did not occur with either allogeneic or syngeneic cells taken only one day after irradiation. It was speculated that this represented acquisition of self-reactivity induced by the radiation. However, as mouse skin is a rather sensitive site for these types of local reaction mediated by various pharmacological agents, considerably more studies with precise controls are needed for a confirmation of this observation.

241. In addition to these previously mentioned speculative aspects of radiation and auto-immunity, it has also been recognized that radiation-induced tissue damage might lead to the release of normal self antigens, which then induce the formation of auto-antibodies (155, 659). These might then play a role in the general pathology of radiation damage, although this has not been conclusively confirmed.

242. Irradiation has also been shown (639, 645, 649, 654, 673) to produce changes in the antigenic structure of tissues. This is also often followed by the appearance of auto-antibodies (646, 654, 659). An important role has been ascribed to these auto-antibodies in the development of radiation sickness (658). In the opinion of one author (645) the complement-fixing auto-antibodies against denatured protein, formed

under the action of external irradiation, are capable of neutralizing the toxic products of tissue disintegration and are a vital factor in protecting the organism against the effects of irradiation. Similarly, with internal irradiation by daily intake of a mixture of rare-earth and alkaline-earth radio-isotopes, rats were shown (697) to develop auto-antibodies for tissues of the kidney and liver. Disturbance in enzymic function of the liver preceded the detection of auto-antibodies, which in turn preceded the development of morphological changes in liver and kidneys.

243. Two hypotheses have been formulated concerning the role of auto-immune processes in the pathogenesis of acute radiation damage. The first is the auto-allergy hypothesis (657, 661), which assumes that the development of an anti-tissue immunological reaction caused by the action of cell-destruction products on the immunological apparatus leads to the appearance of anti-tissue cytotoxic antibodies and autohæmolysin-forming cells in the blood. This in turn leads to the development of general and local increases in sensitivity to autologous, allogeneic, and xenogeneic tissue products. The second hypothesis is the immunogenetic concept of the consequences of radiation damage (674, 675) which assumes the following sequence of events: mutagenic effect of radiation→relative increase in the anomalous cells which have an immunological competence against normal tissue antigens→accumulation of clones of these "forbidden cells" with the development of tolerance to them→auto-immune aggression of the forbidden clones against the normal tissues as in the graft-*versus*-host reaction.

244. These preceding paragraphs have considered the general question of radiation as it may relate to auto-immunity, and possibly in turn to ageing. In general, there is very little information available either in animal models, or from human studies. As was discussed in relation to the acute radio-sensitivity of the immune response at the young adult period, it may still be some time before the effects on the immune system that might be expected from atomic bomb exposure will become evident, and further studies on these patients are continuing. There are however several results, particularly from animal studies, that are consistent with the present hypothesis, that irradiation may lead to a breakdown in the balance of self-tolerance, which in turn may lead to auto-immune disease.

VI. Immunological aspects of radiation-induced carcinogenesis

245. It is a well-established fact that irradiation can lead to an increased incidence of cancer. A general review of cancer induction in animals is provided in annex G. Radiation neoplasia in man has been known for an even longer period of time and there is a vast literature covering the field. The reader is referred to annex H for a detailed discussion of human data.

A. Immunological surveillance and enhancement

246. In this report on radiation and immunity, the connexion with cancer stems from the interactions of the immune response with malignant cells, and therefore we will be concerned solely with those aspects of cancer and radiation which may involve immunological mechanisms or interactions. This will be confined to a detailed examination of a few of the mouse tumours in which the ætiology of the malignancy may involve immune processes activated or suppressed by radiation.

247. The general concept of *immunological surveillance* is based on the observation that tumours can present to the host a foreign antigen which is capable of stimulating an immune response directed against the tumour. It was first proposed by Thomas (563), and then considerably expanded by Burnet (68), that one of the main functions of the body's cellular-immunity system is in fact to control and eliminate potential malignancies. This thesis is essentially based on the factual observations that some tumours are antigenic. It should be noted, however, that although it is well established that the immune response can affect the growth of an established tumour, there is little direct evidence (except for virally-induced tumours in mice) to indicate that immunosuppression will increase the incidence of primary tumours, despite several recent investigations of this possibility. Furthermore, although an elevated incidence of certain malignancies has been observed in immunosuppressed kidney transplant patients and in immunodeficiency disease patients, this has not been found in a large series of immunosuppressed auto-immune disease patients.

248. Several reviews (246, 290, 429, 464) have dealt in depth with this area and the types of tumour-specific antigens might be summarized as follows:

(a) *Antigens associated with viruses*: these are well described in mice and represent a virus-directed product which is ultimately found either within the cell or on the cell membrane. All tumours induced by a given virus carry the same virus-associated tumour-specific antigen, for example, the G+ antigen of the Gross murine-leukæmia virus (293, 529). In man, the Epstein-Barr viral antigens carried on and in Burkitt-lymphoma cells and in nasopharyngeal carcinoma cells appear to be the most likely parallel known at present to the mouse-leukæmia viruses (134);

(b) *Tumour-specific antigens induced by chemical carcinogenesis*: in this instance, a series of tumours induced by the same chemical carcinogen may all have tumour-specific antigens, but with the exception of occasional cross reactions, these are mostly different antigens from one tumour to another. It should be noted that carcinogen-induced tumours may have virus-associated tumour antigens, which may be the consequence of later super-infection of the tumour by latent leukæmia viruses, although this relationship is still uncertain;

(c) *Embryonic antigens*: these are not strictly speaking tumour-specific antigens, but are antigens normally present only in embryonic life and expressed by the tumour in the adult host. The human-colon embryonic antigen carried by all tumours arising in the gastro-intestinal tract is one of the best known examples of this type (210), although some other recent data cast doubt on the colon specificity of this antigen (313). It is not yet clear whether some of the instances of tumour-specific antigens presently classified in groups a and b may not in fact belong in group c.

249. In many of these cases, it can be directly demonstrated that an immune response develops in the host bearing the tumour (244). Alternatively, immunization of normal animals with various forms of killed or altered tumour cells will provoke a state of immunity such that subsequently-transplanted tumour cells will

be rejected. Many of these studies have been performed with serially-transplanted mouse tumours, principally virus-induced tumours, which are quite strongly antigenic. Other studies have been performed with carcinogen-induced primary tumours or radiation-induced sarcomas and in these cases the antigenic stimulus often appears weaker. On the basis of these studies, it is a reasonable hypothesis that the first malignant cell arising in the primary tumour, carrying the tumour-specific antigen, presents an immunogenic challenge to the host's wandering lymphocyte pool of cells. Whether such stimulation is then mediated directly or via antigen-processing mechanisms is not known. It is envisaged that a continuing eradication of emerging, potentially-neoplastic cells must be occurring in the body, and that if a tumour clone is to develop, it must override or evade this potential antagonism. It is important to stress that this type of study has not been extensively performed with spontaneous primary tumours (although some data are available, Stjernsward and Vanky (528)), and that at present we are extrapolating from the data with virus- and carcinogen-induced tumours in the mouse.

250. The relevance of this discussion of neoplasia and immunity to radiation and immunity is based on the following set of premises: (*a*) many tumours are antigenic and therefore may initiate an immune response in the host against the tumour cells (cellular and/or humoral response); (*b*) many carcinogens, both chemical and viral, can induce an immune depression; (*c*) upon induction of a tumour, an interaction develops in which the growth rate of the tumour is pitted against the developing immune response. Various studies have shown that the immune response can both retard the growth rate of tumours, and be of particular importance in limiting the metastatic spread of malignant tumours. The relationship of the immune response to tumour growth is therefore pictured in an analogous fashion to the balance between immunity and infection, and just as radiation depresses immunity and permits a greater spread of infection, so it is proposed that radiation depression of immunity may permit more rapid tumour growth and spread. The essential question relevant to this report is therefore whether radiation-induced depression of immunity is a key factor in the mechanism of the radiation induction of cancer.

B. Radiation and tumours in mice

1. *Effect of radiation on antigenicity and the immune response*

251. Radiation may act on the immune response to tumours by a possible effect on the antigenicity of the tumours, or by altering the host's immune response itself. In regard to the first point, normal lymphocytes exposed to 1,200 roentgens *in vitro* fully retain their antigenicity, as assessed by their ability to stimulate a mixed lymphocyte reaction (164). Irradiated mouse lymphoma cells were assayed by two different methods for growth activity in syngeneic mice (341, 342). No difference was observed between cells exposed to 100 roentgens and controls, but with 1,000 roentgens increased reaction against the tumour was very evident. It appears that the irradiation may have exposed the antigenic sites of the tumour cells to a greater extent, or alternatively may have selectively enriched the tumour population in antigenic cells by selectively removing the less antigenic ones.

252. There are many observations indicating that mouse tumours are antigenic to their syngeneic strain and that irradiation, like many other forms of immunodepression, will permit a more rapid tumour growth or an earlier induction of tumours (172, 208, 292, 610). For example, in a study (475) on methylcholanthrene-induced sarcomas in mice, whole-body irradiation prior to transplantation resulted in marked increase in tumour growth. A dose of 400 rads give a maximum effect, and enhanced growth rate could be detected in mice in which the tumour was transplanted four months later, although the maximum effect was observed with transplantation 24 hours after irradiation. Unfortunately, there are few studies yet available dealing with primary or spontaneous tumours and radiation-induced immune depression. However, a similar result of an earlier appearance of primary carcinogen-induced tumours has been reported in mice immunodepressed by neonatal thymectomy (223).

253. Osteosarcomas which arose in mice following administration of ^{90}Sr have been shown to carry tumour-specific transplantation antigens, in that immunization of recipients with 15,000 irradiated tumour cells will result in a lower incidence of takes of transplanted tumours providing the recipients are also exposed to 400 roentgens one day before transplantation (410). These experiments confirm a previous suggestion (411) that radiation-induced sarcomas may be antigenic, and that this antigenicity may be a factor in the development of the primary tumour. Infection of ^{90}Sr-treated mice by BCG at a time close to the expected appearance of the first bone tumours resulted in a delay of the development and a significant decrease of the total incidence of such tumours, which may have been due to an increased stimulation of the immune system by the BCG.

2. *Radiation and mouse leukæmias*

254. One of the strongest arguments relating radiation-induced immune depression to tumour induction comes from a study of radiation-induced mouse leukæmias. Before considering this argument in detail, it must, however, be emphasized that the model system used is not ideal, as it involves neoplasia of a component cell type in the immune system. The changes that have been attributed to the host immune system might alternatively be explained by direct interference with the potential neoplastic line of cells. However, in the absence of any more suitable model, but with this reservation, it is relevant to consider this model system (see also annex G).

255. In any attempt to propose a pathogenic mechanism for radiation-induced lymphosarcomas and lymphatic leukæmias in mice, two main experimental observations must be considered (277, 278): (*a*) there is a far greater incidence of tumours when the dose is fractionated with successive increments spaced a few days apart; and (*b*) the entire body must be irradiated since shielding of the spleen or bone marrow, or injection of normal bone marrow after whole-body irradiation, drastically reduce tumour incidence (280, 316). Three separate factors appear to be involved: injury to the normal sites of storage of the latent virus with its concomitant release; injury to the thymus followed by regeneration; and injury to the bone marrow which in turn interferes with the thymic regeneration, thereby producing a maturation arrest in which large numbers of blast cells are exposed to oncogenic virus. Lym-

phoma induction can also be achieved by the direct injection of the leukæmogenic filterable agent from irradiated C57 mice into a thymus graft carried by a thymectomized irradiated host (236). If the host is not irradiated, leukæmia will not result, suggesting that something more is required than the active virus in large numbers and the presence of large and medium thymus lymphocytes. Haran-Ghera (235) and Haran-Ghera and Peled (237) have given evidence to suggest that the other essential factor in leukæmogenesis may be irradiation-induced immunological depression. Tests on the immunological reactivity of irradiated mice were performed by evaluating the production of antibodies to *Shigella* antigen. The four weekly whole-body exposures of 170 roentgens used for leukæmia induction resulted in marked immunological depression, with the minimal antibody production in these mice persisting for about one week following irradiation, and coinciding with the timing of the demonstration of release of filterable agent into bone marrow. Inoculation of normal bone marrow immediately after irradiation was, therefore, suggested to re-equip the immune system, and accordingly reduce tumour incidence. An alternative explanation is that it leads to repopulation of the host thymus, thus interfering with the maturation arrest of thymic cells.

256. It therefore appears that in leukæmia induction a transient radiation-induced depression in host immunity (possibly mainly homograft immunity, Haran-Ghera, (235)) is an important factor, combined with the activation or release of a latent virus, in permitting expression of the neoplastic transformation that occurred in the appropriate thymus cell. A similar phenomenon may well pertain to other tumour-induction systems in that host immune depression may permit the proliferation and expression of other non-radiation-induced neoplastic transformations.

257. In these preceding paragraphs we have been considering radiation-induced tumour induction in a mouse strain that rarely develops lymphoid leukæmia unless it is irradiated. In studies with a high-leukæmia-incidence strain of mice, AKR, a novel immunological approach to the ætiology of the tumour was proposed (601). In this strain, all mice eventually succumb to leukæmic development and it has been shown that the Gross virus probably acts as one of the ætiological agents of the AKR lymphomas (600). In an analysis of the immunological status of the AKR mice, it was proposed (601) that an immune attack, rather than immune depression as we have previously been discussing, may play an ætiological role in AKR-leukæmia development. Using a cytolytic plaque assay with AKR embryonic cells (600) it was shown that both spleen and lymph-node cell suspensions from AKR mice taken in the preleukæmic adult period will exhibit an immune type of cytolytic activity against syngeneic AKR cells. As young AKR mice are tolerant to the G antigen, it was suggested that the development of a partial or complete breakdown of tolerance to the G antigen occurs in the preleukæmic period. Secondary, immunologically-mediated damage of virus-infected G+ thymic lymphoid cells may then be the ultimate process that precipitates leukæmia development in the AKR mice. Recent evidence (637) suggests that a comparable sequence of events may occur in the development of mammary cancer following neonatal infection by the Bittner virus. Thymectomy reduced the incidence of mammary cancer in C3H MTV-positive mice (mammary-tumour-virus positive) and thymus grafts to such mice restored a high mammary-cancer incidence. When adult C3H MTV-negative spleen cells were injected into thymectomized C3H MTV-positive mice, a high incidence of mammary cancer was observed. It seems likely that in this tumour also, the injection of non-tolerant spleen cells precipitated tolerance breakdown, leading in some manner to the development and/or emergence of mammary cancers.

258. These latter two experiments do not involve irradiation. However, their basic premise is that loss of tolerance may lead to the development of an immune response which itself is directly involved in the ætiology of the neoplasia. Since irradiation has been shown to break the tolerant state, particularly in situations where tolerance will eventually be lost in any case, it is not unreasonable to consider that radiation-induction of neoplasia might sometimes involve a break in the state of tolerance against a vertically-transmitted oncogenic agent, which would then swing the balance towards an immune attack against those cells expressing the particular tumour-specific antigen. This in turn may lead in some manner to a proliferation or destruction of the target cells which, perhaps by altering the normal proportions of blast and mature forms, greatly increases the proportion of cells that are acutely sensitive to malignant transformation.

259. Another effect of radiation on leukæmia incidence in mice has recently been reported (317). The same radiation dose which enhances leukæmogenesis in an unirradiated mouse will counteract leukæmia development, if given to a mouse which was previously irradiated but has not yet developed leukæmia. This indicates that the preleukæmic interval between recovery from the first dose of radiation and the development of the tumour includes a vulnerable radio-sensitive stage in the preleukæmic cell line. It was proposed that the target cell for transformation may be acutely radiosensitive in this phase. However, in terms of the immune-attack theory of Wahren and Metcalf, it might be proposed that the first dose of radiation has broken a state of tolerance to a vertically-transmitted leukæmia virus. Following this, a phase of a developing immune response to the viral antigen occurs, which may be essential for neoplastic development. A second dose of radiation in this period would largely suppress this newly-emerging state of immunity against the viral antigen, and therefore suppress tumour development.

260. It must also be considered that radiation may have other effects on viral release akin to lysogeny, such as has been shown for lambda phage in bacteria (see for instance reference 127). If radiation has such an effect on vertically-transmitted oncogenic viruses in animals, it may well be of considerably greater ætiological importance than any of the other more speculative immunological considerations.

C. Radiation and immunotherapy

261. Viable tumours frequently carry exposed tumour-specific antigens on their surface membranes which renders these cells vulnerable to an immune reaction against the tumour antigen. This observation suggests that elimination of the cancer cell by an induced immune reaction might be a feasible means for therapeutic elimination of the cancer. This has now become a most intensively investigated area. Most of the current work is concerned with basic immunological

approaches and does not touch on the field of radiation. Approaches to immunotherapy are reviewed elsewhere (11, 520). Two aspects of this field are, however, relevant to this present report: (*a*) the use of irradiation of donor cells as antigens and the effect of host irradiation on tumour immunity, and (*b*) the use of radio-labelled antitumour antibodies.

262. Growing tumours may lead to the establishment of a type of paralysis to the tumour antigens in the host. If an immune response against the tumour is to be elicited, a more potent immunogenic stimulus must be given to the host. Furthermore, if immunization is to be attempted, it must be in a manner such that the serum blocking factors described by Hellstrom and Hellstrom (242) are not increased but rather that cellular immunity is primarily activated. When tumours carry unique tumour-specific antigens, the autologous tumour may have to be used as the immunizing antigen. Since re-inoculation of the viable autologous tumour may lead to its regrowth, the tumour cells must first be exposed to some treatment that destroys their viability without affecting their immunogenicity. X-irradiation appears to meet these requirements in most cases. Lymphoid cells appear to retain their normal immunogenicity after irradiation (164), although some reduction in activity has been reported (353). While some investigations have not shown any effect of irradiated autologous cells alone in inducing tumour rejection (349), others have observed a significantly increased immunogenicity of irradiated isologous tumour cells (341, 342). In one study (226), a sample of fibrosarcoma was removed from rats and exposed to 10,000 roentgens *in vitro*. The irradiated cells were then given back to the autologous animal and the remaining large mass of the primary tumour was locally exposed (*in vivo*) to 2,000 roentgens. A striking regression in growth of the primary tumour occurred in many cases. Injection of irradiated autografts alone had no effect without local irradiation. It was suggested that with large masses of tumour tissue, local irradiation (even 2,000 R) does not kill all cells. A certain proportion will remain. The growth of the surviving fraction, however, may be considerably inhibited by the immune response initiated by the irradiated autologous graft.

263. Tumour-specific cytotoxic antibodies were also produced in man by the immunization of 13 patients with their own irradiated melanoma cells (263). The longest response lasted 14 days, but again the procedure had no apparent effect on the course of the disease in these patients. In a study with the Morris hepatoma in rats, the immunogenicity of the hepatoma cells was considerably increased when the cells were combined with a pertussis vaccine (629). Irradiation of the hepatoma or liver homogenate did not seem to interfere with the immunizing properties of the tumour.

264. In recent studies, attempts have been made to determine whether rat reticulo-endothelial cells are capable of producing a cellular anti-tumour agent against Yoshida-sarcoma cells in tissue culture (491). In these studies, it was found that an effective antigenic cell component was released into the tissue-culture medium from tumour cells after three days of culture in a diffusion chamber. The same cell components were obtained from cultured medium of tumour cells after x-irradiation. Optimal doses of radiation capable of releasing this agent ranged from 2,000 to 4,000 rads. In other studies (412) this same anti-tumour agent, derived from incubation of macrophages in the supernatant fluid from irradiated tumour cells, could be transferred to lymphoid cells when they were cultured in the same tube. These studies therefore indicate the possibility of producing immunogenically-active tumour-specific antigens in culture by irradiation of cultured cells, and also of activating immunocompetent cells *in vitro* and perhaps of then obtaining *in vivo* destruction of tumour with these cells.

265. Immune sera prepared against tumour-specific antigens can occasionally be shown *in vivo* to reduce the growth rate of tumours (217) and in these cases it is most likely that cytotoxic complement-fixing antibodies are involved. However, in many cases this is not found, and antibody-mediated enhancement of the tumour is more likely. It is clear, from these experiments, that anti-tumour antibodies can localize on the surface of tumours *in vivo*. If the antibody carried with it a high source of radiation, then selective radiation killing of the tumour cells might occur. This finds a good precedent in the work of Ada and Byrt (3) who showed that ^{125}I-labelled antigen bound to the surface of antigen-reactive cells specifically killed those cells without affecting normal cells. In man, cancer-specific antibodies have been produced, but there is little evidence that they have any inhibitory or destructive effects *in vivo* (263, 264). In a report on the production of a specific precipitin to a renal cancer in man, Nairn *et al.* (401) suggested the idea that specific antibody to tumours might localize on the surface of the tumour cells and act as a homing carrier for radiotherapeutic or chemotherapeutic agents. This was then demonstrated in mice by Ghose *et al.* (203) who treated Ehrlich-ascites cells *in vitro* with an ^{131}I-labelled antibody to the tumour. On inoculation of these cells into mice, the tumour did not grow. In another series of investigations (128, 129, 325), it was shown by means of radio-labelled antibodies that antibody molecules to human brain tumours could be localized *in vivo*, and further studies on this approach are in progress. Radio-labelled antifibrin antibodies have been shown (350) to localize preferentially in certain cancer lesions, as the deposition of fibrin often occurs in these areas. This indicates a possible means of delivering local radiation to fast-growing tumours.

266. Another recent approach has been the demonstration (202) that antibody-treated Ehrlich-ascites cells are rendered more radio-sensitive than control tumour cells. This may be an effect mediated through antibody fixation on the membrane and interfering with the cell-membrane permeability, making some of the x-ray effects more damaging (596). Doses of radiation that did not greatly influence the subsequent growth rate of normal rabbit-serum-treated tumour cells severely inhibited the antibody-treated cells. It was suggested that this phenomenon may be related to the correlation of "observed durability of the response to chemotherapy in a Burkitt lymphoma" with the observed frequency of preferential binding of a globulin fraction on the tumour cells surface (291).

VII. Effect of variation of condition of irradiation on immunological responses

267. In the preceding paragraphs, much of the available data on the effects of radiation on the different types of immune responses has been considered primarily from the point of view of the nature of the immune response. In this section emphasis will be

given to the different ways in which radiation may be presented to the individual, and their subsequent effects on the immune response.

268. It must be stressed that in most of the cases where experimental studies have clearly shown effects of radiation on some type or component of the immune response, relatively few studies are available on the effects of changing the conditions of irradiation, and in particular dose, number of exposures, or type of radiation. In most studies, the aim has been primarily related to an immunological problem and the type of data that would be most relevant for estimates of risks from radiation is simply not available.

A. Small doses

269. Studies of radiation inactivation of antibody-forming capacity have usually given D_{37} values of around 60-100 rads. Thus doses of radiation in this range, when given to the whole animal, have usually shown some significant degree of suppression of antibody formation. A 75 per cent reduction in antibody-forming plaques was found (286) in mice given 50 rads 10 days prior to antigen. Dixon *et al.* (145) also found a significant reduction in antibody formation with 75 roentgens, and 125 roentgens gave considerable depression. The results of Makinodan and Price (336) show 65 per cent of a normal primary response following 100 rads. In dose-effect studies, which have usually started at either 50 or 100 rads, increasing exposure to radiation yields proportionately more suppression of antibody formation (e.g., table 4 and figure V). The phenomenon of interphase death of lymphocytes *in vivo* discussed in paragraph 152 is probably not involved with doses of radiation to the whole body below 100 rads, although it must be observed that there are few direct data on the effect of different types of radiation or of dose rate on interphase death of lymphocytes.

270. The effect of radiation exposures in the 100-roentgen range may not be solely on the immunocompetent cell. Decreased bactericidal activity was observed in polymorphs isolated from guinea-pigs 3-5 days after whole-body exposures of 100 roentgens (440). Some depression in antibody formation was also observed (177) when mice exposed to 550 roentgens were given macrophages from donors that had received only 150 roentgens (table 2). These two studies therefore reinforce the point that depression of the immune response as a whole by radiation in the 100-roentgen range is not solely due to interference with the proliferation of immunocompetent cells.

271. Radiation-induced enhancement of the immune response has also been observed with relatively low doses. A heightened peak titre, shorter latent period, and a high rate of antibody synthesis were all observed in rabbits given 25 rads two days to two hours after antigen injection (547). Prolonged production of haemolysins was also observed when rabbits were given 25 rads even one month before injection of antigen (548). In a similar system with mice a dose of 50 rads was also shown to give an enhanced response when given one hour before or after antigen (table 3). These results show that single doses of radiation in the range of 25 to 50 rads may either depress or enhance the antibody response, the direction being determined mainly by the time relation between injection of antigen and exposure to radiation.

272. Data on the effects of single radiation doses below 25 rads are very sparse, and in most instances no significant effects were observed on the immune response as measured in the whole animal. However, a change in the morphology and motility of small lymphocytes following *in vitro* exposures of 2-5 roentgens was reported (527), although the possible *in vivo* significance of an effect at this dose might well be doubted.

273. A key problem in attempting to extrapolate from the effects of high doses of radiation on the immune response to the effects of low doses, is that the immune response as a whole is composed of separate components which differ in their radio-sensitivities. Accordingly, at moderate to high doses, several components may be affected, but at low doses only one may be susceptible. The real question is therefore whether *any* of the essential components are indeed radio-sensitive with exposures below 50 to 100 roentgens. The studies mentioned above dealt primarily with the 25 to 100 roentgen range, and it is indeed apparent that some significant, albeit relatively minor, effects are observed with 25 roentgens. However, as the D_{37} value (calculated from experimental curve) for the actual antibody-forming cell series is around 75 rads, very little significant effect on this component will occur with single exposures below 25 roentgens. As mentioned above, the phagocytic cell series is also affected, but only marginally, by 50 to 100 roentgens, and again no significant effect would be expected with exposures less than 25 roentgens. For antibody production, this only leaves the lymphocyte cell series to be considered and, in view of the extreme radio-sensitivity of this cell series as a whole, it is possible that some depression in the antibody response might occur in situations where the full available complement of thymic-derived "*T*" cells are needed in eliciting a primary response. Clearly, further direct studies are needed to provide information relevant to this question. A more appropriate question, however, concerns the effect of multiple or continuous low doses of irradiation on the immune response and, in particular, whether the immune-response potential will eventually decline or adapt to continuous low-level exposure. This will be considered in the next section.

B. Fractionated and prolonged doses

274. It is perhaps appropriate to first consider the hæmatopoietic stem cells, and to note that if all cells of this type were completely inactivated by irradiation, then the immune system would also eventually fail, at least in the ability to mount primary responses as, in this case, a continual input of differentiating stem cells proceeds throughout life. It is quite clear that this possibility is remote except with relatively high doses. With a daily dose of 50 rads after an initial dose of 150 rads, a further 250 rads was required to reduce the stem-cell repopulating activity to 5 per cent of control values which still, however, represents a large reserve of potential hæmatopoiesis. Furthermore, the observation that adult thymectomy alone only leads to a depression in some immune responses several months later, suggests that a large reserve of differentiated immunocompetent cells already exists in the body.

275. The amount of actual data available on the effects of continuous or repeated exposure at low dose levels is again quite limited, but does at least provide an order of magnitude of exposure for which suppression is found. In rabbits given 4-5 roentgens per

day to a cumulative exposure of 356 to 2,039 roentgens, no functional disturbances of antibody formation in response to three injections of paratyphus vaccine (642) were evident. However, with 21 roentgens per day up to a total of 2,000 roentgens a partial inhibition was observed. Also, in monkeys, a daily exposure of 1.34 roentgens (up to a total of 675 R) did not affect the production of antibody to tetanus toxin (641). In another study on mice, rats, guinea-pigs and rabbits (651) given a daily exposure of 1.2 to 4.3 roentgens for 1½ to 2 years, animals were investigated for bactericidal activity in the blood. The strongest disturbance of natural immunity occurred in young animals and particularly with radiation delivered during intrauterine development. Some depression of immunity occurred with cumulative exposures in the range of 300 to 450 roentgens. In a study (533) on pathogen-free mice exposed to 1-4 roentgens per hour, the ability of irradiated animals to produce antibody to some but not all antigens was inhibited by sublethal doses.

276. In a study (671) on the effect of low doses of radiation given each day for five days, pregnant rats with fœtuses at 16 days of embryonic development were irradiated with 4-65 roentgens per day for five days. There was found to be a resulting inhibition of agglutinin production to typhoid vaccine when the immunization was performed at 2-5 months of age. Some reduction of phagocytic activity of blood leucocytes was also observed.

277. In considering data on the effects of fractionated low doses, it is important to bear in mind the studies previously discussed in paragraphs 128, 157 and 182, that stress that dose rate as well as absolute dose is quite important in determining the degree of radiation-induced inhibition of immunity. As many of these fractionated or continuous radiation studies were performed with low-dose rate irradiation, it is difficult to assess the actual effect of a fractionated dose that would have caused considerable suppression if it had been given in a single dose.

278. One of the main issues that is relevant here, is whether repeated small doses or low-level continuous irradiation give rise to an accumulation of damage, or whether restoration following small doses is complete and thus adaptation to repeated irradiation occurs for the immune system. This problem is at the heart of the matter in attempting to assess risk estimates for man in terms of effects on the immune system. Although there are considerable data on the over-all susceptibility of the immune response to higher doses of radiation, there is very little that is of direct relevance to this central question. Some speculation is therefore justifiable.

279. It is most likely that the immune response as a whole would readily adapt to repeated low doses of irradiation. Studies assessing the over-all potential expression of responding cells by comparison with those that actually respond (336) stress the enormous reserve that is held unexpressed. Thus, if an individual normally expresses only 10 per cent of his actual immunological potential, then this cell population could readily tolerate up to a 90 per cent loss in that system from continual irradiation without any apparent loss of immune responsiveness. In this connexion, it is also relevant to consider the hæmatopoietic stem cell which is continually entering into differentiation throughout life and feeding more potentially-immunocompetent cells into the system. Unlike some other tissues, the immunocompetent population of cells is not produced only once in ontogeny but rather depends heavily on continual replacement. Furthermore, as the hæmatopoietic stem cell itself is in large reserve, considerably high levels of radiation would be required to limit the potential input into the immune system.

280. It is essential, however, to remember that the expression of an immune response is not a single one-hit event dependent on antigen directly stimulating one cell. From studies on the relative radio-resistance of thymic-derived carrier cells essential for collaboration in many secondary responses, it would not be expected that much of an effect would be exerted on this cell type by repeated or continuous irradiation. The cell type which is more likely to control the over-all immune response is the macrophage, as this cell type may not be renewed as frequently as immunocompetent cells, and in many instances active processing by this cell is obligatory if the immune response is to proceed. As several studies mentioned above have suggested that some interference with antigen processing may occur at moderately-low single exposures (100 R), it is possible that cumulative damage to these cells might result from repeated or continuous exposures to low doses. Further studies on this aspect are clearly required, with particular emphasis on a comparison of the effect of continuous or repeated irradiation on immune responses which do or do not require macrophage participation.

281. It is important to distinguish this concept of adaptation to repeated low-level irradiation from the implication of acquired radio-resistance in the antibody cell series. This latter view was first proposed in studies with mice (454) but, as has been discussed previously (paragraphs 85 and 86), can be explained by changes in the proportions of interacting cells. Instead, the concept of adaptation implies that cells of the antibody-forming precursor series are all equally radio-sensitive, and are being continually replaced throughout life, and that at any given time there are many more potentially-immunocompetent cells to a given antigen than are needed to produce the usual level of immune response. In the studies of Kennedy *et al.* (286) it was in fact suggested on the basis of plaque-forming response data, that the immune system could suffer at least a thousand-fold depletion of the proliferative capacity of its cells without completely losing the ability to respond to an antigen by the production of plaque-forming cells. This actual number will vary for different antigens, and further studies of this type would be most relevant to this problem.

282. There is one instance in transplantation immunology where fractionated radiation doses appear to be of some value in depressing the host's potential immune rejection of the graft. Local irradiation of organ grafts (kidney or heart) *in situ* appear to aid in prolonging graft persistence. These studies (see paragraphs 208 to 212) usually involve 150 rads given six times at two daily intervals. The actual mechanism of prolongation is obscure, but is likely to be associated with destruction of invading host cells which are continually infiltrating the graft.

C. Whole-body and local irradiation: delayed effects

283. Most of the studies described in this report have dealt with early effects on the immune system

after whole-body irradiation. In some studies, *in vitro* irradiation of cells has been used, and these are often valuable in determining immediate and acute effects on a given cell type. Local irradiation of an organ *in vivo* can, however, be complicated by other problems. In many instances, local radiation applied to various organs of the immune system, such as lymph nodes, spleen, or even extracorporeal radiation, have been shown to lead to lymphopenia and reduction in immunocompetence by depleting the recirculating lymphocyte pool. However, in cases where solid organs are irradiated, the fixed structural cells of the organ are also irradiated, and it is reasonable to question whether long-term effects may be observed in these cases, even though the lymphocyte content of the organ may be completely restored by entry of new cells.

284. The special case of local irradiation of renal allografts in man, dog and goat was discussed above, and in this instance with the doses used (6×150 R) no deleterious effects appear to have been observed in the parenchymal tissue of the kidney, although in many of these experimental situations, prolonged observations were not made.

285. In many centres there is increasing use of cadaveric kidneys for renal transplantation. As the kidney from the cadaver source frequently shows some degree of acute tubular necrosis (ATN), it is pertinent to consider the effects of radiation on the regenerating tubular epithelium. In a recent study of this problem with kidney grafts in dogs, it was shown (354) that therapeutic doses of local graft radiation (600 rads) given immediately following the onset of acute tubular necrosis significantly delay recovery of renal function from the ischemic insult. These authors therefore caution against indiscriminate use of local kidney radiation without signs of immunologic injury to the kidney which would merit its use.

286. Following whole-body irradiation at a dose of 1,250 rads, little direct damage was observed to the cytoplasmic fibril web of the reticular structure in lymph nodes of rats. When doses of up to 8,000 rads were used, the entire structure showed considerable necrosis and destruction (272). With local irradiation of lymph nodes, regeneration of lymphoid content is extremely rapid, presumably because of entry of immigrant unirradiated lymphoid cells. However, following 3,000 roentgens, an extreme secondary atrophy develops several weeks later, apparently following vascular damage and destruction of the original stroma (165).

287. The effect of radiation on the popliteal lymph node of sheep on its output of lymphocytes has been described (230). Chronic fistulæ were established in the different ducts, and the nodes received x-ray exposures of 800 to 2,000 roentgens. A significant fall in lymphocyte output occurred, but was not accompanied by any gross change in the morphology of the cells. Five preparations were also antigenically stimulated 6 to 140 hours after the nodes had received 2,000 rads. The resulting increases in antibody titre and characteristic cellular changes showed that irradiation had not significantly altered the immunological performance of the nodes. This strongly indicates that the functional capacity of the node is dependent on the entry of recirculating lymphocytes and not on a fixed cellular population. Late effects on the node are discussed in paragraph 289. A similar conclusion was reached from a study of the regeneration of lymph nodes from whole-body irradiated mice (643).

288. In this aspect of delayed changes following irradiation, data from the clinical use of radio-therapy in Hodgkin's disease and malignant lymphomas are most relevant. Radio-therapy offers a significant chance for cure of Hodgkin's disease (449). In retrospective studies of the recurrence rate (279), defined as the probability of reappearance of disease in a radiation-treated field as the first new manifestation of disease, a correlation with the median dose was clearly evident. With a median exposure of 500 roentgens there was a 78 per cent recurrence rate, with a median exposure of 1,000 roentgens the rate fell to 60 per cent, and with 4,000 roentgens in weekly fractions of 1,000 roentgens (using megavoltage energy beams) only 2 per cent recurrence was observed in 300 fields at risk. As these figures are based on single fields, it was stressed that the chance of success is an independent variable for each field, and accordingly the use of higher doses (4,000 rads) becomes of even greater statistical importance. With this type of treatment, it is obvious that considerable care must be taken to shield the lungs and other vital tissues. The judicious use of lead shielding, monitored carefully, has proven that this problem can be successfully avoided. But what of late complications in those areas which are irradiated?

289. Severe leucopenia or thrombocytopenia has rarely been a problem (279), presumably because of adequate shielding of some bone marrow which has sufficient hæmatopoietic stem-cell reserve. In terms of survival rates, the data in table 5 strongly vindicate the use of radical (3,500-4,000 R) radio-therapy. This is particularly evident when it is realized that virtually no cures of Hodgkin's disease in stage III had been reported before this study. Subsequently, some evidence of late necrotic changes may occur in lymph nodes in the treated fields. In one study (634) some calcification was observed in intrathoracic lymph nodes 1-14 years following irradiation of the mediastinum at exposures between 1,000 and 6,000 roentgens. This calcification is probably due to post-irradiation tissue necrosis. However, it must be stressed that this possibility of some minor post-irradiation changes in lymph nodes most certainly does not outweigh the enormous value of carefully-administered radical radio-therapy for this disease. Hall and Morris (230) observed that the irradiated lymph nodes of sheep eventually showed a definite increase in the thickness of the capsule and of the connective tissue trabeculæ. They suggested that the lymph node may eventually lose its capacity to transmit recirculating lymphocytes. In an earlier report (10) with irradiated rabbit popliteal lymph nodes marked fibrosis was seen three weeks following irradiation.

D. Radio-isotopes

290. In various experimental studies radio-isotopes have been used to deliver radiation at localized sites in the lymphatic system. This includes such methods as the application of ^{32}P-impregnated polythene strips to the surface of the spleen (191), intra-atrial implantation of a β-emitting source (31), and intra-lymphatic infusions of radio-isotope-labelled agents (159, 567, 620). Perhaps one of the greatest dangers in applying these types of treatments to man is that it is relatively difficult to calculate effective dosages to organs in the body. In one study (80) with endolymphatic radio-therapy (ERT) for therapy of malignant lymphomas,

some attempts were made at dosimetry. ERT may use either ^{198}Au, ^{90}Y, ^{32}P, but more frequently ^{131}I. With 50 millicuries of ^{131}I injected, it was estimated that a dose of 842 rad mCi^{-1} was given to the lymph node, and of only 10, 2 and 8 rad mCi^{-1} to lungs, liver and spleen, respectively, but of 48 rad mCi^{-1} to the thyroid. Possible complications from ^{131}I accumulation (after excessive doses) in the thyroid can be minimized by administration of Lugol's solution preceding ERT (80).

291. Several experimental studies on the effects of various radio-isotopes on immune responses have been reported. Moderate inhibition of immunity after chronic uptake of small doses of ^{90}Sr was observed even a year later. A single subcutaneous injection of 0.5 mCi kg^{-1} of ^{210}Po, or a single intraperitoneal injection of 0.05 mCi kg^{-1} of ^{90}Sr to guinea-pigs did not affect the primary response, but led to a marginal reduction in secondary immunization (666). Single intraperitoneal injections of tritium oxide in doses of 0.3 Ci kg^{-1} (total dose 400 rad) to dogs (693) led to depression in immunological activity which correlated with the clinical manifestation of radiation disease. Depression of phagocytic activity and agglutinin formation was observed in rabbits given simultaneous subcutaneous injections of ^{60}Co or ^{131}I together with secondary immunization (698b). Intravenously-administered ^{32}P colloidal chromic phosphate in a dose of 780 microcuries to rabbits (636), was calculated to yield 14,000 rads during the 14 days between isotope and antigen injection. This resulted in marked depression of antibody formation. This effect could be counteracted by multiple antigen injections, which might indicate that the major effect of intravenously-injected isotope was on the spleen, and that by multiple injections non-splenic sites then participated in the response. However, in another study (81), the injection of ^{32}P chromic phosphate showed some effect on all organ systems when injected intravenously, whereas a selective destruction or change only in lymphoid tissues occurred following intralymphatic injection.

292. The literature also contains a large amount of data concerning the effects of various incorporated radio-isotopes (^{210}Po, ^{89}Sr, ^{90}Sr, ^{32}P, ^{131}I, ^{198}Au, ^{65}Zn and an unseparated mixture of nuclear-fission products) on immunogenesis when experimental animals are vaccinated with various other bacterial antigens: *Salmonella breslau* (683), *Proteus vulgaris* (669), typhoid-dysentery vaccine (698b) and brucellosis vaccine (666). It has also been shown that antibody formation is decreased when animals damaged by ^{210}Po, ^{131}I, ^{45}Ca or ^{65}Zn are immunized with tetanus and diphtheria anatoxins or gamma globulin (691). In a number of investigations it was found that antibody formation was reduced when animals damaged by ^{32}P, ^{131}I, ^{137}Cs and ^{144}Ce were immunized with rickettsial and viral antigens (smallpox-vaccine virus and influenza virus) (640, 695, 696). Reduced antibody formation in animals damaged by ^{45}Ca, ^{90}Sr and ^{137}Cs was observed when the animals had absorbed total doses of the order of 220-270 rads (698). A depression in the formation of antibacterial and antiviral antibodies in rats damaged by ^{144}Ce was observed when the isotope had been introduced intra-abdominally, even at relatively low total absorbed doses to the critical organs—liver, skeleton and spleen—apparently because of severe damage to the reticulo-endothelial cells (696).

293. According to some authors (698) the changes in immunogenesis which result from incorporated radio-isotopes have several phases: periods of depressed antibody formation alternate with phases of normalization and stimulation. It is also important to point out that internal irradiation is accompanied by a very marked suppression of the secondary immunological response in a number of cases; this suppression is more marked than the depression of the primary immunological reaction (666, 696). It is assumed that when animals are irradiated internally, the long period over which the dose is accumulated slows down the restorative processes. Under these conditions, continued exposure to radiation causes a marked suppression of the immunological response when the animal is revaccinated. Internal irradiation with ^{144}Ce may stimulate the formation of plasma cells (685, 687) and there have even been cases of mitosis among them. The sub-microscopic organization of the plasma cells undergoes only slight changes (some disturbances in the structure of the nucleus and the mitochondria), which confirms the structural hypothesis concerning radio-sensitivity to the effect that resistance to radiation is due to the presence of enough organoids in the cells to keep reparative processes at a high level. The irradiation both accelerates differentiation of the plasma-series cells and stimulates the development of the endoplasmatic reticulum (686, 687). It has been shown by electron-microscopic immunocytochemistry (using peroxidase as an antigen) that, despite the normal ultrastructural organization of the plasma cells, an antigen-antibody reaction no longer develops in ultra-thin sections after irradiation. A generalized discussion of these data is available in a monograph by Klemparskaya *et al.* (660).

294. In various other studies radio-active gold, bismuth, silver, yttrium and iodine have been used, either as the "naked" radio-active material itself, or coupled to a carrier. Probably the main point with which to conclude this section, is to stress again that the major problem with this type of irradiation is the great difficulty in controlling radiation dose, and thus although it may be valuable in experimental research studies, it should be considered hazardous in clinical studies.

E. Indirect effects

295. It is well recognized that lymphocytes, particularly thymic cortical lymphocytes, are very sensitive to lymphocytolysis by x-irradiation, by corticosteroids and some other steroid hormones. This raises the theoretical possibility that destructive effects observed on the immune system through the use of external agents such as radiation, may in fact operate by causing a release of endogenous steroid hormones which in turn actually cause the destructive effect on the lymphocyte. A distinction between the direct action of radiation and steroid-mediated destruction could be made (*a*) by assessing effects of *in vitro* radiation of lymphoid cells, and (*b*) by the use of adrenalectomized animals. Actual data on this subject are again sparse and, where available, have been obtained without consideration of the separate components of the immune response.

296. *In vitro* studies (238) have shown that the antibody-forming response can be inhibited by corticosteroids only very early after antigenic challenge. Resistance to steroid inhibition develops rapidly with time, and as this steroid-resistant phase coincides with the lag phase of cell proliferation, steroid inhibition is clearly active only on non-dividing lymphoid cells, prior to their antigen-induced proliferation. This would in turn imply that doses of radiation which are known

to mediate suppression of the immune response by inhibition of cell division, are clearly not operating through steroid mediation. Indeed, in a study (149) on atrophy of lymphoid organs in unoperated and adrenalectomized mice, no difference in involution was observed with exposures from 25 to 200 roentgens.

297. The possibility of steroid effects mediating lymphocytolysis is more likely with exposures around 10 roentgens. X-ray exposures in this range will produce stimulation of adrenocortical secretion, as judged by depletion of either adrenocortical sudanophilic material or total adrenocortical cholesterol (148), and by increased cortical secretion (439). On the other hand, some *in vitro* effects of radiation on lymphocytes have been observed with two to five roentgens (527). Again it might be concluded that although further studies are necessary, there is little likelihood that steroids play an important role in mediating radiation-induced immune depression.

298. It it also possible that abscopal effects may exert a positive influence on the lymphoid system rather than a negative effect. It has been shown (339) that exposure of the head of rats to 1,000 roentgens will increase the rate of incorporation of thymidine into DNA in the thymus. With 250 roentgens it was found that the effect is detectable within two hours, reaches a maximum at 19 hours, and then disappears after four days. No change in DNA incorporation into spleen was observed in these animals. This observation may well relate to another study which demonstrated that neonatal thymectomy of mice results in early degranulation of acidophilic cells of the anterior pituitary (457) and it was suggested that the thymus is a target gland of the hypophysis. It is therefore possible that thymic cell turnover is directed and controlled by a neuro-endocrine factor probably at the hypothalamic-hypophyseal level and that irradiation of the head affects this system.

F. Comparative studies in animals and man

299. That radiation has profound destructive effects on the immune response of experimental animals is quite clear but, because of the paucity of data in man, it is essential to question whether direct species comparisons are possible in order to extrapolate from the experimental findings to a realistic risk estimate for man. This is of particular importance in evaluating those situations where considerable benefit to the patient from radio-therapy must be compared to the long-term risks.

300. Much of the experimental data on radiation suppression of immunity has been obtained in small laboratory animals, such as mice. We are therefore attempting to extrapolate from an animal with approximately 3 10^8 potential immunocompetent cells to man with approximately 10^{12} cells of this type. This absolute difference is probably one of the main factors which might argue against the feasibility of direct extrapolation, in the sense that, whereas a single exposure of 700 roentgens in the mouse may depress the primary immune response to an antigen to 5 per cent of control, the degree of depression may not be nearly as large in man. Several factors will be important in determining whether or not this is so. For each antigen, the absolute number of initial responding cells, as a proportion of the actual potential number capable of responding, must be related to the degree of eventual response required to deal with the particular type of antigenic challenge. Thus, if 90 per cent of cells are destroyed with a given dose of radiation, an immune response which requires most of the original potential response to be expressed may be approximately as severely compromised in man as in the mouse.

301. This factor will certainly vary from antigen to antigen but it does not even imply that what is true for a particular antigen as studied in mice need also be true for man. As stressed previously, the secondary immune response as a whole appears far more radio-resistant than the primary response, simply because there are more cells available to react with that antigen. This is most relevant for species comparisons, as the natural experience of cross-reacting antigens often determines whether a state of immunity may have been developed to a particular antigen. For example, natural antibodies to various bacteria may be present in man but not in mice, so that first direct challenge in these two species may in fact respectively measure a secondary *versus* a primary response.

302. Where direct measurements of radio-sensitivity of the immune response have been made in different laboratory animals, similar results were almost invariably found. For example figure XIII shows the radio-sensitivity of the immune response as a function of the time relative to antigenic challenge for mouse,

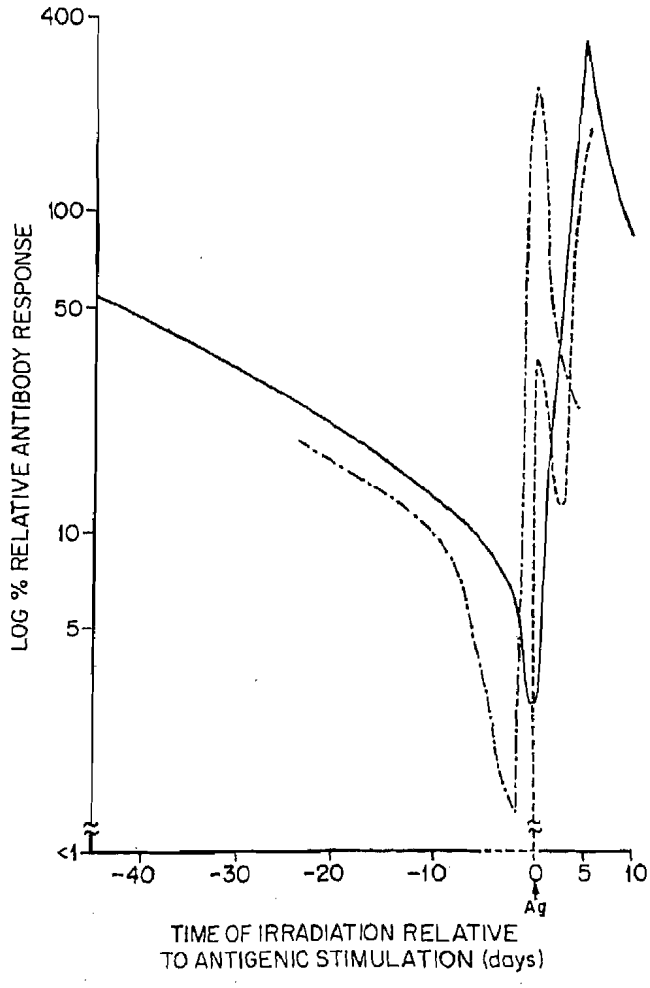

Figure XIII. Radio-sensitivity of the immune response as a function of time of irradiation relative to antigenic stimulation, for three animal species (336, 510, 550): mouse (710 R) (—); rabbit (500 R) (— · —); and rat (500 R) (- - -)

rat and rabbit. Similar results were observed in all cases for both radiation depression and enhancement. In terms of the actual radio-sensitivity of the immunocompetent cells, the data available would strongly predict that no essential difference will be observed among species, including man. Direct assessment of this will be possible with the use of *in vitro* techniques for studying the immune response of human cells.

303. One of the other major problems in comparing radiation studies in different species, particularly the radio-therapeutic studies in man, is the wide range of dose rates and radiation quality used in these studies. A further example of the dramatic effect of dose rate on immune depression is shown in figure XIV, in which

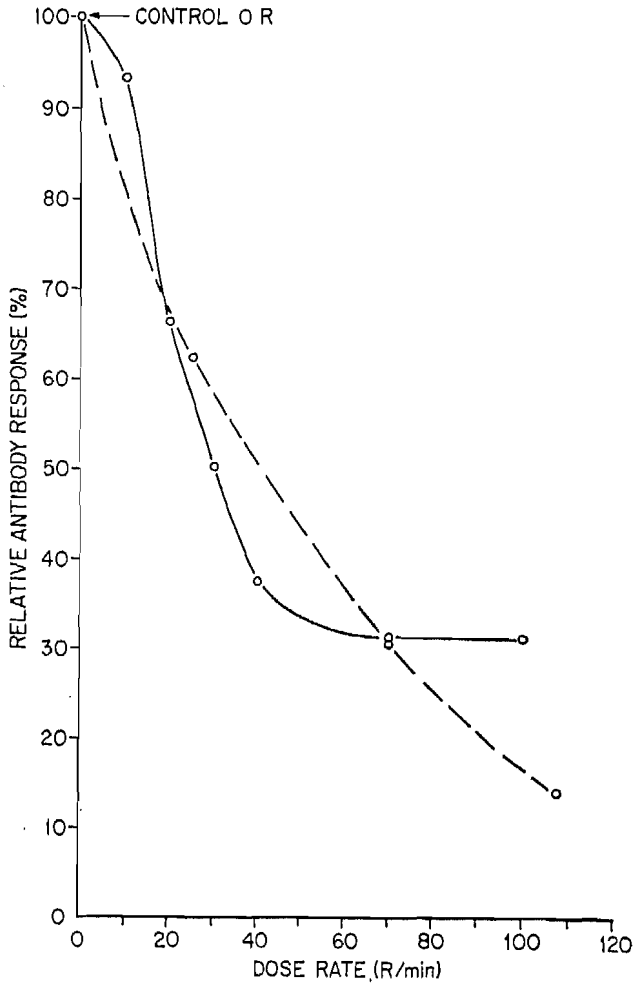

Figure XIV. Effect of exposure rate on the relative antibody response of mice (—) given a total exposure of 700 R, and of rats (- - -) given a total exposure of 500 R (197, 336, 510)

mice were given a cumulative exposure of 700 roentgens, and rats one of 500 roentgens. As a curve of this type is not available for man, and since so many factors enter into the calculation of the actual dose rate received by the relevant tissue, extrapolation of effects from animals to man simply on the basis of cumulative dose is very likely to be inaccurate. Despite this possible problem, however, studies with experimental primates have shown that whole-body irradiation in the range of 550 to 930 roentgens did permit initial successful takes of allogeneic bone marrow in at least 95 per cent of cases (116). This is in the same dose range as for similar experiments in mice, and would therefore seem to indicate that extrapolation from animals to man, at least for this type of immune response, may not be subject to any wide errors.

304. Clearly we need more direct data on the radiosensitivity of human immune responses before it can be concluded that the wealth of experimental animal work can be safely extrapolated for estimates of human risk. Such basic data on the radio-sensitivity of the various components of the immune system could be obtained from *in vitro* studies of antigen processing, of lymphocyte-mediated cellular immunity, and of antibody induction and proliferation. With this as a basis, it might then be more practical to consider other possible factors that might modify direct extrapolation, such as nature of antigen (primary or secondary response depending on previous cross antigenic experience), dose rate, etc.

305. Despite some uncertainty at present on this matter, it should be stressed that in many radio-therapeutic studies such as with Hodgkin's disease, and for eradication of leukæmia followed by marrow transplantation, the benefit to the patient of successful radiotherapy (and marrow transplantation if feasible) may well outweigh the perhaps minor but uncertain risks in regard to long-term effects of radiation on the immune response.

VIII. Summary and conclusions

A. Proposals for further investigation

306. There is virtually no well-controlled careful assessment of the radio-sensitivity of the antibody response in man. As this may be extremely relevant to certain aspects of the problem of radiation and neoplasia, some effort should be made to ascertain radiation sensitivity *in vitro* of the different human lymphoid cells involved in different stages of immune responses, with due consideration to effects of different forms of radiation, dosage, and dose rate.

307. It is clear from the many experimental studies that assessment of the true radio-sensitivity of the cells involved in antibody formation *in vivo* may be complicated by many factors. These include differential sensitivities of the various components, for example, macrophages *B* and *T*, lymphocytes and plasma cells, and differential effects on the catabolism of different antibody proteins. Accurate measurements of the radiosensitivity of the various human lymphoid cells involved in the development of an immune response should be made *in vitro*. With the various cell-separation systems available and the methods at hand for inducing primary-antibody formation *in vitro* and for quantitating numbers of antibody-producing cells, it should be possible to carry out the following determinations: (*a*) *macrophages*: with certain antigens, macrophages are required for the primary induction of antibody formation. Through the use of a macrophage-free test system, assays could be performed on the effect of addition of macrophage preparations which were previously subjected to different radiation exposures; (*b*) *antigen-reactive cells and antibody-forming precursor cells*: in similar fashion, both thymus-derived and bone-marrow (bursa?) derived cells could be separately irradiated at various doses and then recombined with the appropriate unirradiated cell type and studied for ability to collaborate towards antibody production *in vitro*. In man, bone-marrow cells and thymus cells (obtained

from fragments at surgery), could be assayed, together with *T* cells and *B* cells derived from normal human blood and fractionated by some of the immunological techniques now available; and (*c*) *plasma cells*: antibody-plaque-forming cells from any primary induction system with human lymphoid cells could be assayed for radio-sensitivity *in vitro*.

308. In studies with *Shigella* antigen and mice, the radio-sensitivity of the macrophages has been stressed. This type of study in experimental animals should be extended to other antigens, since, if this is a major factor with bacterial antigens, then susceptibility to infection following sublethal irradiation with doses above 100 rads might primarily involve interference with macrophage function. Active immune responses against pathogenic bacteria might then be induced in man by the use of appropriately modified forms of the bacterial antigen (for example, small soluble proteins rather than whole bacteria or flagella) which do not require macrophage processing. This would apply even in situations where irradiation had been previously encountered by the individual.

309. In the field of transplantation there are at least two areas in which radiation can be of considerable value. In organ transplantation, it is clear that immunosuppression from whole-body irradiation with sublethal doses is not feasible. However, both extra-corporeal irradiation of the blood, and local graft irradiation have been shown to be of value, particularly in acute reactions. Further experimental studies with these techniques, preferably in large animals, should be continued, and in particular should evaluate alternative schedules of irradiation. The use of other techniques, such as intralymphatic injection of radio-active colloids in suppression of allografts, also need further evaluation, with much emphasis on a more accurate determination of doses received by various cells and tissues.

310. In marrow transplantation, several promising approaches to the elimination of the secondary disease syndrome must be actively pursued if elimination of leukæmic cells by radiation, followed by marrow transplantation, is to be a practical form of therapy. Such approaches include comprehensive multifactorial studies, fractionation of immunocompetent cells from hæmatopoietic stem cells, and elimination of immunocompetent cells by appropriate anti-serum pretreatment. The possibility also exists that host thymic factors (? epithelial in origin) are involved in inducing differentiation of donor marrow stem cells into immunocompetence. This host component might be vulnerable to radiation suppression and thereby result in a depression of the initiation of secondary disease. Further studies of this phenomenon are needed to determine whether this could lead to a practical approach to the elimination of secondary disease.

311. If the concept of immunological surveillance is applicable to most forms of cancer, it might be expected that irradiated individuals would show an increased susceptibility to all types of cancer, approximately in proportion to their normal incidence although, if serum blocking factors are antibodies, then depression of this factor could result in an effective increased efficiency of cell-mediated tumour regression. These aspects are however quite speculative at present, as the original observation of an increased incidence of reticulo-endothelial and lymphoid tumours that occur in immunosuppressed kidney transplant patients is not observed in immunosuppressed auto-immune patients. However, as there is now available a considerable amount of background knowledge (480) on production of antibodies in man to defined antigens under controlled circumstances, a careful examination of various groups of irradiated subjects for antibody production might therefore be undertaken. This should include patients receiving intralymphatic radio-isotopes, as well as all known survivors previously subjected to whole-body irradiation. The basic question is whether, if immunodepression were demonstrated, this result would help in assessing the probability or risk of subsequent tumour development. Studies on cell-mediated immunocompetence would perhaps be of even greater value.

312. The concept of loss of the tolerant state after irradiation leads naturally to a consideration of possible auto-immune consequences. If the normal absence of anti-self reactivity is due to a continuing equilibrium between a potential aggressor cell and the right balance of self-antigen (perhaps in combination with natural antibody), then an alteration in the equilibrium might lead to antibody production as a result of radiation effects on the population of lymphoid cells. As few relevant data are available, further examinations of the sera of surviving patients who have received whole-body irradiation (from atomic bombings or, for example, from treatment for ankylosing spondylitis) for various auto-antibody levels will be of great interest. Again, there is considerable background information available on the incidence of various auto-antibodies in normal subjects of differing ages (623). It already appears that incidence of auto-antibody formation increases with age, and whether this is indeed a part of the ageing process itself is of great interest. Particularly as radiation is also thought to have some accelerating effects on the ageing process, auto-antibody incidence and titre estimations would be of considerable value. If radiation-induced life-span shortening is associated with, or mediated by, effects on the immune system, then it is likely that increased manifestations of auto-immunity may occur predominantly in the sub-population of those exposed to radiation in young life. Accordingly, more intensive surveys for cellular as well as humoral auto-immune activities would be most warranted in exposed individuals.

313. The potential involvement of radiation in immunotherapy of neoplasia is of great interest. Several relatively new approaches are available, which will require extensive evaluation. If a tumour gives rise to an impairment of the host's immune response to its own tumour antigens, a drastic immunogenic stimulus will be required to overcome this state. This might well be aided by a break of the tolerant state brought about by sublethal irradiation of the recipient, followed by administration of a sample of the autologous tumour pre-irradiated *in vitro* in order to stimulate additional lymphoid elements. This type of approach should be monitored not only by examination of the growth of the primary tumour but also by attempting to directly assess the state of anti-tumour immunity (cellular and humoral) of the host by the various *in vitro* assays which are currently under development in several laboratories.

314. Eradication of tumour cells might also be approached by the use of tumour-specific antibodies which would localize on the tumour-cell surface. If the antibodies were heavily labelled with a radio-

isotope, a lethal radiation dose to the cell might thereby be delivered. However, the major problem may rather be in effectively contacting tumour cells in a solid tumour with the antibodies.

B. Radiation, resistance to infection, and antibody formation

315. One of the best illustrations of the injurious effects of ionizing radiations on immunity is that showing decreased resistance of irradiated animals (usually in the 200-600-R exposure range) to the pathogens of infectious diseases. This has been demonstrated countless numbers of times with many different pathogens of bacterial, viral, rickettsial and fungal types. In general, species resistance is maintained after irradiation, although in some studies there are examples of partial elimination of congenital resistance to certain infectious agents.

316. Decreased resistance to infection varies considerably for different infections, species, types of infections (acute or chronic) and radiation parameters. Part of this variation may depend on the type of assay system, for example it appears that radiation-induced decreased resistance to infection occurs primarily after several days, rather than immediately, and challenge with a very acute infectious agent at the time of radiation will not show any decrease in resistance. Challenge with an agent that induces a more chronic and prolonged infection will be more likely to show decreased resistance.

317. Radiation-induced decreased resistance to infection is primarily mediated by the decrease in the host's specific immune response, although other non-specific factors may also be of importance, particularly macrophage handling of antigen and granulocyte functions. As this review is primarily concerned with the immune response, evaluation of the susceptibility of its components cannot be very readily ascertained by simple whole-animal studies with challenge of infectious agents. Accordingly, a more detailed examination of the separate components of immunity has been made.

318. Phagocytosis of antigens and antigen degradation are relatively radio-resistant with doses of the order of 1,000 rads. Some changes in granulocyte activities have been reported even with relatively low doses (100 rads) but the significance of this for the eventual immune response is probably minor. In several studies, however, although irradiated macrophages successfully phagocytosed antigen, they did not appear to be capable of processing it in a manner which is obligatory for the initiation of the immune response. This effect was observed even with doses of 150 rads. Antigen-handling in lymphoid follicles appears to be particularly important for the development of the secondary response and radiation inhibition of this function may be a factor in antibody depression.

319. Depending upon dose, dose rate and time of irradiation relative to antigen injection, the immune response may show either a shortened lag phase and higher antibody levels (particularly with relatively low doses), or a lengthened lag phase and reduced antibody response. This increase in the antibody response appears whenever the radiation dose is low enough (observed with 25 rads) and antigenic stimulation delayed enough to allow the steady-state population of precursor cells to recover. The radio-sensitivity of the hæmatopoietic stem cells and the precursor immunocompetent cells, as indicated by their D_{37} values, is in the range of 60 to 150 rads. It is during the lag phase of the immune response that cell co-operation appears to occur in some antibody responses, and although the actual precursor cell of the antibody producer is most sensitive, conflicting data on the thymic-derived cell (T) exist. It appears that in primary responses, if the T cell must proliferate to produce sufficient numbers for collaboration, then it will appear radio-sensitive, whereas in its carrier function in secondary responses proliferation may not be as important, and radiation in doses of up to 2,000 rads does not seem to interfere with this function.

320. The logarithmic phase of antibody production is only moderately radio-sensitive, because of the mixture of both proliferating immature plasmablasts and the highly radio-resistant mature non-dividing antibody-synthesizing cells. Finally, no significant depression of antibody secretion is observed in the populations of cells irradiated (with doses up to several thousand rads) in late logarithmic, plateau and decline phases, most of which are mature plasma cells.

321. The secondary antibody response has often been described as radio-resistant in studies which assess the over-all antibody production in whole animals. However, many of the differences between the primary and secondary responses can be accounted for by the numbers of potentially available cells which can be called upon for the particular immune response. Thus a comparable reduction by radiation in the percentage of cells in a primary and secondary response will still leave in absolute numbers many more surviving cells in the secondarily-stimulated animal.

C. Radiation and transplantation

322. Although it is clear that the lymphocyte population which is involved in cellular immunity is *in general* relatively radio-sensitive, there are few direct assessments of the actual radio-sensitivity of all components involved in graft rejection. Furthermore, there are indications that some of the lymphocytes that mediate cytotoxic cellular immune responses are relatively radio-resistant.

323. It is now quite definite that prolongation of foreign organ grafts cannot be obtained solely by whole-body irradiation of the recipient, at least without using lethal doses of radiation, which would then require simultaneous marrow transplantation. Local irradiation of the graft *in situ* (e.g., kidney), usually administered in fractionated doses, has been used as giving some definite advantage for graft survival in the early stages. Indiscriminate use of radiation may, however, be of considerable disadvantage since, if radiation is administered in a form such that radiation-induced augmentation of the immune response occurs, this might lead to accelerated graft rejection, which might also negate any further attempts at transplantation.

324. Various other methods of irradiation have been attempted in order to suppress the recirculating pool of lymphocytes which is of prime importance in graft rejection. These include extracorporeal irradiation of the blood (ECIB), intravascular insertion of radio-active implants, intralymphatic injection of radio-active colloids, and application of a radio-active strip to the

surface of the spleen. As these latter approaches are mainly experimental and suffer from some problems of irradiation of other tissues, and because it is very difficult to calculate doses at various points in the body, further investigations are needed before these techniques can be used extensively in man. ECIB, however, has been found to be of advantage with renal allografts, particularly in acute rejection crises. The combined use of radiation therapy and other immunosuppressive régimes (drugs, anti-lymphocyte serum) often leads to considerably greater immunosuppression and graft prolongation.

325. Lethal doses of whole-body radiation have been attempted only in bone-marrow transplantation. If these patients could indeed be restored by marrow transplants, then a direct application of this technique to leukæmia might be possible. Although it is by no means certain that there is a systemic factor involved in the induction of the malignancy, it must be pointed out that if this did exist, donor-derived marrow cells could eventually become malignant and the major problem of marrow transplantation would indeed not be the immunological problem. However, there is a major immunological obstacle to marrow transplantation in man, namely, the graft-*versus*-host reaction (secondary disease) which is now clinically well documented. Initial takes of marrow appear to be satisfactory, indicating that irradiation has prevented early host rejection. The key immunological problem now is to prevent the subsequent secondary disease complications, and several recent promising reports in this field have been published, although care must be taken in extrapolating from animal graft-*versus*-host reactions, as the content of *T* cells in marrow clearly differs from species to species.

D. Radiation, tolerance and cancer

326. In considering the interaction between a developing primary tumour and the antagonistic host immune response, it is likely that any factors which affect the host immune response may alter the current balance, and tend to favour or inhibit the growth of the tumour, again depending in turn on the relative proportions of cell-mediated immunity, humoral immunity and blocking factors. Experimental studies have demonstrated that immune suppression will lead to an increase in the growth rate of transplanted tumours, and will permit greater and more rapid metastatic spread of tumours. This in turn affects the time of observation of macroscopic tumour development and the survival of the host. Whether immune suppression actually leads to an increase in the number of primary malignant clones is not yet clear. It is on this critical point that the concept of immune surveillance rests, and further work is still needed, particularly with spontaneous primary tumours, rather than the more highly antigenic viral induced tumours. Alternatively, however, if the case for human virus induced tumours becomes fully established, the role of immune depression in the ætiology of tumours must be carefully evaluated.

327. Radiation-induced augmentation of immune tolerance has been demonstrated with various systems. In the field of transplantation, non-lethal doses (300-500 rad) have been used in this manner to facilitate the induction of skin-graft tolerance to weak histocompatibility antigens. On the other hand, a state of persisting immunological tolerance can be broken by radiation and converted into an active immune response. This may well have relevance to certain types of tumour induction.

328. If radiation can break the state of induced immunological tolerance, it may also do so with self-tolerance. This could lead to the development of auto-immune disease. Experimental or clinical information on this aspect is very sparse, and limited studies with atomic-bomb survivors have not at present indicated any increased incidence of auto-antibodies in this population. However, it is also quite probable that such increased incidence may only be observed in that sub-population of individuals who were exposed at a young age, and further studies on this aspect should continue.

329. In conclusion, it must be emphasized that accurate determinations of the radio-sensitivities of the various cell types involved in immune responses in man are not at present available. It appears reasonable to extrapolate from animal studies in relation to the actual radio-sensitivities of the cell types, although it is important to note that the expression of an immune response frequently involves an interaction of cell types, and that the proportions of these cells in the tissues of man may not be the same as in other species. Even less is known in man about the possible role of radiation effects on immunity in relation to cancer and auto-immune disease.

330. These considerations indicate that accurate risk estimates for man on the effect of radiation on immune responses cannot at present be made. Further studies on the radio-sensitivity of individual cell types and long-term studies on immunological changes in relation to cancer and auto-immunity may eventually lead to the realistic assessment of these risks.

TABLE 1. CLASSIFICATION OF IMMUNE RESPONSES

	Humoral-antibody formation		*Cellular immunity*	
Reactive cells ...	Plasma cells and *B* lymphocytes		*T* lymphocytes	
Ontogenic control of differentiation	Bursa of Fabricius (avian), bursal equivalent (mammal) *and* thymus		Thymus	
Primary mediator of measured immune response .	Antibody-secreted immunoglobulin		Lymphoid cells (possibly through cell-surface-bound immunoglobulin) and macrophages	
Secondary mediators of response	Complement components, histamine, serotonin, SRS		Migration inhibition factor Transfer factors Lymphotoxin, etc.	
Clinical and experimental forms of immunity ...	Serum antibody IgM, IgA, IgG etc.	Immediate hypersensitivity Anaphylactic / Arthus type reaginic	Transplantation immunity	Delayed hypersensitivity

TABLE 2. ANTIBODY PRODUCTION BY 550 R IRRADIATED MICE FOLLOWING INOCULATION OF MACROPHAGES FROM NORMAL AND IRRADIATED DONORS INCUBATED WITH *Shigella* ANTIGEN (177)

Radiation exposure to macrophage donors[a] *(R)*	*Treatment of recipients*	*Agglutinin titre* 5 days		8 days	
		Number of animals	log_2	*Number of animals*	log_2
0	Macrophages[b]	20	2.0	47	5.7
150	Macrophages	15	0.5	34	2.8
300	Macrophages	15	0.4	31	2.2
450	Macrophages			12	1.5
600	Macrophages			12	0.8
750	Macrophages	5	0	10	0.8
	Shigella (0.1 ml of 0.1 per cent suspension)	12	0.5	36	0.8

[a] Animals were exposed to x rays two days after thioglycolate injection. Two days later the peritoneal cells were harvested.

[b] 15 10^6 macrophages incubated *in vitro* with *Shigella* antigen.

TABLE 3. THE EFFECT OF THE TIME OF ANTIGEN INJECTION ON THE IMMUNE RESPONSE TO SHEEP ERYTHROCYTES OF SUBLETHALLY IRRADIATED MICE: PLAQUE FORMATION IN THE SPLEEN AS A FRACTION OF THE CONTROL[a]

(*95 per cent confidence limits*)

Irradiation dose (rads)	*Antigen 1 hour before irradiation*	*Antigen 1 hour after irradiation*	*Antigen 24 hours after irradiation*
50	1.645 (1.354-1.930)	1.698 (1.390-1.956)	0.774 (0.617-0.916)
100	0.914 (0.751-1.087)	1.086 (0.900-1.282)	0.533 (0.417-0.623)
200	0.600 (0.408-0.729)	0.697 (0.564-0.837)	0.142 (0.102-0.185)
300	0.133 (0.095-0.159)	0.107 (0.079-0.139)	0.032 (0.026-0.038)

[a] Plaque formation determined 3 days after antigen injection.

TABLE 4. EXPECTED RESULTS ON THE EFFECT OF TOTAL-BODY X-IRRADIATION ON PRIMARY (1°) AND SECONDARY (2°) ANTIBODY RESPONSES BASED ON THE POPULATION-DENSITY FEED-BACK-CONTROL THEORY[a] (336)

X-ray exposure (R)	*Relative suppression[b] (survival percentage)*	*Number of surviving immunocompetent units[c]*		*Number of immunocompetent units differentiating into antibody-synthesizing cells*		*Responses (per cent of normal)*	
		1°	*2°*	*1°*	*2°*	*1°*	*2°*
0	0	100	4,000	100	200	100	100
100	65	65	2,600	65	200	65	100
200	18	18	720	18	200	18	100
300	5	5	200	5	200	5	100
400	1.5	1.5	60	1.5	60	1.5	30
500	0.3	<1	12	<1	12	<1	6

[a] It is assumed that the ratio of immunological expression to immunological potential is 1.0 in a primary antibody response and 0.05 in a secondary antibody response. This is based on the following two observations: (*a*) there can be as many as 10 to 100 times more immunocompetent units responsive to an antigen in the spleens of maximally primed mice than in those of nonprimed mice, and (*b*) the difference between primary and secondary antibody responses against foreign red blood cells can be as small as twofold.

[b] Data obtained from Makinodan, Kastenbaum and Peterson (333).

[c] For convenience it is assumed that there are 100 immunocompetent units in nonprimed individuals. If so, there should be 4,000 immunocompetent units in maximally primed individuals.

TABLE 5. RESULTS OF X-RAY THERAPY IN HODGKIN'S DISEASE (279)

X-ray therapy and stage of disease	*Total number of patients*	*Number of deaths*		*Patients continuously free of disease*	
		With disease	*Without disease*	*Number*	*Duration (months)*
IB and IIB					
Limited fields	10	4	0	2	
Extended fields	10	0	1	7	
IIIA					
1,500 R	6	2	0	3	30,39,43
3,500-4,000 R	5	1	0	4	14,24,43,47
IIIB					
1,500 R	9	5	0	0	
3,500-4,000 R	17	7	1	7	15,15,15,22 23,25,51

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Annex G

EXPERIMENTAL INDUCTION OF NEOPLASMS BY RADIATION

CONTENTS

Introduction

1. From the time of the initial observations that radiation causes tissue damage and subsequently cancer (66, 67), animal experiments have been designed and carried out (119, 120) in an attempt to understand better the mechanisms of radiation-induced injury and predict the carcinogenic effects of radiation in man. The older literature has been reviewed by Colwell and Russ (28), Furth and Lorenz (53), Furth and Upton (54), and Lacassagne (101, 102). More recently, the Committee has reviewed the subject (182) and reviews have been published by Casarett (19), Moskalev and Streltsova (135), Upton (185, 187) and Van Cleave (201).

2. It is not the purpose of this annex to review exhaustively the literature but rather to evaluate the present understanding of some of the basic principles of experimental radiation carcinogenesis which could lead to a better predictability of radiation effects in man. Direct assessments of the reliability of existing standards for radiation protection in man will not be made, but the review will indicate what useful data exist or could be developed from experiments on animals. It is recognized that radiation is but one of several important ætiological agents known to induce or accelerate neoplasms in man and while many of the principles discussed in this review are of general significance to carcinogenesis, studies on viral and chemical carcinogenesis have in general not been reviewed.

I. The role of animal experiments in predicting radiation carcinogenesis in man

3. While medicine has frequently turned to animal experiments for assistance in solving problems of human disease, the validity of animal models for studying human disease has often been questioned. It is well known that inherent differences exist among the many neoplasms in experimental animals used in the study of radiation carcinogenesis. Not only are the life spans and tumour latencies different, but the type of tumours induced or accelerated by radiation vary markedly from species to species and even from strain to strain of the same species. Such variability leads to considerable difficulty in extending conclusions from experimental animals to man. Nonetheless, the development of neoplasms appears to be qualitatively similar in man and in those experimental animals that have been studied. Thus, the epidemiological data on radiation carcinogenesis in man, when evaluated in the light of conclusions developed from experimental studies, can lead to a better understanding of the risk to man from exposure to radiation. There are three basic methods for developing experimental data which will be useful for evaluation of such risks in man; these are outlined in paragraphs 4-7.

4. The most useful method entails the study of neoplasms in experimental animals that respond to radiation in a manner qualitatively and quantitatively comparable to their counterpart in man. Unfortunately, directly comparable neoplasms are not well documented in radiation carcinogenesis since considerable amounts of data are required from humans to validate the authenticity of the animal data. Perhaps the best documented model system is osteosarcoma induction by ^{226}Ra in beagle dogs (40, 122) but even here considerable uncertainty is involved in producing quantitative inferences applicable to man. Another animal model system is radiation-induced myelogenous leukæmia in RF mice (192), which has many of the qualitative characteristics of human chronic granulocytic leukæmia. No quantitative relations have been demonstrated and some qualitative characteristics of the murine disease, such as its dependence on the microbial environment (209), have not yet been documented in man.

5. Another method of developing experimental data which are of value in estimating the risk of radiation to man is the establishment of useful generalizations as described by Mole (134). Qualitative generalizations are particularly useful when all types of neoplastic diseases show the same patterns in all species. There are three such generalizations apparent from existing data, although some exceptions have been noted in the literature: high-LET radiations (that is, those radiations which deposit large amounts of energy per unit length of track, e.g., fission neutrons, alpha particles) are more effective in inducing neoplasms than low-LET radiations (e.g., x rays, gamma rays); the incidence of such neoplasms generally increases with dose up to some maximum incidence; and for low-LET radiations, lower dose rates are less effective in inducing neoplasms than higher dose rates. These generalizations appear independent of species and hold true for most, if not all, of the radiation-induced neoplasms that have been adequately studied.

6. Quantitative generalizations are not as easily apparent and none are immediately obvious from experimental animal data. Measurements of RBE (Relative Biological Effectiveness, the inverse ratio of the absorbed dose from one radiation type to that of a reference radiation required to produce the same degree of a stipulated effect) vary considerably; the reduced effectiveness of low-dose-rate radiation is also variable; and dose-effect relations are not only quantitatively different but the shapes of the dose-response curves appear to vary from one neoplasm to another. Such quantitative generalizations require either that the characteristic measured not vary with species and type of neoplasm or that it vary according to some fixed relation with some other quantifiable variable such as body weight, metabolic rate, rate of synthesis of DNA, etc. Were such a relationship established, the neoplastic response in man could be predicted by relating it to the non-neoplastic response. Thus every attempt should be made to develop such quantitative relationships.

7. Perhaps the most useful application of experimental animal data is to clarify the mechanisms of radiation carcinogenesis. As early as 10 years ago, currently popular theories on the mechanisms of radiation carcinogenesis were well developed and discussions about how to clarify their relative importance were common (see discussions in reference 69). Although considerable effort has been directed to the problem over the last decade, the mechanisms of radiation carcinogenesis remain obscure. The design, execution, and analysis of definitive studies on radiation carcinogenesis have proved to be extremely difficult. To be conclusive, many of these studies require large numbers of animals which must be examined minutely for pathology at death and the results analysed statistically to adjust for competing probabilities of other causes of death (see paragraph 22). Frequently, scientists designing animal studies with large sample sizes use only survival as an end-point and those carefully analysing pathology at death design experiments with inadequate sample sizes. Few have properly corrected the data for intercurrent mortality before drawing conclusions. However, when combined, consistent reports of an effect obtained with small sample sizes can give weight to useful qualitative generalizations.

8. One of the principal general theories of carcinogenesis is the two-step mechanism proposed by Berenblum (6). Such a mechanism has been considered for chemical carcinogenesis for many years, but only recently have data begun to suggest a similar mechanism for radiation carcinogenesis. Croton oil, a potent promoter of chemically-induced skin tumours, effectively enhances the induction of skin tumours by some (44, 176), but not all (16), types of radiation. It has also been identified as a promoter of murine thymic lymphosarcoma following exposure to moderate doses of x rays (86). The enhancement of radiation-induced thymic lymphosarcoma in mice treated with urethan has been reviewed by Vesselinovitch (205). Berenblum *et al.* (7) have recently shown that x rays, in doses not normally leukæmogenic, serve as an initiating treatment when followed by adequate doses of the promoter urethan, the increase in effect being generally related to increase in radiation dose. A similar initiating effect of radiation has been described for production of lung tumours in mice treated with urethan (23, 108, 227). Interactions of radiation with other carcinogens have resulted either in an increased incidence of tumours (75, 109, 136, 169, 200, 224, 232, 236), or in no change, or in decreased incidence (99, 167, 200, 224), depending on the carcinogen and tumour system studied and the dose and dosage schedule used.

9. Another general theory of interest to the mechanism of radiation carcinogenesis involves the interaction of energy at the intracellular level. Through a series of theoretical considerations and examination of experimental data, Rossi (160) and Kellerer and Rossi (98) have concluded that a number of radiobiological effects are due to primary lesions within the cell nucleus produced by as few as one particle, in the case of neutrons having energies up to about 14 MeV, or by two or more electrons, in the case of x and gamma rays, and, further, that the elemental lesion results from dual damage within a site by inactivation of two loci. These conclusions would predict a much greater reduction in effect at low doses for x and gamma rays than for neutrons, and an RBE that increases inversely with x-ray dose as has been demonstrated for breast tumours in rats (161) (see paragraphs 35 and 41). It is not clear how such events relate to the general mechanisms of radiation carcinogenesis discussed above (paragraph 8) but the implications for dose-effect relationships and RBE for radiation-induced neoplasms must be considered.

10. Theories of the mechanisms of radiation carcinogenesis which best fit the existing data involve both initiating and promoting events. Initiating mechanisms concern immediate events occurring during the interaction of radiation with cellular macromolecules. The principal initiating mechanisms are release or activation of oncogenic virus and induction of somatic mutations, mechanisms which may be, but are not necessarily, exclusive. The principal promoting mechanisms are increase in cell replication and decrease in immune competence, both of which may be operating concurrently but independently.

11. The role of leukæmogenic viruses in radiation-induced murine leukæmia has been reviewed repeatedly (42, 95, 113, 188) and has been thoroughly discussed by the participants of the 1966 Conference on Murine Leukæmia (29). The early work of Kaplan with C57BL thymic lymphosarcoma and of Upton with RF myeloid leukæmia clearly established a viral mechanism as one of the important factors in the induction of

murine leukæmias by radiation. The significance of numerous host and environmental factors (193) in the development of murine leukæmia suggests the importance of viral interaction with a number of other factors. Strain susceptibility in mice may be more closely related to factors other than virus release, since the release of C-type particles has been demonstrated in resistant strains of mice after x-irradiation at doses which do not induce significant amounts of leukæmia (64). An infectious virus capable of inducing osteogenic sarcoma in mice has been isolated (49), suggesting that there may also be a relationship between radiation-induced osteosarcoma and virus release. On the other hand, ^{90}Sr-induced osteosarcomas of CBA mice have few demonstrable virus particles (145, 181) and low antigenicity (147), suggesting that oncogenic virus may not always be involved in the ætiology of these tumours. The importance of virus activation and release for other radiation-induced neoplasms has not been identified and visible viruses ("C-type particles") have not yet been demonstrated after irradiation or in neoplastic tissue of germ-free rats, although germ-free mice have an abundance of such particles (92). Young adult rats are susceptible to leukæmia induction following radiation if injected with rat-adapted passage-A Gross virus but not if treated with radiation or the virus alone (216).

12. Radiation carcinogenesis has also been explained on the basis of radiation damage to nucleic acids and its effect on information contained in the genetic material of the cell (45, 68). The relationship of such somatic mutations to radiation-induction of neoplasms has been recently reviewed by the Committee (183). In summary, the support for this theory derives from the observation that radiation induces both chromosome aberrations and neoplasms and that chromosome changes have been demonstrated in most of the tumours studied. There are few or no quantitative data that would permit correlation of incidence of chromosomal abnormalities (or gene mutations) with incidence of neoplasia following exposure to radiation. Moreover, such a direct quantitative relationship may never be found if the complex, multi-step, pathogenesis suggested for radiation-induced diseases is correct (25, 34).

13. Interest in the immunological reactions associated with neoplasia has been heightened by the discovery of tumour-specific antigens (i.e., surface antigens not present on normal cells of the adult host). Cells carrying these antigens are capable of stimulating an immune response. These observations have led to the concept of immunological surveillance (see annex F, paragraph 247), which proposes a continuing eradication of emerging, potentially neoplastic cells. A thorough review of the current status of the relation of immunity and tolerance in oncogenesis has been published in the Proceedings of the IV Perugia Quadrennial International Conference on Cancer (168). Recent data on tumour-specific antigens and their relation to immunotherapy of cancer have also been reviewed (2).

14. The relation between radiation-induced immunosuppression and radiation-induced neoplasia is reviewed by the Committee in annex F of the present report. There is abundant evidence to show that immunosuppression is not the sole factor in radiation carcinogenesis and the relative contribution of immune suppression to the complex chain of events resulting in radiation-induced neoplasms has not been determined. As with viral and chemical inducers of neoplasia, radiation results in a transient immunosuppression during what is thought to be a critical period in development of neoplastic cells. The selection of radiation-induced murine leukæmia to study this hypothesis may have been unfortunate since the neoplasm arises out of the same tissue that produces antibodies. Increased cell proliferation of the lymphopoietic organs follows radiation-induced immunosuppression and the importance of this to radiation leukæmogenesis has been reviewed (95) (see paragraph 16). The relative importance of these two mechanisms cannot be determined with this model system.

15. Indirect evidence supporting the importance of immunosuppression has been reviewed by Cole and Nowell (26) and includes such general phenomena as immunosuppression induced by carcinogens, including radiation; increased tumour incidence following immunosuppression; and increased ease of transplanting tumours in immunosuppressed animals. It is interesting that the principal neoplasm observed in immunologic deficiency states in experimental animals (26, 100) and man (151) is lymphoma and that the greatest increase of all types of tumours following immunosuppression for organ transplantation in man is reticulum-cell sarcoma (151). Nonetheless, it has been clearly demonstrated that immunosuppression by radiation and other treatments permits more rapid growth of neoplastic cells, results in more metastases, and permits transplantation across weak histocompatibility barriers (2, 26, 100, 168; see also this report, annex F). Therefore, depending on the antigenicity of radiation-induced tumours, immunosuppression may play a significant role in the rate of growth and rate of metastasis of such tumours, and may determine the tumour latency and final incidence, and the rate of survival of irradiated animals.

16. Another important promoting mechanism of radiation-induced neoplasia is increase in cellular proliferation. Some aspects of the relation between cellular proliferation and neoplasia have been reviewed by Reiskin (156). In summary, numerous chemical carcinogens cause a reduction in DNA synthesis and cell division, followed by recovery characterized by increased cell division. Mean duration of the DNA synthetic period remains normal but the number of cells synthesizing DNA increases. These observations are further supported by the generalization that rapidly proliferating tissues are more responsive to carcinogens than their more slowly proliferating counterparts. There are some tissues which are not covered by this generalization, and the exceptional behaviour of some of them, such as intestinal epithelium, may be explained by loss of potentially neoplastic cells through physiological mechanisms (110). Treatments which induce cell proliferation frequently increase the incidence of neoplasms in irradiated tissue as well. Data on the following radiation-induced tumours support the importance of cell proliferation for expression of radiation-induced neoplasms: mammary-gland neoplasms of the rat (52); bone tumours in the shaft of fractured long bones in some (225, 226) but not all cases (59); ^{90}Sr-induced bone tumours in mice following estrogen treatment (144); thyroid tumours in rats following goiterogen treatment (39, 109); thymic lymphoma alone (95, 166) and following urethan treatment (7); kidney cortical adenomas following uninephrectomy (27) and necrotizing doses

of x rays (117); lung tumours following urethan (8) and following exposure to radon in the presence of irritating dusts (21, 152); hepatomas following injection of carbon tetrachloride (24); liver tumours following hepatic deposition of thorotrast (175); and stomach tumours following necrotizing doses of x rays (71).

17. Attempts to clarify the relative importance of these various hypothetical mechanisms have included studies with chemical and cellular protection against the late somatic effects of radiation. Most of these experiments (13, 73, 103, 116, 127) have given conflicting results largely because of small sample sizes, inadequate description of tumour sites and lack of correction for causes of competing mortality. The data, however, do show that isogenic bone marrow is highly effective at preventing radiation-induced thymic lymphoma (97) but less effective at preventing the development of other late-occurring tumours (31). Chemical protective agents are also able to reduce the incidence of radiation-induced thymic lymphoma following single exposures to x radiation (115, 233) and of other leukæmias with fractionated radiation (139). Chemical protection does not appear to be effective against the induction of non-reticular tumours resulting from whole-body exposure to radiation but interpretation of these data is obscured by competing probabilities of other causes of death because these tumours occur later in life. When local irradiation is used, however, there appears to be protection against radiation-induction of kidney (43) and breast tumours (174). More data will be required, however, before experiments of this sort are able to clarify the mechanism of radiation carcinogenesis.

18. In summary, it seems quite possible that all the mechanisms identified above play some role in radiation carcinogenesis, but the relative contribution has not been assessed and may vary from case to case.

II. Importance of radiation carcinogenesis for life-shortening effects of radiation

19. The general principle that radiation-induced life shortening is due not to the induction of specific diseases but to the advancement in time of all causes of death is derived from analysis of two large, carefully designed and executed, studies on the late somatic effects of radiation in mice (105, 106, 194) where the mean age at death for every disease was reduced by x-irradiation. Corrections for intercurrent mortality were made for some of the data (percentage incidence of some specific diseases) but adjustments for mean age at death of the corrected data were not presented. It has been suggested that this consistently-reduced mean age at death for all diseases is a statistical artifact (see paragraph 22), and the importance of serial killing to provide end-points free from alterations due to survival has been emphasized (3).

20. Preliminary analysis of the survival of RFM male mice exposed to 300 roentgens of x rays at 5-6 weeks of age suggests that virtually all the life-shortening effects of the x-irradiation can be ascribed to induction of neoplasia (210). In this experiment it was also shown that radiation did not significantly alter the cumulative survival curve for mortality from reticulum-cell sarcoma when the data were properly corrected for mortality from other causes. It was also noted that the mean ages at death for mice dying with this or other non-radiation-induced diseases were not significantly different when corrected data were used for the calculations but were reduced in the irradiated groups when such corrections were not made (208). Although radiation can reduce the life span of animals by inducing non-neoplastic diseases, the greater importance of radiation-induced neoplasms has been noted in animals continuously exposed to neutrons (132) and ^{60}Co-gamma radiation (60) and in animals carrying internally-deposited radio-nuclides (18) when doses (and dose rates) were low. Additional data from larger experiments will be required to verify the observation that specific diseases, principally neoplasms, are responsible for life-shortening after exposure to moderate to low doses of radiation.

III. Statistical analysis of specific disease incidence in survival experiments

21. Failure to analyse properly data from survival experiments seriously weakens the conclusions that can be drawn. The common rules, including clear statement of hypothesis, proper experimental design (particularly, adequate sample sizes), adequate accumulation and recording of data and statistical analysis of the data with particular reference to testing for significance, must be applied before accepting or rejecting the hypothesis on which the experiment is founded. These rules have been particularly difficult to apply to animal survival experiments because of the duration and biological variability inherent in such experiments, but the rules must be applied nevertheless. The use of computer data storage and retrieval systems coupled with statistical analysis and testing by computer (see reference 63, for example) permit easier handling of these complex data.

22. An additional problem in analysis of survival experiments is posed by competing probabilities of other causes of death. It has long been recognized that the final incidences of late-occurring diseases are seriously affected by mortality rates from early-occurring diseases. Despite this, data obtained at necropsy are usually presented as the observed incidence of a specific disease and the mean age at death of animals dying with that disease. Such incidences are also used for computing RBE and dose-reduction factors due to protraction of radiation and for describing dose-response curves despite recommendations to the contrary (47, 132). The extreme variability of these radiation parameters is due in part to the use of such uncorrected data. Various actuarial techniques are available for correction of mortality from a lethal disease (e.g., 35, 93) and should be used. The Committee has based its conclusions almost exclusively on data which have not been obscured by intercurrent mortality. In most cases this has been accomplished by using data corrected for competing risks, data for diseases occurring early in life, such as thymic lymphoma where perturbations from other diseases are relatively minor, or data from serial-sacrifice experiments. Occasionally, uncorrected data for late-occurring tumours are referred to if the mortality patterns for all causes of death are similar in the groups being compared.

23. Another possible source of error in statistical analysis is the use of the actuarial techniques designed for assessing causes of death (paragraph 22) with non-lethal diseases such as benign tumours. Changes in mortality pattern due to lethal diseases can be shown to alter age-specific incidence rates of non-lethal diseases

such as ovarian cysts and small pulmonary adenomas. Use of appropriate statistical techniques for correction of competing risks in the case of both lethal and non-lethal diseases has been described by Hoel and Walburg (72).

IV. Special problems of internal emitters

24. Considerable animal experimentation has been carried out in the last decade in an attempt to predict the effects of internally-deposited radio-isotopes in man. In assessing the experimental data, there are several difficulties in interpretation which must be considered. The principal problem is the determination of dose to the susceptible cell population. Different isotopes localize in different tissues to different extents—depending on route of introduction, species, age, and other physiological and environmental variables. In addition, deposited radio-isotopes are involved in the normal metabolic and replacement mechanisms of the body which proceed at varying rates in different hosts and environments. Without measurement of dose to the tissue at risk it is difficult to determine such quantitative factors as dose-response curves, dose-reduction factors for protracted radiation, and RBE (see reference 18 for discussion). Recent attempts have been made to measure the dose to various regions of an organ (principally bone). Both theoretical considerations (121, 228) and direct measurement of linear path length with packed lithium-fluoride thermoluminescent dosemeters (177) and quantitative auto-radiography (70) have been investigated. Considerably more data on dose distribution of internal emitters and structural characteristics of tissues from experimental animals and man are required before a confident comparison of effects in experimental animals and man can be developed.

25. An additional problem is that the susceptible cells are exposed to continuous irradiation at variable dose rates depending on the replacement or metabolic changes and physical half-life of the radio-isotope studied. The dose-response relationships of radiation-induced neoplasms are often difficult to determine for internal emitters because the dose and dose-rate effects cannot be isolated from one another, although it has been shown in some cases that the total accumulated dose is more important than the initial dose rate (146, 202). It is clear that when an animal is continuously irradiated (externally or internally) until death, the cumulative dose received by a tissue contains a component of "wasted radiation", i.e., radiation in excess of that required to produce the effect being measured (9, 49, 128). In addition, survival time may be affected by the radiation administered during the development of an ultimately fatal pathological process (49, 134). Because of these indeterminates, it becomes essential to define the parameters involved in assessing quantitative radiation factors. For example, the RBE for external whole-body irradiation describes the relative effectiveness of different qualities of radiation. An RBE for internal emitters, on the other hand, depends not only on radiation quality but also on differences in distribution, dose rate, etc. Dose-effect curves and dose-reduction factors are often calculated on the basis of the "mean skeletal dose", but it appears that for osteoblastic osteosarcomas it is the dose delivered to cells on the surface of the bone that is important (see paragraph 28). It would be helpful if the data from experiments on late somatic effects of internal emitters were presented in a uniform manner by different investigators and if more accurate determinations of dose to the tissue at risk could be performed. As will be discussed subsequently, generalizations drawn from data on neoplasms induced by external radiation seem to apply equally well to data on neoplasms induced by internally-deposited radio-nuclides, suggesting that when dosage patterns are comparable to those for external radiation, internal emitters produce similar results (18).

26. An equation relating known experimental data from animals to the observed effects of ^{226}Ra in man has been used to extrapolate data for other internally-deposited radio-nuclides from animals to man. Thus the ratio of the accumulated absorbed doses of radiation from radio-nuclide X to those from ^{226}Ra that give equal effect in an experimental animal is equated to the ratio of the corresponding doses of radiation in man:

$$\frac{\text{dose from X in animal}}{\text{dose from } ^{226}\text{Ra in animal}} = \frac{\text{dose from X in man}}{\text{dose from } ^{226}\text{Ra in man}}$$

Parameters in the experimental animal can be determined, leaving "dose from X in man" as the unknown for which the equation is solved (41, 49). This equation carries the assumption that the ratio of absorbed doses which produce equal effects for different radio-nuclides will be the same in man and in the experimental animal, independent of promoting host factors which might act differentially, an assumption that at present remains unproved.

V. Tissues at risk

27. As noted in a previous Committee report (182), neoplasia is apparently induced if sufficient radiation is administered to almost any tissue. Induction of neoplasms in various tissues has been recently reviewed (187). The most frequently studied tumours induced by whole-body exposure to external radiation are thymic lymphosarcoma and granulocytic leukæmia in mice and tumours of the endocrine system (particularly of the female) including tumours of the breast, pituitary, thyroid, adrenal and ovary. Considerable attention has also been paid to tumours induced by internally-deposited radio-nuclides including bone, lung and liver tumours. Most of these tumours are common after exposure to doses of radiation in the 100-1,000-rad range. Recent data support the concept that even highly-resistant tissues can be stimulated to form neoplasms. Carcinoma of the œsophagus in mice following ^{60}Co wire implantation (56), bronchial adenocarcinomas in rats following high x-ray doses to the lung (65), intestinal neoplasms in mice following whole-body x- and neutron-irradiation (33), ovarian neoplasms in dogs following fractionated x-ray doses (4), and tumours of the central nervous system following implantation of radio-active pellets (88, 220, 221) have been documented. None of the tumours of the central nervous system have arisen from adult neuronal tissue. As previously noted (182), although neoplasms arise most commonly in proliferating tissues, among different tissues no simple relationship between rate of cellular proliferation and tissue sensitivity exists (85). However, for any one tissue, an increase in cell proliferation appears to increase the incidence of radiation-induced neoplasms (see paragraph 16).

28. The extensive literature on tissues at risk of tumour induction by radiation from internal emitters has been repeatedly discussed (10, 18, 109, 123, 135, 229). In general, neoplastic effects depend on the distribution of the radio-nuclide and its radiation energy, susceptible cells within range of the radiation providing the site of origin of the neoplasm. Particular interest has been focused on the tissue of origin of osteogenic sarcomas. The subject has recently been reviewed (84, 204). The conclusion of these reports and of recent experimental data (211, 215) is that the principal cells at risk are the endosteal pre-osteoblasts and/or osteoblasts, although the site varies with the site of osteonecrosis and its resultant bone resorption and osteoblastic activity (78, 226). The clearest description of events preceding development of a grossly observable bone tumour following treatment with ^{90}Sr has been provided by Nilsson (141). The initial event is cell death followed by increased bone resorption and increase in number of osteoblasts, then by an increase in non-neoplastic fibroblastic and osteoblastic proliferation within the resorption cavities or along the endosteal linings and finally by the development of microscopically and then macroscopically-visible osteosarcomas, both fibroblastic and osteoblastic. This pathogenesis demonstrates the importance of localization of radio-nuclides and, for induction of osteosarcomas, explains the greater effectiveness of surface seekers compared to those radio-nuclides that have a more diffuse distribution in bone.

29. The relative importance of leukæmia and osteosarcoma for radio-nuclides deposited in bone has also received considerable attention. There appears to be a dependence on total accumulated dose, quality and dose rate of radiation, as well as a strain and species sensitivity. Radio-nuclides emitting short-range alpha particles are more effective in producing osteosarcomas than leukæmia (57, 84). Single doses of long-range beta emitters, which expose the endosteum to large doses of radiation in short periods of time, principally cause osteosarcomas (11, 76, 111, 126, 141). On the other hand, leukæmoid reactions and myelogenous leukæmia predominate when long-range beta emitters are administered continuously (57, 76, 111, 126, 231) or in single low-dose injections (12, 142), presumably because of the resultant low dose rates. Rats, dogs, and pigs have a high sensitivity to induction of leukæmia. Mice and rabbits, on the other hand, have a low sensitivity (84, 111) although non-thymic lymphoma can be induced by ^{90}Sr in some strains (87, 143). Even mice, which characteristically have a high incidence of myelogenous leukæmia after whole-body x-irradiation, appear refractory to induction of these leukæmias following ^{90}Sr injection (30).

30. A similar interest exists in defining the sensitive cell population in skin-tumour induction. Although there is no correlation between general skin damage and tumour induction for some radiation (81, 154), careful studies have demonstrated a close correlation between damage to the hair follicles and the induction of skin tumours (1, 17). It has also been shown that development of carcinomas in the mucous membranes of the head of mice injected with ^{90}Sr is preceded by enhanced mitotic activity and dysplasia in the *stratum germinativum* of the epidermis (140).

VI. Dose-effect relations

31. Characterization of the dose-response curves for radiation-induced neoplasms is essential for predicting effects of exposure to radiation at levels too low to examine experimentally (see paragraph 39). The theoretical curves which might be expected and cannot be excluded by presently available experimental data are linear, quadratic, sigmoid, or some other with a slowly-increasing response to increasing dose as shown in figure I (85). The shape of a dose-response curve can be altered not only by differences in radiation quality and related experimental variables but especially by the end-point used as the indicator of response. For example, use of final uncorrected incidences of a late-occurring tumour may give a decidedly different dose-response curve from use of incidences of animals carrying the tumour in mid-life, as determined by serial-killing experiments. Attempts to verify one or another of the initiating mechanisms of radiation carcinogenesis by the shape of the dose-response curve do not appear reasonable in the light of the many promoting influences which appear to be operating from the internal and external environment of the experimental animal.

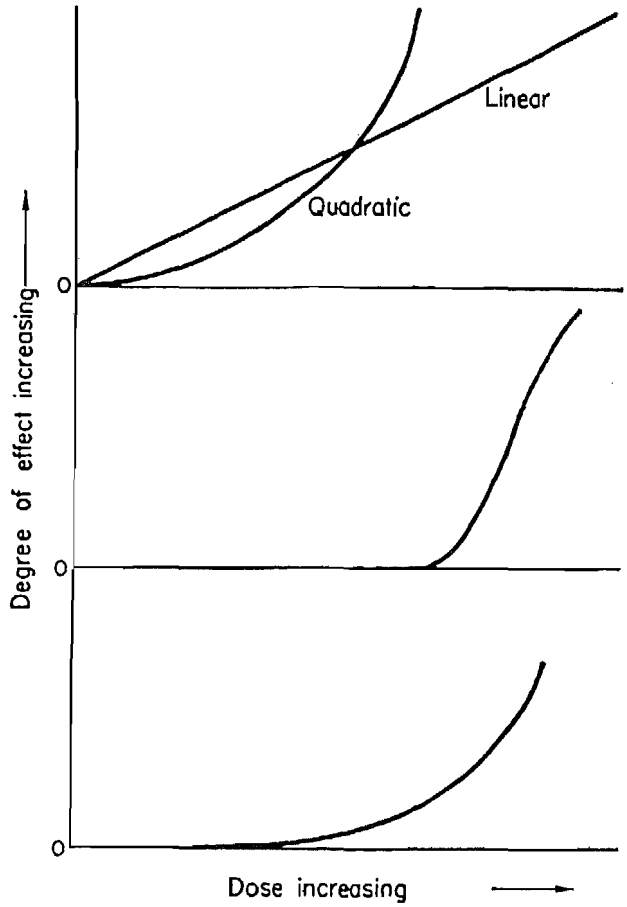

Figure I. Different kinds of dose-response relationship

32. Of particular interest to the selection of appropriate end-points is the question of induction *versus* acceleration. It is generally agreed that if a neoplasm occurs spontaneously with high incidence (e.g., breast tumours in Sprague-Dawley rats), then radiation acts principally by changing the time of onset or latency of the neoplasm. The continuing increase with time in probability of death from a specific neoplasm is often interrupted by death of the animals because of unrelated diseases. Therefore, the final incidence is determined not only by the mortality rate

from the neoplasm of interest but by that from other unrelated diseases as well. Even when neoplasms occur spontaneously late in life with a small but continuously increasing probability, the earlier occurrence and increased incidence of these neoplasms in irradiated populations may be due to acceleration rather than induction. Thus, some measure of acceleration, e.g., the mean age at death corrected for competing probabilities of other causes of death may be a more appropriate end-point than the corresponding final incidence.

33. Whether tumours are induced or are accelerated, one apparent effect of increasing dose is to decrease latency, where this term is defined as "time from application of radiation to observation of neoplastic changes". Such an effect has been noted for rat skin tumours following irradiation with alpha particles and with electrons (17), as well as with fast neutrons and x rays (91); mouse skin tumours after exposure to low-energy beta particles (80); induction of œsophageal cancer in mice following implantation of ^{60}Co wires (212); rat mammary neoplasia following whole-body x-irradiation (172); radiation-induced thymic lymphosarcoma in mice (55, 188); radiation-induced myelogenous leukæmia in mice (192, 234); myelo- and lympho-proliferative diseases of swine following feeding with ^{90}Sr (77); ^{90}Sr-induced bone tumours in mice (141); osteosarcomas induced by alpha-particle radiation in dogs (40) and mice (79); ^{224}Ra-induced ossifying fibromas in mice (59); squamous-cell carcinomas of lung in dogs following inhalation of $^{230}PuO_2$ (150), and skin sarcomas and basal-cell carcinomas in rats following implantation of radioisotope-impregnated Mylar disks (15). When death with a tumour of moderate to long course is the end-point, decreasing latency with increasing dose is not always seen (49). With this end-point, considerable inaccuracy in determination of latency can be expected since the neoplasm originated (and could be observed in radiographs) long before it was observed macroscopically. In all of the examples cited above, except the last, the complications associated with mortality from neoplasms having a long course were not a factor in assessing the latency since determination of early neoplastic growth was made without death of the animal or by serial killing. Where mortality occurred, the course of the neoplasm was short and it appeared early in the life span of the animal.

34. The general character of the dose-response curve consists of an increase in incidence of neoplasms with increasing dose of radiation to a maximum followed by a decline in incidence. The doses at which (*a*) the rise in incidence becomes detectable, (*b*) the maximum incidence is reached, and (*c*) the decline in incidence begins, differ for different neoplasms, species, types of radiation, etc. (figure II). With whole-body irradiation, the limiting factor is often death due to hæmatopoietic failure, and the decline in incidence seen with higher doses can be explained in part by decreased numbers of animals at risk (199). Nonetheless, the fall in incidence of myelogenous leukæmia at higher whole-body doses (192, 234) can in part be explained on the basis of cell killing (62). Certainly there is a decline in incidence of neoplasms with exposure of local areas to high doses of radiation. Such a decline has been seen in the induction of skin (17, 80) and breast tumours (173) by partial-body exposure to radiation from an external source, in the induction of kidney tumours following high doses of radiation to the exteriorized kidney (118) and in the

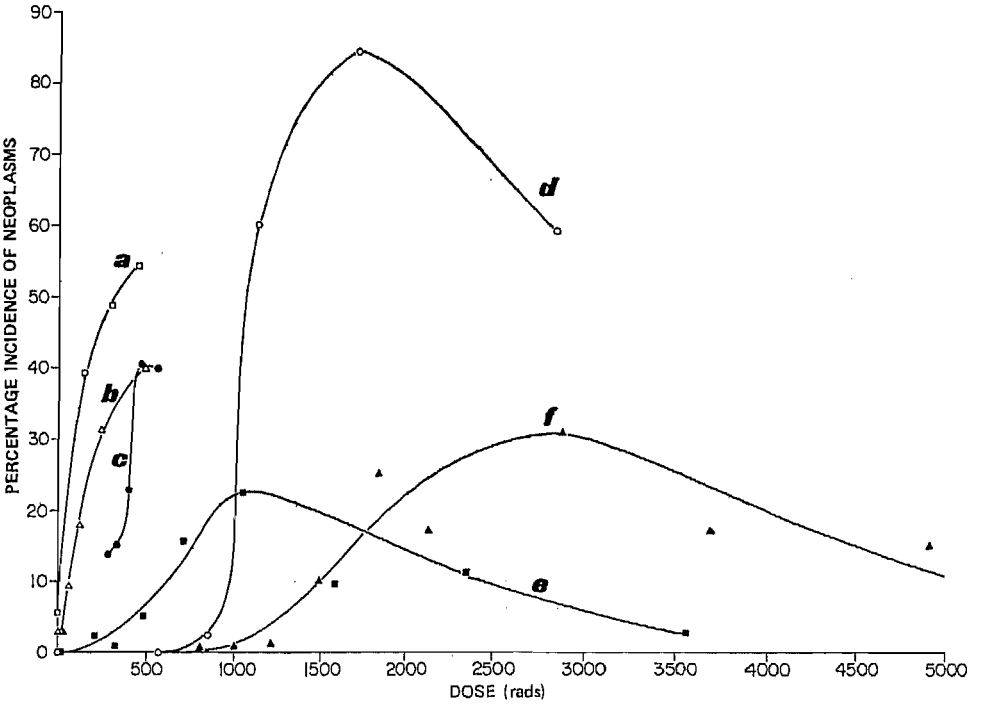

Figure II. Dose-response curves for different types of tumours following exposure to external radiation: (*a*) myeloid leukæmia induced in mice by x rays (199); (*b*) mammary tumours at 12 months in rats by gamma rays (172); (*c*) thymic lymphoma in mice by x rays (96); (*d*) kidney tumours in rats by x rays (118); (*e*) skin tumours in rats by alpha particles (percentage of incidence × 10) (17); (*f*) skin tumours in rats by electrons (percentage of incidence × 10) (17)

induction of bone tumours by internally-deposited radio-nuclides which give rise to high doses of radiation, principally to bone (141). These data can best be explained by cell killing rather than by reduced numbers of animals at risk since little or no mortality of the irradiated animals occurred or the animals were part of a serial-killing study. The doses at which the effect of cell killing begins to reduce the incidence of such tumours are an order of magnitude greater than for myelogenous leukæmia.

35. The dose-response relationship for low doses of external radiation at high dose rates has been described as linear for some neoplasms and curvilinear for others. Data from cell killing and chromosomal-mutation-induction experiments (38) indicate that more than a single event is required to produce the end-points measured. At high dose rates there are often indications that the dose-response curves are linear for high-LET radiation but exhibit positive curvature for low-LET radiation. This is in agreement with the Kellerer-Rossi arguments (see paragraph 9) i.e. with the assumption that two critical events are initiated by a single high-LET particle but predominantly only by two low-LET particles. However, an analysis by Rossi and Kellerer (161) of the dose-incidence curve for mammary neoplasms in the Sprague-Dawley rat indicates that there is lack of linearity at low doses of high-LET radiation. These data suggest also that each tumour arises because of unspecified radiation effects on more than one cell and that extrapolations to low doses are unwarranted. The complications of a multi-step process, however, preclude drawing firm conclusions and these arguments must be tested by examination of more extensive data on radiation carcinogenesis before the validity of the theory can be asserted. It is apparent, however, that repair does not play a significant role in determining the shape of the dose-response curves for single exposures to high-dose-rate radiation, although it is important in the case of low-dose-rate radiation (see paragraph 47).

36. Few experiments have been large enough to provide statistical proof concerning the exact form of the dose-response curves for radiation-induced neoplasms. An apparently linear relationship has been described for the acceleration of mammary neoplasms in Sprague-Dawley rats from x-ray exposures of 25-400 roentgens (172) and preliminary data from a large experiment suggest that the frequency of thymic lymphoma induced in RFM mice by gamma-ray exposures between 10 and 300 roentgens may rise linearly with exposure (190). On the other hand, the x-ray induction of kidney tumours in rats is almost certainly non-linear in the low-dose range (118), and the radiation-induction of skin tumours in rats (17) and mice (80) is probably curvilinear, the response varying with the second or fourth power of the dose. The dose-response curve for myelogenous leukæmia in mice has been described as curvilinear, the response varying with the square of the dose (192). In contrast to the linear dose-response curve seen for thymic lymphosarcoma in RFM mice, the curve for C57BL mice strongly suggests a curvilinear response for the same disease (96). Although the C57BL strain is normally considered to be highly susceptible to induction of thymic lymphosarcoma, it is in reality more resistant to induction of this disease by single, brief x-irradiation than is the RFM strain. These data from experiments with low-LET radiation suggest that the more resistant the tissue to tumour induction, the more likely that the dose response will be curvilinear or sigmoid, and the more sensitive the tissue to radiation, the more likely that linear dose-response curves for tumour induction will be observed. Likewise, linear dose-response curves are seen where the spontaneous incidence of neoplasms is moderate to high, further suggesting that linearity of the dose-response curve is related to sensitivity of tumour induction.

37. As the character of the radiation changes, so does the dose-response curve. X or gamma rays are much less effective at low doses and low dose rates than neutrons. Life-shortening data (197), which can be related principally to the induction of neoplasms (210) demonstrate such differences. For life-shortening due to neutron-irradiation of female RF mice (197), the dose-response curve below 150 rads appears to be linear for both high and low dose rates. The character of the dose-response curve for gamma rays is not as clear, the response to the high dose rate appearing to be linear (as is the case for the thymic lymphosarcoma in this strain), whereas that to the low dose rate appears to be non-linear with marked reduction in effect. For acceleration of mammary tumours in rats, the dose-response curve is non-linear with doses of neutrons up to 50 rads and shows a saturation effect from 50 to 250 rads (207). Considerably more data will be required before the shape of the dose-response curve for neutron-irradiation below the point of saturation can be clearly determined. Change in dose rate also alters the shape of the dose-response curve, but the exact nature of the curve at low dose rates cannot be determined from the limited data available. For both thymic lymphosarcoma and myelogenous leukæmia in RF mice (198) a reduced dose rate of gamma rays results in a dose-response curve similar to that for a high dose rate but with lower peak incidence.

38. The shapes of the dose-response curves for internal emitters, while of importance for predicting effects of such type of exposure at low doses and dose rates, are difficult to interpret because as the activity decreases so does the dose rate. It is clear that for induction of bone tumours by long-range beta emitters, particularly ^{90}Sr, the dose response cannot be linear (125). This effect is attributed to recovery from damage produced by the low-LET radiation when dose rates are sufficiently low. The induction of bone sarcomas by high-LET radiation (i.e. alpha emitters) appears to increase linearly with dose in some cases, but to follow threshold or sigmoid relationships in others. Thus, at low doses, non-linearity was demonstrated for the combined studies of ^{226}Ra, ^{228}Ra and ^{228}Th in dogs (122, 124), and for ^{226}Ra plus ^{228}Ra in humans (162). On the other hand, ^{226}Ra-induced bone sarcomas in CF-1 mice appear to have a linear dose-response curve down to very low doses (49). A similar response was detected with external partial-body x-irradiation (49). The presence of a linear response in this strain of mice may be related to the high (2 per cent) spontaneous incidence of the disease (49). It has also been shown that the RF (30) and the CBA strains (49, 141) are significantly less susceptible to ^{90}Sr-induced bone sarcomas than the CF-1 strain of mouse. As was the case for external irradiation, the induction of tumours in resistant tissue by internally-deposited radio-nuclides appears to be non-linear. The induction of osteosarcomas in most animals (excluding the sensitive CF-1 mouse strain) is a good example of

such non-linear dose-response curves, as is lung-tumour induction by inhaled radio-nuclides (20, 104).

39. There have been many attempts to demonstrate the presence of an absolute threshold for radiation effects. It appears that there is a threshold for very resistant tissues since it requires doses of more than 800 rads in a single brief exposure of the exteriorized kidney to produce kidney tumours in rats (118) and an exposure of more than 35,000 roentgens with local continuous irradiation at high dose rate to produce œsophageal tumours in mice (56). While it has not been disproved that there is an extremely low but slowly increasing response below these effective doses, it seems more likely that induction of these neoplasms occurs only after sufficient tissue destruction and regeneration have occurred, suggesting the existence of a threshold. For more susceptible tissues, however, the threshold, if it exists, is at sufficiently low doses to require massive, expensive experiments to determine its presence. Such efforts should have low priority.

40. On the other hand, the concept of a "practical threshold" has been introduced by Evans (46). This concept is based on the fact that, as dosage decreases, the latency or tumour appearance time increases in some monotonic fashion such that there will be some value of the dose below which the tumour appearance time exceeds the life span. This concept is supported by the suggestion that most, if not all, neoplasms show a decreasing latency with increasing dose (see paragraph 33) and that animals irradiated as adults often die of other diseases before developing long-latency neoplasms because the latency exceeds the remaining life span (see paragraph 51). However, as discussed in paragraph 33, the relationship of dose to latency varies with the end-point for determination of effect. In addition, an accurate determination of the dose at which latency equals the remaining life span requires extrapolation into low-dose ranges where no data exist, which raises much the same problem encountered with the absolute threshold. Further, different values for the practical threshold result when the data are plotted on a semi-log as compared to a log-log plot. It has also been suggested (22) that an extrapolated least-square fit to the MIT human radium data provides a better estimate (and a considerably lower "practical threshold" dose) than the method selected by Evans. Thus, while a "practical threshold" based on latency has some validity, it also appears to have many of the same indeterminants that plague the absolute threshold.

VII. Relative biological effectiveness (RBE)

41. It has been known for many years that low-LET radiation is less effective than high-LET radiation in producing a variety of biological effects including carcinogenesis. Relative biological effectiveness (RBE) was first used by Failla and Henshaw (48) to compare the biological effectiveness of different radiations. Subsequently, RBEs were also used in radiation protection as weighting factors in adding doses of radiations with different qualities (137). It was recognized that the usage of RBE in radiation protection was incorrect (82) and it was recommended that RBE be used exclusively for its original purpose in radiobiology and that the term quality factor be used in the field of radiation protection (83). Thus RBE is now defined as the inverse ratio of the absorbed dose from one radiation type to that of a reference radiation required to produce the same degree of a stipulated biologic effect (138). It has been noted recently that in some instances the RBE increases as the dose decreases because variations in RBE are dependent on the shapes of the dose-response curves. Thus it is necessary to define the curves for both radiations before the RBE can be estimated (figure III). The relationship of RBEs to dose-response curves and their implications for theories of energy interactions at the molecular level have been outlined (98, 160, 179) and are discussed above (paragraph 9).

42. There is no single value of RBE which can be used to predict the relative effects of radiations of different quality in man. A variety of RBEs for different neoplasms in animals, varying from less than 1 to as high as 80, have been reported. It seems certain that much of this variation is due to the use of inappropriate data (e.g., uncorrected incidences) or differences in level of dose or dose rate studied. There are only a few cases where high-LET and low-LET radiations have been compared over a wide range of doses.

43. One of the most extensively studied radiation-induced neoplasms is thymic lymphoma in female RF mice (198). It has been demonstrated that at high doses and high dose rates the RBEs of fast neutrons (196, 198), 14-MeV neutrons (36) and 60-MeV protons (36) are approximately equal to one. This appears to be true over a dose range of approximately 150 to 400 rads of x rays. Between 150 and 25 rads of x rays the RBE for neutrons increases to between 7 and 10 (37). No data exist for RBE below 25 rads of x rays and the shapes of the dose-response curves are unknown at these doses. If both curves become linear, the RBE will remain between 7 and 10. If, as anticipated from theoretical considerations, the neutron curve is linear and the x- or gamma-ray curve is curvilinear at these doses, then the RBE will increase further. The influence of dose rate on RBE has also been studied extensively with this neoplasm and it is clear that neutrons are more effective at lower dose rates than x or gamma rays over a wide dose range. Between gamma-ray doses of 700 and 25 rads, the RBE for fast neutrons increases from between two and four to more than seven, depending on dose rate (196, 198). Since the dose-response curve for neutrons appears to be linear and that for gamma rays curvilinear, higher RBEs might be expected at lower doses.

44. Mammary tumours in Sprague-Dawley rats have also been studied extensively. These data are more difficult to interpret since most of the x- and gamma-ray data come from one laboratory (172) and the data for fission neutrons from another (206). Attempts to determine RBE values from these experiments, which use different techniques and different end-points, have led to values as high as 80 (207). These high values were obtained from the ratio of gamma to neutron doses (400 rad/5 rad) which produce roughly 90 per cent incidence of mammary tumours in Sprague-Dawley rats maintained to death. The accuracy of such determinations is open to question since final incidences uncorrected for competing risks were used and since the final incidence in unirradiated control rats is high (about 50 per cent). Since irradiation results in a dose-dependent acceleration of mammary neoplasia in this strain of rats, the percentage of rats with mammary neoplasms 10 to 12 months after exposure represents the dose-effect relationships more accurately than the survival data. In either case

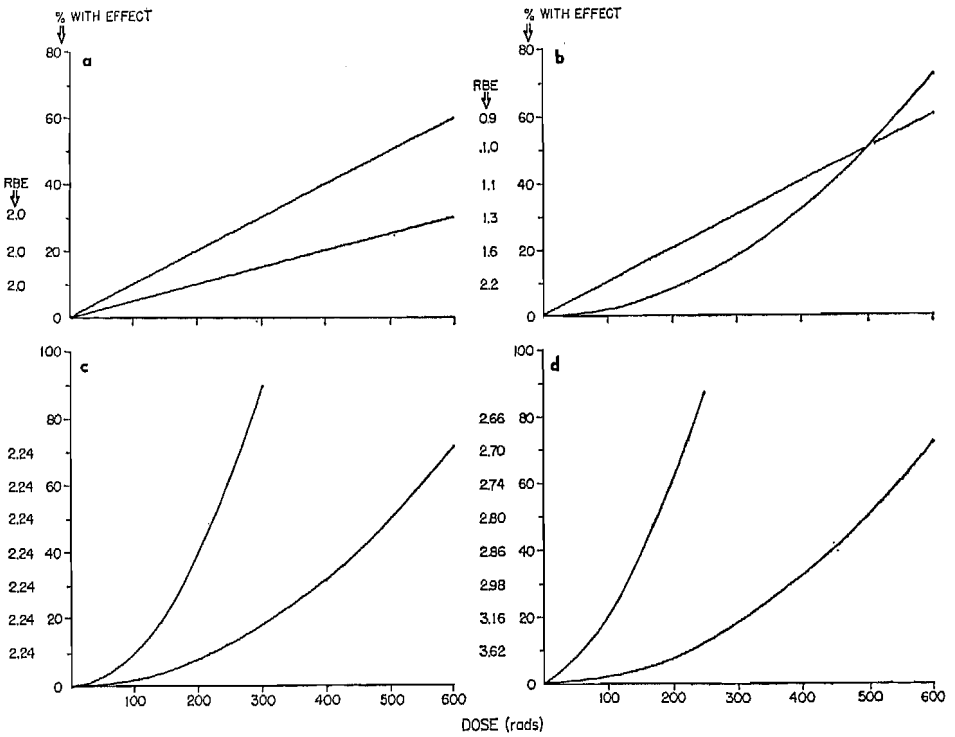

Figure III. Influence of character of dose-response curves on RBE: (*a*) first order (linear) polynomial curves with the same intercept but with different slopes; (*b*) standard radiation, second order (quadratic) polynomial; test radiation first order polynomial; (*c*) second order polynomial curves defined by only two terms, the intercept (which in this case is zero) and the second order term, i.e., $y = cx^2$, but with different second order term constants; (*d*) standard radiation, second order polynomial defined by only the intercept and the second order term; test radiation, second order polynomial defined by the intercept, first and second order terms, i.e., $y = bx + cx^2$

the RBE approaches a value of one between 350 and 400 rads of x rays and increases with decreasing dose (161, 207).

45. Considerable information is also available on the induction of rat skin tumours by cyclotron-accelerated alpha particles and mono-energetic electrons. At lower doses the dose-response curve appears to be curvilinear for both types of radiation, although the alpha particles are more effective and have a more rapid rise in response than the electrons (figure IV). Over the range of doses studied, the RBE of the alpha particles compared to electrons is approximately three and can be considered to increase slowly as the incidence decreases—from 2.3 at 2.0 per cent incidence to 4.3 at 0.2 per cent incidence. Thus within the range of doses for which data for different neoplasms are available, the RBE for high-LET radiation appears to move from one at high doses and high dose rates toward a maximum of 10 at doses between 25 and 100 rads. Estimates at lower doses are not possible since data for calculation of RBE or estimation of RBE by shapes of the dose-response curves are lacking.

46. The relative effectiveness of internal emitters for induction of neoplasia depends not only on the RBE of the particle emitted, but even more strongly on differences in localization, metabolism, transport and size of animal, all of which influence the resultant dose and dose rate to target tissues. For this reason it is not possible to derive quantitative values of RBE for internal emitters for the purpose of extrapolating to man. It can be said, however, that alpha emitters are more effective in producing osteosarcomas (40, 58) and lung tumours (165) than the lower-LET radiations, and the localization patterns of radio-isotopes in bone and the lung play an important role in determining their effectiveness (121, 159, 165).

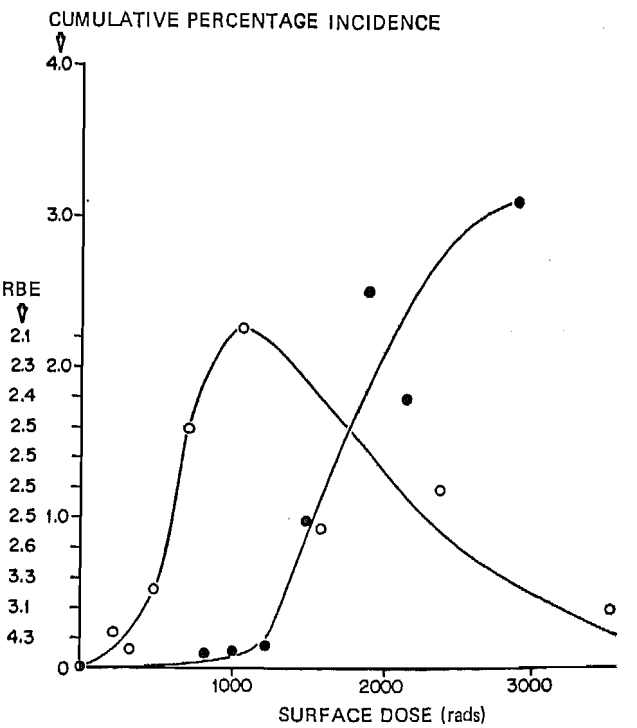

Figure IV. Cumulative percentage incidence of skin tumours at 76 weeks *versus* surface dose of alpha (●---●) and electron (o---o) irradiation (17)

VIII. Effect of dose rate

47. It has been known for many years that the dose of ionizing radiation required to produce a given biological effect varies with the dose rate but there are few data on late somatic effects of radiation at low dose rates. Much of the data available relate to life-shortening, which has been reviewed recently by Grahn and Sacher (61). The much smaller body of data on dose-rate effects in radiation carcinogenesis has been reviewed by Upton (185) and the greater dependence on dose rate of low-LET radiation compared with high-LET radiation has been discussed (189). These dose-rate effects can be explained in part by the same mechanism used to explain changes in RBE with dose. If induction of neoplasms by radiation requires that two events occur within a small volume within a sufficiently small amount of time to preclude repair, then low-LET radiations delivered at low dose rates would be expected to result in a low probability of two events occurring simultaneously, and sufficient time might elapse between the two events for repair to take place. With high-LET radiations, the intense ionization patterns yield such a high probability of two events occurring in a small volume at the same time, that even low dose rates would be expected to decrease the effectiveness of high-LET radiations relatively little compared with low-LET radiations.

48. A reduced efficiency of x and gamma rays for production of mouse leukæmia at low dose rates has been reported (164). Dose rates as high as 0.35 rad min^{-1} produce a lower incidence of leukæmia than do dose rates normally employed in "high"-dose-rate exposures (5-100 rad min^{-1}) (129). Thymic lymphosarcoma is more sensitive to this dose-rate effect than is myelogenous leukæmia (196, 217) and induction of both diseases by neutron-irradiation at low doses shows less dependence on dose rate than does induction by x or gamma rays (186, 188, 192, 196, 198). The effect of protraction of dose on the induction or acceleration of other tumours by radiation is less clear, principally because uncorrected incidences have so far been used as end-points. Thus the report that lower dose rates cause an increase in hepatoma incidence (148) can best be explained by increased survival time in the lower-dose-rate group. A significant reduction in ovarian tumour incidence was seen in RF mice irradiated with ^{60}Co gamma rays at low dose rates (184). Although no mortality data accompany the incidence data, it was demonstrated that survival at the lower dose rate was greater than at the higher dose rate (197); thus the reduced incidence cannot be explained on the basis of shortened life span. Since the induction of ovarian neoplasms by radiation has a complex pathogenesis involving the primary event of oöcyte destruction and secondary hormonal stimulation leading to tumour development (see paragraph 53), it is not possible to determine which part of the complex chain of events is affected by the decreased dose rate when tumour incidence is the end-point. No difference in incidence of mammary adenofibroma was noted in Sprague-Dawley rats after gamma-irradiation at exposure rates of 10 R min^{-1} and 0.03 R min^{-1} but the incidence of adenocarcinoma was lower in rats exposed at 0.03 R min^{-1} (170).

49. Numerous studies on fractionation of radiation dose have been performed to determine the amount of reparable injury sustained by the cell, and its translation into radiation-induced disease. Other than the peculiar response associated with induction of murine leukæmia, which is increased by fractionation of radiation dose depending on the schedule of administration (89, 95, 218), most neoplasms occur with lower incidence when doses are substantially fractionated. Such an effect has been reported for skin tumours (81, 157, 235), for ovarian tumours (222), and for the induction of lung tumours promoted by urethan (23, 227). On the other hand, it has been reported that fractionation does not alter the final incidence of mammary neoplasms in rats (230) even when a dose of 500 rads of gamma rays is given in 32 exposures over a period of eight weeks (171). However, since the final incidence of mammary tumours is high in controls, the best measurement of effect is the acceleration caused by the radiation (see paragraph 32). An examination of the data (171) (figure V) shows that at 400 days

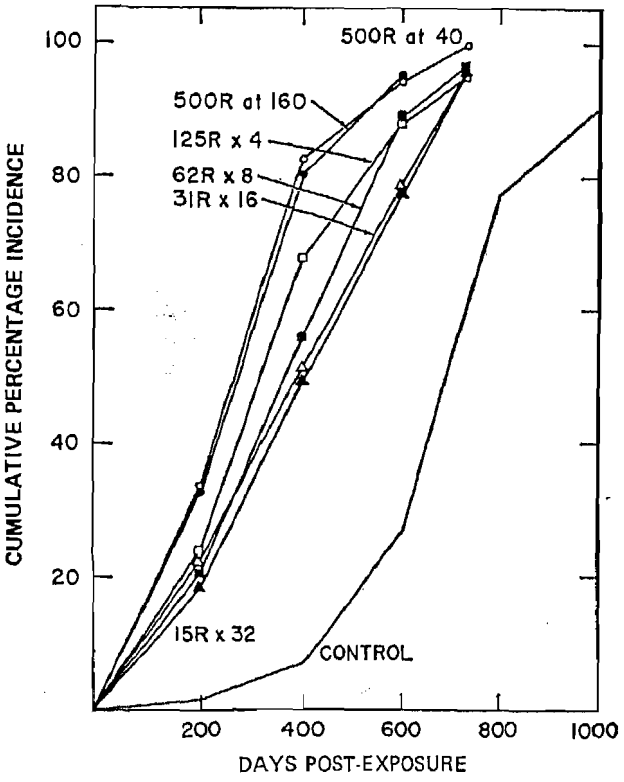

Figure V. Cumulative incidence of mammary gland neoplasia in rats, expressed as a percentage of surviving animals at a given time (rats at risk) having one or more tumours of any histologic type. "At 40" and "at 160" indicate age of rats at time of exposure; "125 R × 4" means that 4 fractions of 125 R were given twice weekly beginning on the fortieth day of age (171)

the cumulative percentage of rats with mammary neoplasms is inversely proportional to the amount of fractionation, with the 32-exposure group showing about 40 per cent reduction. Thus it appears that radiation damage which leads ultimately to neoplasia shows evidence of repair, as do most biological effects of low-LET radiation.

50. The determination of dose-rate effects with internal emitters is a more difficult problem but it is clear that reduction in dose rate reduces the efficiency of tumour induction. Such an effect is seen for induction of bone sarcomas in beagle dogs injected with ^{90}Sr (122). There is a "low-risk" region where bone tumours are not seen despite an accumulation of total

dose in excess of that required for induction of these tumours at higher dose rates. A similar dose-rate effect is seen (*a*) in mice when injected doses of ^{90}Sr are fractionated, giving a reduced maximum dose rate and a decreased number of osteosarcomas (49); (*b*) in mice exposed to different but constant dose rates by continuous ingestion of ^{89}Sr (214), where a reduced dose rate results in reduced incidences of bone sarcoma and hæmatopoietic disease; and (*c*) in lactating mice where the dose rate of ^{90}Sr and the bone tumour incidence are reduced when compared to their non-lactating counterparts (146). In studies on lung-tumour induction by surgically-implanted intrabronchial pellets of ^{106}Ru (104), no difference in lung-tumour incidence was noted between animals exposed at 2,250 rad d^{-1} and those at 422 rad d^{-1}. This is, however, a relatively small dose rate reduction and it would be useful to repeat the experiment with a wider range of dose rates.

IX. Dependence of sensitivity on age

51. The relation of age to the life-shortening effects of radiation (much of which can be ascribed to induction of neoplasms) has been reviewed by Upton (191). In mice, age susceptibility to life-shortening following a single brief exposure to radiation increases to a maximum in juvenile animals, declines until middle age, and increases again in old age (107, 131) (figure VI). The reduced effectiveness of radiation in adult as

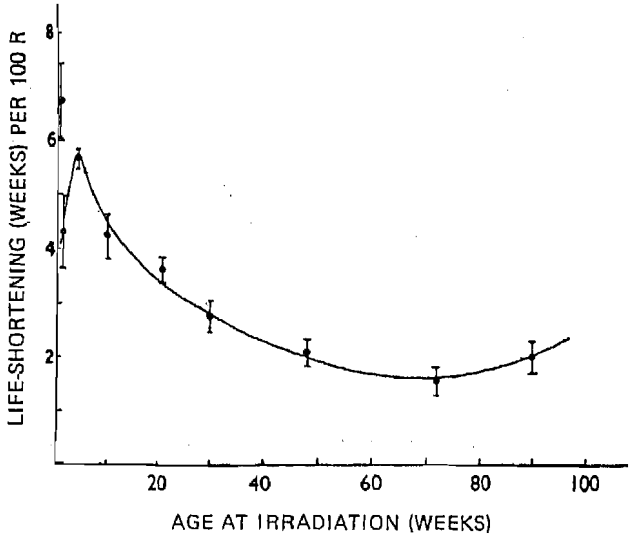

Figure VI. Life-shortening per 100 R exposure as a function of age at irradiation in SAS/4 mice which survived at least 30 days after whole-body exposure to radiation in the LD_0 to LD_{100} range (107)

compared to juvenile animals may be due largely to the failure of adult irradiated animals to survive the long induction period required for expression of the late somatic effects responsible for life-shortening. On the other hand, there is evidence that the susceptibility itself varies with the age at irradiation.

52. The induction of neoplasms is likewise affected by the age at irradiation. Thymic lymphosarcoma, myelogenous leukæmia, and ovarian tumours are particularly sensitive to age effects in mice. In RF mice the sensitivity to induction of thymic lymphosarcoma is low just after birth, increases to a maximum at six weeks of age and declines thereafter, demonstrating low sensitivity after thymic atrophy is far advanced. Myelogenous leukæmia in RF mice also demonstrates a low sensitivity to induction at birth with a slower increase to a later maximum at 10 weeks of age and a slower decline thereafter (195) (figures VII and VIII). Thymic lymphosarcoma in other strains also has

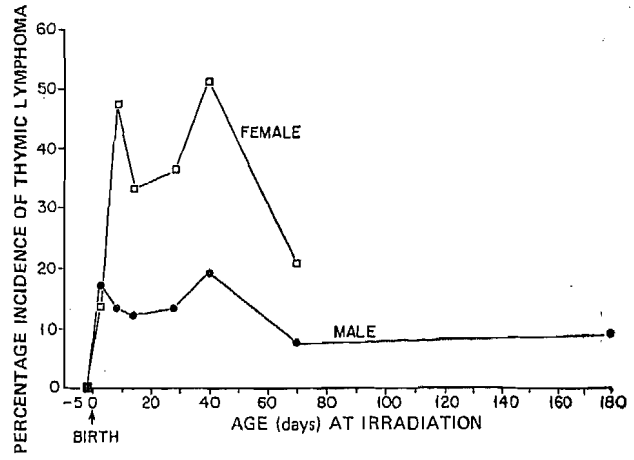

Figure VII. Incidence of thymic lymphoma in relation to sex and age at irradiation (x rays, 300 R) (195)

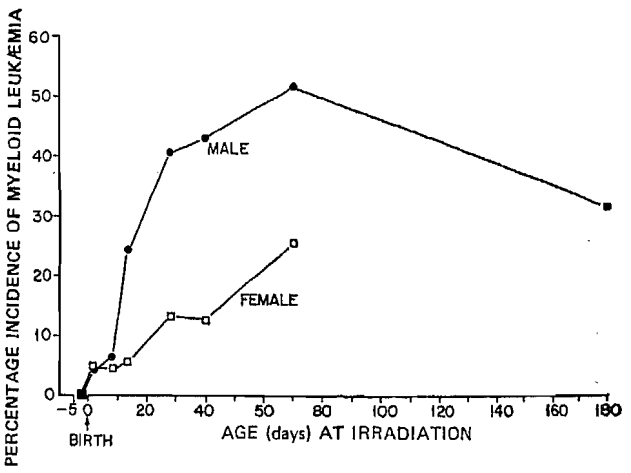

Figure VIII. Incidence of myeloid leukæmia in relation to sex and age at irradiation (x rays, 300 R) (195)

a maximum early in life with subsequent decline (94, 107). Since the latent period and course of murine leukæmias are relatively short, the decline with age cannot be due to insufficient residual life span for expression. X-ray exposures of 50-400 roentgens to RF mice *in utero* at fœtal stages of development when blood formation is localized predominantly in the yolk sac (9½ days after conception), liver (12½ to 14½ days after conception) and marrow and spleen (17½ days after conception) failed to induce leukæmia (185, 191), although these doses are effective in inducing both thymic lymphoma and myelogenous leukæmia in mice of this strain irradiated after birth (198).

53. The incidence of ovarian tumours is dependent on the age at irradiation. Total-body irradiation of young adult mice produces degenerative changes in the ovary with loss of ova of all stages. This radiation

damage enhances the production of gonad-stimulating hormones of the pituitary. While the relative role of this endocrine imbalance as opposed to the direct effect of radiation on cells of the ovary is not clear, the former must be important since intact ovarian endocrine function inhibits the development of tumours in irradiated ovaries (see reference 51). A gamma exposure of 200 roentgens which produces complete sterility in mice exposed at the age of 2 or 12 weeks failed to destroy all ova in ovaries of mouse fœtuses irradiated on the fourteenth or fifteenth days after conception (223). Likewise, doses of radiation which induce a high incidence of ovarian tumours in young adult mice fail to induce such tumours when mice and rats are exposed before birth (155, 195). Immediately after birth, the sensitivity to induction is high (195), reaching a maximum when animals are exposed at 10-20 weeks of age (107, 155) and declining thereafter (32, 107, 155, 158). It is not possible to determine whether the decline in sensitivity with age of irradiation is associated with insufficient life span for expression or with reduced sensitivity of the tissue.

54. The importance of age at time of irradiation has also been demonstrated for the induction of other neoplasms. Kidney adenomas are more easily induced in neonatal than in three-month-old mice (27). Male Sprague-Dawley rats exposed to fast-neutron doses of 215-230 rads show an age-related susceptibility to induction of certain neoplasms (90). Rats irradiated at one month of age were more susceptible to osteochondromas than their three-month-old counterparts. While skin tumours were induced in both groups, those irradiated at one month had a predominance of fibromas while those irradiated at three months had a predominance of basal cell carcinomas. Rats irradiated at three months were more susceptible to cortical carcinomas of the kidney than their one-month-old counterparts.

55. Age-related susceptibility to radiation-induction of tumours by internal emitters has also been reported, and is related to differences in uptake or metabolism of the radio-nuclides. Bone-tumour incidence in young mice (203) and rats (180) is higher than in older animals after treatment with ^{90}Sr; this can be explained on the basis of higher uptake and higher initial dose rate in the younger animals. Similarly, bone-tumour incidence in young rats after treatment with 1 μCi g^{-1} of ^{144}Ce was higher than in old rats (114). These results were caused by a lower dose resulting from dilution effects in the rapidly growing rats. Adult rats developed bone tumours at lower injection doses. When dose corrections are made, the differences tend to disappear indicating that the bone cells of young animals are not much more susceptible to induction of neoplasms than those of older animals. Similar results have been noted in man when ^{224}Ra was injected into juveniles and adults; the bone-sarcoma incidence in juveniles was no greater than four times that in adults (178).

56. Fœtal exposure to ^{90}Sr ingested by the mother may be an important factor in the subsequent development of hæmatopoietic neoplasms in miniature swine (149) and ^{32}P administered to pregnant female mice resulted in a significant incidence of leukæmia in female offspring (74). This last result is in contrast to the results obtained with external irradiation (paragraph 52).

X. Differences in sensitivity between strains and between species

57. The role of genetic constitution in long-term survival and induction of neoplasms has been reviewed by the Committee (182) and has since been reviewed by Upton (187). Most of the data on experimental radiation carcinogenesis come from studies with rodents where considerable variation is noted in unirradiated animals, even among different inbred strains of the same species. The spectrum of tumours induced by whole-body irradiation and the amount of life-shortening attributable to these tumours vary considerably with genetic constitution. For example, while the induction of neoplasms by radiation in rodents is well established, none is evident in female burros receiving gamma exposures of 300-550 roentgens despite the occurrence of life-shortening (14). As stated earlier (paragraph 27), almost any tissue will produce a neoplasm if exposed to sufficient radiation. It has also been demonstrated that the different sensitivities attributable to genetic constitution can be overcome if sufficient radiation is administered (212, 213).

58. Different inbred strains of mice differ in their sensitivity to radiation-induction of thymic lymphosarcoma and myelogenous leukæmia (188), but when sufficient radiation is applied in a proper pattern (i.e., fractionated radiation timed to maximize the number of blast cells), some of the differences tend to disappear (153). Species differences exist even among rodents: some strains of rats (130) and guinea-pigs (163, 219) are susceptible to radiation-induced leukæmia, but the Chinese hamster is strikingly resistant (130). Since the Committee last reviewed the subject, additional examples of strain and species differences in sensitivity to induction of various neoplasms have been described. Rats are more susceptible than hamsters to induction of adenocarcinomas of the lung by local external x-irradiation (65); rats are more susceptible than mice to induction of kidney tumours following whole-body x-ray exposures of 500 roentgens (5) but may be less susceptible to large local doses (118); beagle dogs do not show the marked sensitivity of mice for radiation-induction of ovarian neoplasms (4). Different species and strains of animals respond differently to induction of corneal tumours by ultraviolet radiation, with rats, mice, and hamsters being sensitive and guinea-pigs relatively resistant (50). In addition, albino strains of rats are less sensitive than pigmented strains.

59. Considerably less species variation is seen in the spectrum of neoplasms induced by internally-deposited radio-nuclides. Thus, in most species, bone-seeking radio-nuclides cause bone tumours, leukæmias, and squamous-cell carcinomas from tissues in close proximity to the bone. Neoplasms may be induced in other organs when radio-nuclides are deposited there either through direct introduction (e.g., inhalation) or by translocation through physiological mechanisms (e.g., liver with plutonium, pigment epithelium of the eye with radium). This qualitative similarity may be related to the high doses required for induction of many of the neoplasms induced. As was noted in paragraph 57, different sensitivities attributable to genetic constitution can be overcome if sufficient radiation is administered. The qualitative similarity in induction of bone sarcomas by bone-seeking radio-nuclides (122, 125) has been analysed for quantitative similarities among species (133). Although one method of dose calculation (i.e., number of beta particles divided by

body mass) suggests that the radio-sensitivity to tumour induction of the entire endosteum is independent of species, the average skeletal dose required to produce 50 per cent bone sarcomas does vary with the species. In addition, extreme variability in sensitivity among inbred mouse strains suggests that quantitative similarities among species may not exist (30, 49).

XI. Summary and conclusions

60. Comparisons of experimental animal data with human data have been frequent and the Committee is reviewing the most recent data on radiation carcinogenesis in man in annex H of this report. The data reviewed here suggest that the animal systems studied thus far are quantitatively inadequate for determining risk estimates in man. In addition, many of the most commonly studied animal tumours such as thymic lymphoma and ovarian neoplasms of the mouse and mammary neoplasms of the rat appear to have an induction sensitivity far in excess of that seen in man (annex H).

61. On the other hand, there appear to be several qualitative generalizations which may help to interpret the few human data now available:

(*a*) Virtually any mammalian tissue with the possible exception of adult neuronal tissue will give rise to neoplasms if exposed to sufficient radiation;

(*b*) The data from gamma- or x-irradiated animals suggest that for low-LET radiation, while both linear and curvilinear dose-response curves are seen, linear curves in the dose range of less than 100 rads occur principally when the target tissue is highly susceptible to induction of neoplasms by radiation. In man, target tissues which show such high sensitivity to tumour induction by radiation have not been identified except possibly in the fœtus (see annex H). If, as the data suggest, most human tumours induced by radiation arise from relatively resistant tissues, then it could be predicted in the light of experimental animal data that the dose-response curves for such neoplasms will be non-linear in the low-dose range;

(*c*) It is clear, both from theoretical considerations (see figure III) and from animal data, that the RBE for high-LET radiation can vary with dose. A comparison of the dose-response curves for neoplasms induced by high- and low-LET radiation will indicate increasing RBEs with decreasing doses. Estimates of the RBE may be particularly difficult to determine in man where the data at low doses are few, and conclusions about the dose dependence of RBE cannot be drawn until the dose-response curves are defined;

(*d*) Another important consideration is the reduced effect of protracted irradiation as compared to an equal dose administered in a short period of time. While considerably more data are required, the animal data available indicate that both protracted continuous irradiation and fractionated irradiation produce less carcinogenic effect than a single administration of the same total dose, suggesting that such an effect might be expected to occur in man as well;

(*e*) While some exceptions are noted, resistance to radiation-induced tumours is higher in adult than in juvenile rodents and, although some strains of rats and mice are highly susceptible to radiation-induction of leukæmias, fœtal irradiation has failed to induce a significant amount of leukæmias in those strains. The significance of this observation to fœtal irradiation of man must await further studies (see annex H);

(*f*) A difference among species and among strains of the same species in resistance to radiation-induction of neoplasms has been noted, suggesting the existence of considerable genetic control. Such genetic control of tumour induction by radiation makes clear the need for caution in the extrapolation from experimental animals to man. On the other hand, the development of valid qualitative generalizations which appear to apply to mammals of many different species gives hope that quantitative inferences may ultimately be possible.

XII. Areas of major emphasis for future studies

62. While some qualitative generalizations have been derived from animal data which may be useful in understanding the risk of neoplasia following irradiation in man, considerably more data from experiments with a variety of animal species will be required before they can be useful for supplementing quantitative estimates of risks of neoplasms in man. It is particularly important that attention be paid to the determination of the populations of cells that are at risk of neoplastic transformation and the determination of the physical dose to such cells. Where such determinations can be made, it is then essential that experiments be designed in such a way that their results may help in better evaluating quantitative risk estimates for humans. Considerable work is in progress in these three areas for the induction of bone tumours by internally-deposited alpha-emitting radio-nuclides, but other tumour systems must also be studied.

63. Further, the Committee recognizes that there are several broad areas in which we require more information. Among these are studies relating to the mechanism of radiation carcinogenesis. Support or rejection of the various initiating and promoting mechanisms or evaluation of their relative importance will require considerably more data from a much broader spectrum of *radiation-induced* tumours. For example:

(*a*) The role of virus activation as a mechanism in radiation-induction of neoplasms of the hæmopoietic system needs to be extended from the C57BL mouse to other strains of mice and other mammalian species;

(*b*) Similarly, efforts must be made to determine whether viruses which can produce tumours of non-hæmopoietic tissues are activated by radiation;

(*c*) Additional attention should also be given to radiation-induced cell destruction and any subsequent changes in cell repopulation which might temporarily increase the population of susceptible cells. Since different tissues have various susceptibilities to radiation induction of neoplasms, they can be examined for such corresponding changes in cellular kinetics;

(*d*) Likewise, damage and repair of DNA with the production of gene and chromosomal mutations, as well as damage to other biologically important macromolecules, must be related to the production of neoplasia before radiation-induced somatic mutations can be accepted as a cause of the neoplastic effects;

(*e*) Radiation-induced changes in other cell constituents, particularly cellular and nuclear membranes, should also be examined and their relationship to neoplastic transformation determined;

(*f*) The role of radiation-induced immune disorders in neoplasia should be examined with radiation-induced tumours of different tissues;

(*g*) Further clarification of the mechanism of radiation-induced neoplasia may result from studies on disturbances of the neuro-endocrine system by radiation;

(*h*) The effect of radio-protective agents, including both chemical agents and cellular replacement, on radiation-induction of neoplasms may also help to clarify these mechanisms;

(*i*) Another broad area of particular importance to the ultimate understanding of radiation carcinogenesis in man is the role of genetic constitution as a determinant of the susceptibility to induction of cancer by radiation. Studies on the mechanism of gene action in inbred animals are critical to this understanding;

(*j*) Further clarification of the relative importance of dose and dose rate for induction of neoplasms by internally deposited radio-nuclides should be sought through studies on the effect of repeated administration of short-lived radio-nuclides, a procedure analogous to fractionated external irradiation.

64. More data are required to confirm and extend some of the conclusions reached in this report. Most of the experimental animal data derive from studies on a few very sensitive rodent tumour systems. It is essential to study the reduced efficiency resulting from protracted irradiation (dose-rate effect) in other radiation-induced tumour systems, especially less sensitive ones, in a variety of strains and species. In the same sense, it is important to provide data on change in RBE with dose of radiation for a variety of tumour systems, since the applicability of the Rossi-Kellerer theory (see paragraph 9) to the mechanism of radiation-induced damage leading to neoplasia is based on examination of only one such system. The suggestion that shortening of life span by moderate to low doses of radiation (below 300 rads) is primarily due to induction of lethal neoplastic diseases is based on very few data. Expansion of these data is necessary to verify this generalization which can be extremely important for setting risk estimates for human populations. And lastly, the Committee feels that the apparent discrepancy between the rodent data and human data on induction of leukæmia by fœtal irradiation must be studied in additional species which have different rates of maturation of the lymphopoietic system during gestation. All of the studies suggested in this paragraph can be carried out now with existing knowledge and techniques. However, it is essential that such studies utilize the best of statistical approaches to experimental design, as well as careful and proper pathological diagnosis. Analysis of the data must include corrections for competing risks, especially where studies involve late-occurring radiation-induced tumours, which now are those of principal interest.

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Annex H

RADIATION CARCINOGENESIS IN MAN

CONTENTS

Introduction

1. It is generally accepted that cancer is the major long-term somatic effect of radiation on human beings. The Committee discussed the subject of human cancer induced by radiation in its 1958, 1962 and 1964 reports (161-163). In view of the substantial increase in knowledge about radiation carcinogenesis in man since the Committee's latest report, this annex will review this subject again.

2. The carcinogenic effects of radiation, as indeed the effects of any environmental factor implicated in the causation of human cancer, are best evaluated by human population studies. Because of the great differences in susceptibility to cancer induction between human beings and other species, studies with experimental animals provide information of more qualitative than quantitative significance. The mechanism of carcinogenesis in general and specifically the role of radiation in carcinogenesis are certainly not well enough understood to deduce from first principles the extent of radiation effects on human beings. It is therefore essential to obtain empirical information from epidemiologic studies.

3. In the evaluation of such studies, the following inherent difficulties must be borne in mind:

(*a*) Populations of sufficient size who were exposed to a sufficiently high dose of radiation are few, and their number has been decreasing as radiation hazards have become increasingly understood;

(*b*) In retrospective, or case-history, studies, quantitative estimates of radiation dose received are often very difficult to obtain, especially when radiation exposure has occurred repeatedly. The fact that a number of years are required for the development of cancer after irradiation makes it particularly difficult to determine radiation exposure that has occurred years earlier;

(*c*) The long latent period for cancer induction is also a drawback in prospective or cohort studies unless, at the initiation of the study, an exposed population or a cohort can be selected on the basis of exposure in the distant past;

(*d*) When there is a low natural incidence of cancer of a specific type, a large population must be followed in order to obtain an adequate number of cancer cases;

(*e*) In most studies cancer frequencies are measured, for practical reasons, in terms of mortality. This practice requires great caution, since mortality statistics can be an unreliable measure of incidence, as when cancer of a specific site has unreliable death notification or shows a low fatality;

(*f*) The data on patients who were exposed to medical irradiation also must be evaluated with caution, since the effects of irradiation may well be confounded both with the effects of the primary disease that prompted the therapeutic irradiation, and with the effects of other treatments given to the patients. In addition, such data are biased in most instances toward specific sex and/or specific age group, thus making it difficult to apply the results to the general population;

(*g*) The relative susceptibility of different organs and tissues is of great interest, and this can best be ascertained if different tissues and organs receive the same amount of radiation. Uniform whole-body irradiation, however, has practically never occurred except for fœtus exposure;

(*h*) Comparison of the results of different investigations are made difficult by the fact that the doses received were often from radiations of differing qualities delivered at differing rates.

4. In the present annex, the risk of cancer induction by radiation will be expressed as absolute and/or relative risk. The absolute risk of a certain type of cancer at a stated dose of radiation of a certain quality is the excess incidence due to that dose of radiation. In practice this is estimated from the difference between the incidence rates of the exposed and the non-exposed population. The absolute risk may for instance be expressed as the excess number of cases per million per year for a given dose. The relative risk for a given dose is the ratio between the incidence rates in a population exposed to that dose and that in a non-exposed population which, ideally, should be comparable to the exposed population with respect to all factors affecting the incidence of the effect studied, except radiation.

5. Relative risks are preferred to absolute risks in epidemiologic studies in assessing whether there exists a causal, rather than a mere fortuitous, association between exposure and the disease (93). Once the association is accepted as being casual, absolute risk is a better index of the impact that a successful preventive programme might have. Therefore, the absolute risk has been the estimate of risk of radiation effects adopted by the Committee in its 1964 report and by the International Commission on Radiological Protection (69). Another consideration is that if, under any circumstances, equal doses of radiation increase the risk in proportion to the natural occurrence of cancer (either in different populations for a given form of cancer, or for different forms in a given population), relative risks may provide more general estimates of the effects of radiation. If, on the other hand, the radiation risks are unrelated to the natural probability of cancer occurrence, and the excess risk is a function of the dose of radiation only, then the absolute risk is a better estimate of the effects of radiation. In the present annex radiation risks will be given both in absolute and in relative terms.

6. Estimates of risk per unit dose derived from epidemiological investigations are valid only for the doses at which they have been estimated and they can be applied to a range of doses only if there is a linear relationship between dose and incidence since extrapolations beyond that range may lead to gross errors. Particular care should be exercised in estimating risks from data on people exposed to mixed neutron and gamma radiation. Radiobiological experiments indicate that the RBE of neutrons varies with dose (see annex G) so that, if these results are applicable to human beings, the incidence of various effects cannot be proportional to absorbed dose for both gamma rays and neutrons and estimates of risk in terms of incidence per unit dose need to be clearly qualified.

7. Another serious problem at the present time arises from the fact that present knowledge of cancer induction by radiation is based on the experience of a limited number of years after exposure, thereby making risk estimates for an entire life span impossible. Because of this incompleteness of follow-up period, information is lacking, particularly about the later part of human life during which the natural incidence of cancer greatly increases over rates at younger ages.

8. In terms of man-year experience, the cohort followed by the Atomic Bomb Casualty Commission (ABCC) with the collaboration of the Japanese National Institute of Health (JNIH) is of far greater significance than the other cohorts under study. However, even the experience of this cohort at present gives only part of the information as to the whole risk of cancer induction. The proportion of cancer deaths to deaths from all causes ranged roughly from 10 to 20 per cent in the past 20 years in Japan. If the average figure of 15 per cent is applied to the ABCC cohort, 15,000 cancer deaths would be expected by the time all persons in the cohort had died. Although the extensive follow-up of the ABCC has revealed about 4,000 deaths due to cancer for the period 1950-1970, these deaths constitute only 27 per cent of the deaths to be eventually expected in the absence of radiation.

9. Jablon and Belsky (71) and Jablon *et al.* (75) have reported that the children who were exposed at ages less than 10 years show now, many years later, an unusually high risk of developing cancer at various sites. Children exposed to irradiation at, for example, 5 years of age and then followed for 20 years, will only be 25 years old at the termination of the follow-up period. At that age the natural risk of cancer is still extremely low. Therefore, a long follow-up is particularly advisable, although practically difficult, for

people who are irradiated at young ages. A follow-up of half a century or so may be needed to measure the whole risk of cancer.

10. The above consideration may not necessarily apply to all forms of cancer. If the risk of cancer induction is assumed to follow a unimodal distribution, follow-up is necessary only until the risk, having passed its peak, approaches the level of natural occurrence. At present, leukæmia is the only type of malignancy belonging to this category. The excess of other types of malignancies due to irradiation of the cohorts that are currently being followed up may still be increasing with time after exposure and it is entirely unknown whether the excess risk reaches a peak with time. Nor is it known what the magnitude of the peak, or the modal induction period, etc., are.

11. Since the 1964 report, a substantial amount of new information on radiation carcinogenesis in man has emerged. This will be reviewed here by type of malignancy. The over-all incidence of malignancies, including those about which statistical information is still too limited to warrant separate discussion, will then be reviewed.

12. The physical radiation quantities that are significant in radiation epidemiology have been variously defined and named. In this annex the recommendations of the International Commission on Radiation Units and Measurements (68) are followed. The quantity employed to specify the radiation field at any position in free air is the tissue kerma in free air (K). This quantity has been variously termed "T65D dose", "air dose", "first collision dose" or simply "dose". The quantity employed to specify energy absorption in irradiated tissues is the absorbed dose (D). This quantity has been variously termed "tissue dose", "radiation dose" or "dose". Both kerma and absorbed dose are measured in rads.

I. Leukæmia

A. A-BOMB SURVIVORS (ABCC-JNIH STUDY)

1. *Material and methods*

13. The cohort of A-bomb survivors and their controls in Hiroshima and Nagasaki (Japan) that was selected by the ABCC for the Life Span Study Sample consists of the residents of both cities who had stated in the 1950 National Census that they were in Hiroshima or Nagasaki at the time of the respective A-bomb explosion (12). All those who were within 2,500 metres of the hypocentre at the time of the bombing (ATB) were included in the sample. A comparison group, consisting of those located between 2,500 and 10,000 metres from the hypocentre, was matched by age, sex, and city to the survivors within 2,000 (not 2,500) metres. A second comparison group, similarly matched to the survivors within 2,000 metres, consisted of persons either not in the cities (NIC) or who were more than 10,000 metres from the hypocentre ATB. As a whole, this cohort amounts to about 100,000 individuals, categorized in table 1 by sex, city, and exposure. Information on nearly 100 per cent of the mortality experience of this cohort was obtained from the Japanese family registration system.

14. An attempt was made to procure autopsies on all deaths in the sample of 100,000 being traced for mortality occurring after 1961; the autopsy rate was about 40 per cent (70).

15. From the Life Span Study Sample of 100,000, a sub-sample of 20,000—the Adult Health Study Sample—was drawn to obtain information about conditions that do not lead to death or that do so only after many years. Biennial physical examinations were made on this sub-sample of 20,000. The sample consists of the following four groups: all survivors between 0-1,999 metres ATB with acute symptoms due to irradiation, those between 0-1,999 metres without such symptoms, those between 3,000-3,499 metres, and those beyond 10,000 metres or not in the city. To the first group, that small number of survivors who were closest to the hypocentre and had acute symptoms, equal numbers of individuals were sampled from each of the other three groups and matched by sex, age and city.

16. The risk of cancer induction was formerly related to distance from the hypocentre. While precise estimates of the absorbed doses received by the survivors are not yet available, not only have estimates of the tissue kerma in free air as a function of distance been published for both cities (5, 54), but estimates of the kerma to which the individual survivors belonging to the major ABCC samples were exposed are now available (101). These latter estimates take into account the attenuation due to shielding by the structures surrounding each survivor.

17. The previous kerma estimates (123) which were used by the Committee in its 1964 report have been more accurately re-estimated by Auxier *et al.* (5) with good agreement with the new and independent estimates of Hashizume *et al.* (54). Table 2 compares the kerma-distance curves from the old (T57D) and new (T65D) estimates. At Hiroshima, the new (T65D) kerma estimates 1.0 kilometre from the hypocentre are half the old estimate (T57D), and they are less than a third at 1.5 kilometres. For Nagasaki, the kerma estimates are essentially unchanged. The probable error of the new kerma values is estimated to be about $\pm$ 30 per cent in Hiroshima and $\pm$ 10 per cent in Nagasaki (5). In Nagasaki, about 90 per cent of the kerma is due to gamma radiation; in Hiroshima, gamma rays and neutrons each account for about half of the total kerma.

18. An exhaustive search for the location and shielding histories of each survivor of the ABCC cohort was made. On the basis of this information, and by utilizing kerma-distance curves and the appropriate shielding attenuation factors, Milton and Shohoji (101) were able to estimate the kerma to which the majority of the survivors had been exposed. For about 3,800 survivors estimates could not be made, usually because the survivor was at a distance where the kerma was high but the shielding configuration made it impossible to estimate the attenuation (71).

19. The reliability of kerma estimates for the survivors appears uncertain. As possible sources of error, a number of factors affecting kerma-distance curves, shielding histories, methods of estimating attenuation due to shielding, etc., must be considered. It must also be clearly borne in mind that absorbed doses, particularly to deep tissues, are difficult to obtain from the kerma estimates available, and the fact that a substantial neutron contribution was received by the survivors at Hiroshima introduces additional complications owing to the higher biological effectiveness of neutrons relative to gamma rays.

20. Regarding the material and methodology of the ABCC study, the following conclusions may be drawn:

(*a*) The study cohort of ABCC is generally unbiased with respect to sex, age and pre-existing disease, an advantage compared to other irradiated populations, such as medically treated groups;

(*b*) The mortality study of ABCC is greatly strengthened by the autopsy programme, a very rare feature of studies on radiation carcinogenesis;

(*c*) The morbidity study of ABCC gives valuable information about cancers with long survival times;

(*d*) The survivors were exposed to short-term (instantaneous), whole-body irradiation. The dosimetry shows uncertainties as discussed.

2. *Leukæmia morbidity*

21. In the 1964 report, the review of leukæmogenesis in A-bomb survivors was largely based on the report of Brill *et al.* (18) and showed that little doubt existed about the leukæmogenic effect of A-bomb irradiation. However, numerous problems (e.g., the precise nature of the dose-effect relationship, the relationship of radiation effects to sex, age, time, etc.) remained unsolved.

22. Since the publication of Brill *et al.*, the results of several studies have been published by the ABCC (16, 45, 62, 66, 67). The reports of Ishimaru *et al.* (66, 67) in particular have extensively covered various aspects of leukæmogenesis according to the new kerma estimates (T65D) for each survivor, and have thus provided significant new information about the relation between A-bomb irradiation and leukæmia induction.

23. In the Master Sample of 113,169 survivors (the Life Span Study Sample plus two additional small samples), 117 new cases of leukæmia were found during the 16-year period, 1950-1966. These were primarily detected through the leukæmia registries in Hiroshima and Nagasaki and were confirmed by at least two hæmatologists of the ABCC.

24. The annual incidences based on 88 cases of leukæmia at Hiroshima and 29 at Nagasaki are shown in figures I and II. It is worth noting that the data show a significant excess of leukæmia in the group exposed to kermas ranging from 20 to 49 rads (median 30 rads) at Hiroshima but not at Nagasaki. Regression analysis indicates that between median kermas of zero and 400 rads the rise of the incidence is not inconsistent with a linear kerma-effect relationship, the regression coefficients being 3 and 1.6 cases per million per year per rad at Hiroshima and Nagasaki, respectively.

25. The risk of leukæmia induction for a given kerma is therefore greater at Hiroshima than at Nagasaki. The difference between the two cities is most likely explained by (*a*) uncertainty in the air-dose curve, especially for Hiroshima since the Hiroshima-type of A-bomb was neither produced nor tested again after the Hiroshima explosion and (*b*) differences in the quality of the mixed radiation received in the two cities.

26. The differences between the incidences in the two cities for equal kermas have been used by Ishimaru *et al.* (67) to estimate the RBE of neutrons with

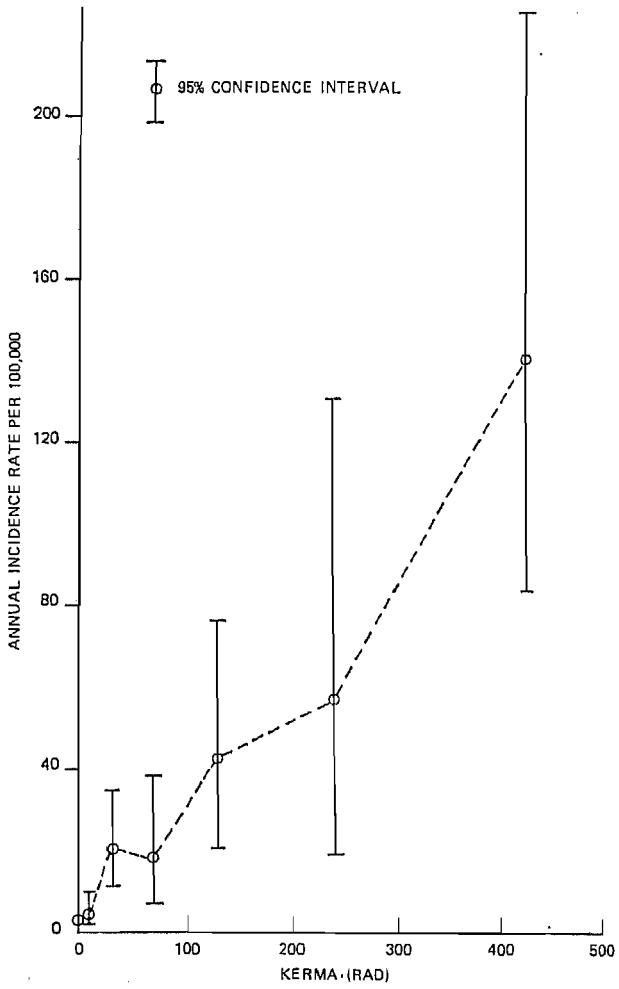

Figure I. Annual incidence rate of leukæmia (all forms) per 100,000 A-bomb survivors in ABCC master sample as a function of exposure at Hiroshima, Oct. 1950-Sept. 1966 (67)

respect to the induction of leukæmia by selecting that value which, applied to the neutron contribution to the kerma, would bring the incidence curves in the two cities to coincide. The closest fit was obtained with an RBE value of five. It has been pointed out (122), however, that the RBE is unlikely to be the same at all doses (see also annex G of this report). Poston *et al.* in fact showed that the data from Hiroshima and Nagasaki on leukæmia induction are consistent with RBE values that vary from four below 100 rads to one at about 400 rads. The implications of assuming that the RBE varies with dose have been mentioned in paragraph 6 and will be further discussed later in this annex.

27. It is worth noting that the data of Ishimaru *et al.* show a significant excess of leukæmia in the group exposed to a kerma as low as 20-49 rads at Hiroshima. However, no leukæmia case is observed at Nagasaki among the survivors exposed to less than 100 rads. The reason for the discrepancy may be due to chance fluctuations resulting from the smaller size of the Nagasaki sample or from differences in the quality of the radiation received in the two cities.

28. Table 3 shows the leukæmia incidence by specific type, kerma, and city. While the excess incidence of leukæmia is primarily seen among survivors having received a kerma of 100 rads or more, no excess is

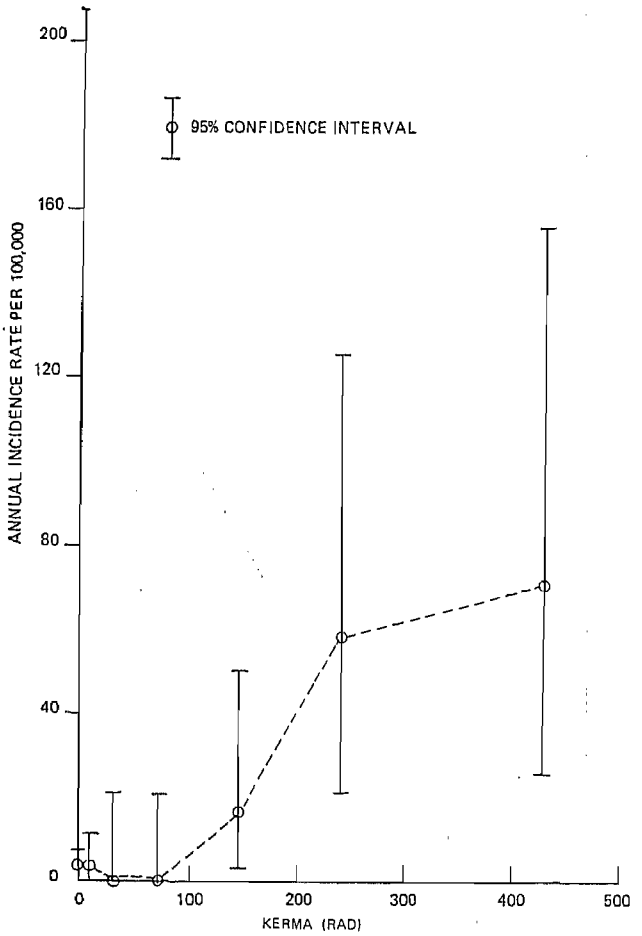

Figure II. Annual incidence rate of leukæmia (all forms) per 100,000 A-bomb survivors in ABCC master sample as a function of exposure at Nagasaki, Oct. 1950-Sept. 1966 (67)

observed, even at 100 rads or more, for chronic lymphocytic leukæmia. In Hiroshima, high risks are noted for acute granulocytic, acute lymphocytic, other acute types, and for chronic granulocytic leukæmia. Although the number of cases is small, the excess in Nagasaki is primarily confined to acute granulocytic and acute lymphocytic leukæmias. This difference in the distribution of excess leukæmias between the two cities may be noteworthy in considering the possible difference between the effects of gamma rays and neutrons. Among younger persons (less than 15 years of age ATB), the risk of acute lymphocytic leukæmia is especially increased.

29. Males seem to be more susceptible to leukæmia induction than females in terms of both relative and absolute risks. Figure III shows a higher relative risk among males than among females in the 5-99 and 100+ rad groups in each of the two cities. Since the natural occurrence of leukæmia (133) is higher in males than in females, the absolute risk must also be greater in males than in females (the male to female ratio is 1.3 for Japan).

30. When the relative risk of leukæmia is examined by age at exposure, both the 0-14-year and the 15-39-year age groups have clearly higher relative risks than the 40+ year age group, as shown in figure IV (only Hiroshima data are presented since the Nagasaki data do not distinguish the 15-39-year and 40+ year age groups). As seen in figures V and VI, leukæmia incidence rates are similar for different age groups in

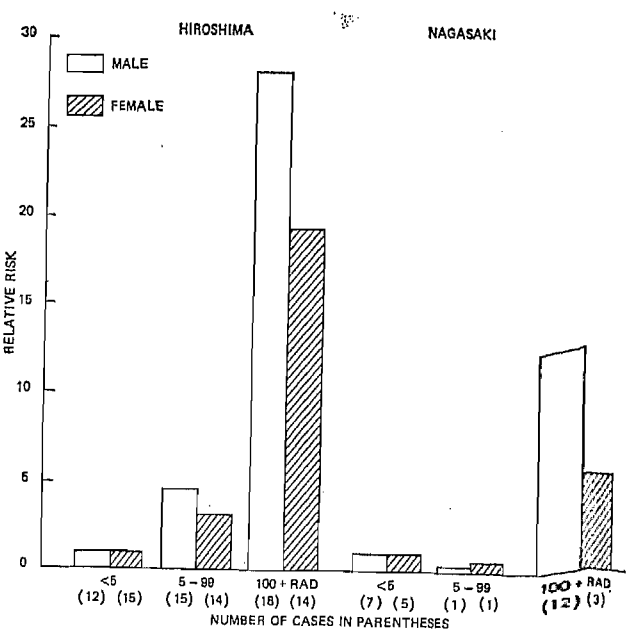

Figure III. Relative risk of leukæmia of A-bomb survivors by kerma, sex and city, 1950-1966 (66)

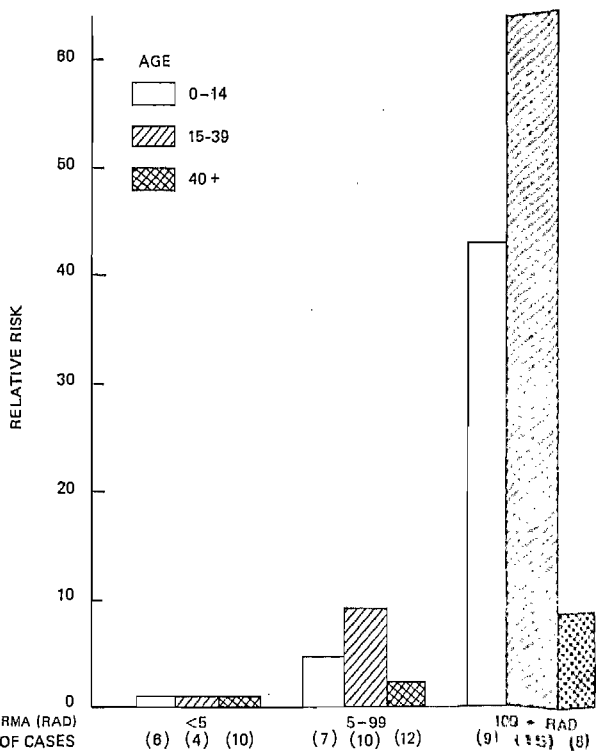

Figure IV. Relative risk of leukæmia of A-bomb survivors at Hiroshima, by kerma and age at exposure (66)

Japan, in sharp contrast to England and Wales and the United States (37)—these three countries being those that have provided the major sources of information regarding radiation leukæmogenesis in man. Thus, the high sensitivity in the younger age groups, as observed on the basis of relative risks, must also be true in terms of absolute risks.

3. *Leukæmia mortality*

31. Table 4, compiled from a report by Beebe *et al.* (10) shows the mortality experience of A-bomb sur-

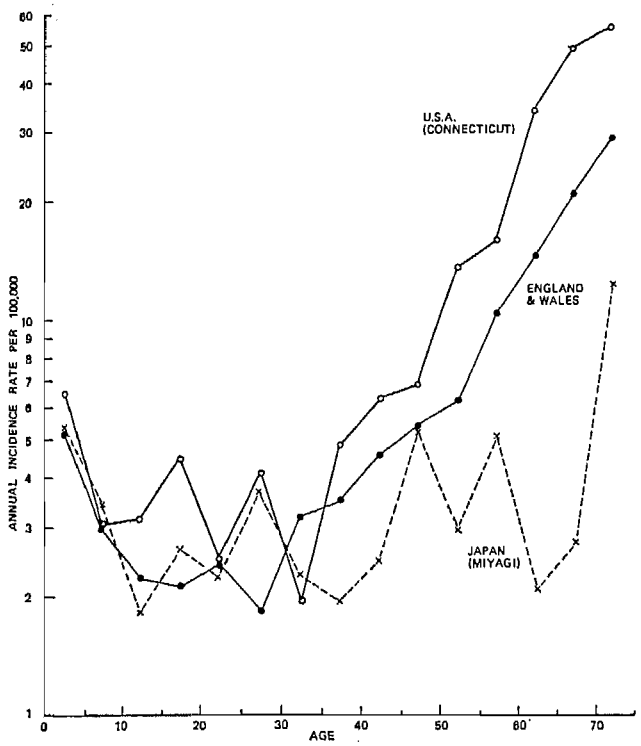

Figure V. Annual incidence rate of leukæmia per 100,000 males in Japan (Miyagi), the United States (Connecticut) and England and Wales in 1959-1960 or 1960-1962 (37)

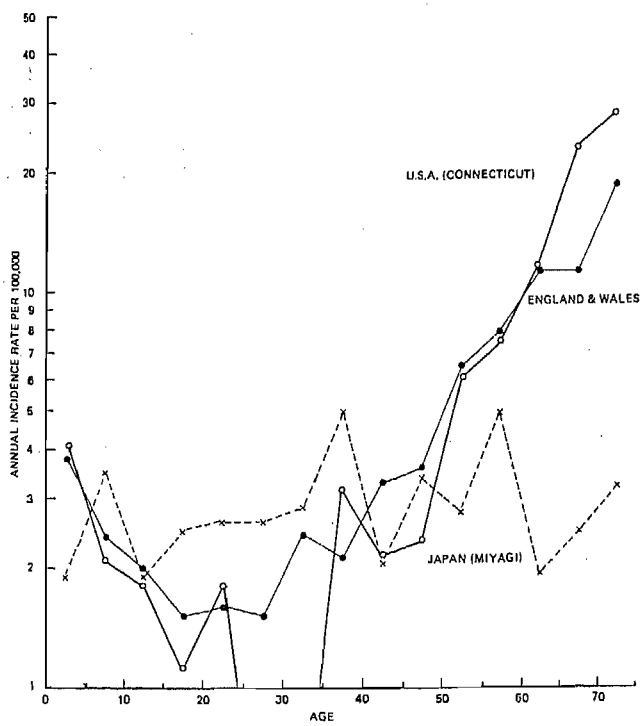

Figure VI. Annual incidence rate of leukæmia per 100,000 females in Japan (Miyagi), the United States (Connecticut) and England and Wales in 1959-1960 or 1960-1962 (37)

vivors in the ABCC cohort (modified Life Span Study Sample) in relation to selected types of cancer for the period 1950-1966. In this tabulation, data from Hiroshima and Nagasaki are pooled together.

32. As seen in table 4, leukæmia mortality clearly increases with increasing dose. For the 1950-1966 period, 116 deaths from leukæmia were observed, which comprised 4.8 per cent of the total malignant deaths of that period. Of the 116 leukæmia deaths, 64, or 55 per cent, may be ascribed to radiation.

33. A more recent mortality study made by Jablon and Kato (73, 74) in the ABCC cohort (modified Life Span Study Sample) has added the new mortality information obtained from 1967 to 1970. Among the five major types of malignancies selected by the authors for analysis—leukæmia, lung, breast, gastro-intestinal tract, and cervix and uterus—the first three show significant excess, and only these three types of cancers are presented in detail in table 5. In this study, the expected numbers were computed from the Japanese national rates by applying to different dose groups the rates specific for age, sex and calendar year. The national mortality rates may be different from those of the unexposed Hiroshima and Nagasaki populations because of geographical differences and because the survivors belong to an essentially urban population. In fact, the ABCC cohort showed a mortality from all causes lower by 8 per cent than the national average. Therefore, two other types of expected numbers of deaths were also estimated, based on the mortality experience of the practically non-exposed populations within the cohort: the 0-9-rad group, and 0-9-rad and NIC groups combined. In the absence of detailed information, these expectations could not be adjusted for sex, age, or calendar year. However, as seen in table 5, the three expected values are in fact very close in all dose groups and for all causes of death.

34. For leukæmia, the Hiroshima survivors show a higher risk than those of Nagasaki, which, as mentioned earlier, may be explained by the different quality of radiation in the two cities. In Hiroshima, the increase of risk is significant even in the 10-49 rad group, but, in Nagasaki, only in the groups receiving more than 100 rads. The excess number of leukæmia deaths in the exposed population (all survivors except the 0-9 rad group) may be estimated as 56.6 at Hiroshima and 18.5 at Nagasaki, when compared to national rates, or 51.8 at Hiroshima and 14.4 at Nagasaki when compared to the 0-9 rad group in the period from 1950-1970.

35. At Hiroshima, the leukæmia mortality rate rose with kerma by about two cases per million per year per rad between 0 and about 450 rads or by about 40 cases per million per rad over 20 years. This is very close to the corresponding figure of 48 cases per million per rad over 16 years of observation that can be obtained from the morbidity study.

36. Because the radiation received at Hiroshima consisted of both gamma rays and neutrons, it would be useful to know the RBEs of neutrons with respect to the induction of leukæmia. Unfortunately, these RBE values are not yet known. Since, however, the neutron contribution to kerma at Hiroshima varied with distance, it must follow that any value (fixed or varying with dose) of the RBE different from one, when applied to the neutron contribution to the dose, must result in a departure of the dose-effect relationship from linearity. For instance, assuming arbitrarily an RBE decreasing from 10 at 5 rads of neutrons to 1 at 100 rads implies that the risk from low-LET radiation varies between 2 cases per million per year per rad at 400 rads to 0.7 case at 60 rads. This could explain why no excess of leukæmia cases is observed

at Nagasaki in the groups exposed to less than 100 rads which received virtually no neutron contribution.

B. A-BOMB SURVIVORS (OTHER STUDIES)

37. In contrast to the ABCC-JNIH study in which investigation was confined to a sample population from the A-bomb survivors, other studies have investigated radiation effects in the unsampled, "open" population of all the survivors living in Hiroshima and Nagasaki. Leukæmia cases among survivors in these cities were ascertained through the leukæmia registry and the size of their parent population living in Hiroshima and Nagasaki was estimated on the basis of periodic census surveys.[1] These studies then have the advantage that radiation effects can be evaluated on all survivors rather than on a sample only. However, a serious disadvantage is that the number of survivors in these cities has become increasingly difficult to estimate accurately with the passage of time.

38. Since the 1964 report of the Committee, several investigators have studied the time trend of leukæmia occurrence (60, 65, 111, 156). Figure VII from

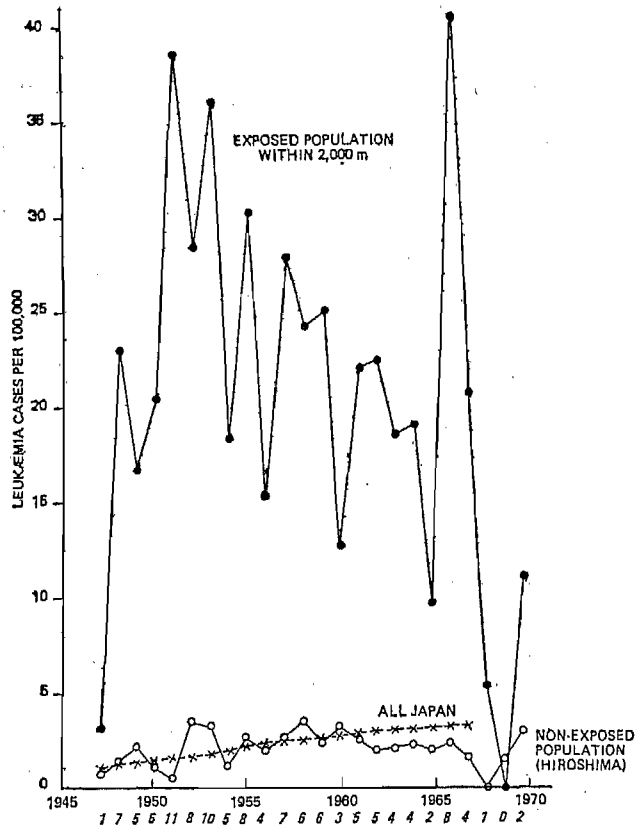

Figure VII. Leukæmia incidence rate in A-bomb survivors in Hiroshima, 1947-1970. In italics, number of cases within 2,000 metres (110, 111)

Ookita's report shows the trend among the Hiroshima survivors in the 1947-1970 period. It is clear from the figure that the Hiroshima survivors within 2,000 metres of the hypocentre ATB had an increase in the rate of leukæmia incidence compared to both the incidence rate in the non-exposed Hiroshima population and the mortality rate from leukæmia of all Japan. The survivors exposed within 2,000 metres were chosen for comparison since radiation dose beyond 2,000 metres was negligible according to the kerma-distance curves.

39. The incidence of leukæmia reached a peak in 1951, six years after exposure, and decreased gradually thereafter with considerable chance fluctuations, particularly in recent years, when the number of cases became small. The observed time trend essentially agrees with that observed among ankylosing spondylitis patients treated by x-irradiation (26), except that the latter showed a more rapid decrease in incidence after a peak was reached 4-5 years following the exposure. The A-bomb survivors had a clear excess risk of leukæmia for even as long as 20 years after exposure.

40. Tomonaga *et al.* (156) analysed the distribution of cell-specific types of leukæmia cases occurring among the A-bomb survivors throughout the country. During the period 1946-1965, 241 cases were found among the survivors exposed within 2,000 metres from the hypocentre. Among these leukæmia cases, the ratio of acute to chronic granulocytic leukæmia was 1.5 in Hiroshima and 2.6 in Nagasaki (the authors did not further classify acute leukæmias into cell-specific type). Among individuals exposed at or beyond 2,000 metres, the corresponding ratios were substantially higher, i.e., 4.9 in Hiroshima and 8.2 in Nagasaki. In the general population of Japan, this ratio was found to be 5.8 in 3,545 leukæmia cases recorded in a nation-wide survey (166).

41. The decrease in the ratio of acute to chronic granulocytic leukæmia among A-bomb survivors, particularly at Hiroshima, was interpreted by Tomonaga *et al.* (156) as a possible specific effect of A-bomb irradiation (neutron irradiation in particular) on the induction of chronic granulocytic leukæmia. A similar decreased ratio was also noted in studies on occupationally exposed populations (103, 165).

42. Consistent with the fact that radiation rarely, if ever, causes chronic lymphocytic leukæmia, no cases of that form of leukæmia were observed among the survivors who were within 2,000 metres of the hypocentre in either city (156).

C. ANKYLOSING SPONDYLITIS PATIENTS TREATED WITH X-IRRADIATION

1. *Material and methods*

43. Court Brown and Doll investigated the mortality experience of ankylosing spondylitis patients in the British Isles treated by therapeutic x-irradiation. Their 1957 report (26) on leukæmia induction was discussed in both the 1962 and the 1964 reports of the Committee. Their studies have since been extended, examining not only leukæmia but also other selected causes of death, including cancer of various sites (28).

44. The 14,554 patients with ankylosing spondylitis treated by x-irradiation in any of the 87 co-operating radio-therapy centres in the United Kingdom during the period 1935-1954 were followed until the end of 1959. The follow-up period was 5-25 years with an average of 10-11 years. The authors also showed the results of an extended but incomplete follow-up to the end of 1962. The duration of the extended follow-up period was 8-28 years, or 13 years

[1] As of 1950, the number of survivors who had been within 2,000 metres of the hypocentre ATB amounted to about 29,000 at Hiroshima and to 8,000 at Nagasaki (62a).

on the average. The study population included only adults, predominantly males (84 per cent). The patients were successfully followed by the end of 1959 with a follow-up rate of 98 per cent.

45. The causes of practically all deaths during the follow-up period were obtained, and the deaths were classified according to the 1957 International Classification of Diseases (ICD). The number of deaths thus recorded was compared with the expected number of deaths derived by applying the national mortality rates specific for age, sex, and calendar year to the person-year experience of the study population.

46. The radiation doses received by the patients were carefully estimated on the basis of the information on radiation exposure available in the medical records of a stratified sample of approximately one of every six patients. The x-ray treatments were from one course of fractionated exposures over a period of about a month to eight courses for periods of up to eight years. Both spinal bone-marrow exposures (roentgens) and whole-body integral exposures (megagramme roentgens) were estimated. The spinal exposures were estimated both as mean exposures to the marrow throughout the entire length of the spine, and as maximum exposures at a point in the spinal marrow.

47. The x-ray treatment of ankylosing spondylitis involved substantial direct irradiation of many organs and tissues in addition to bone and bone marrow. However, precise estimates of the radiation doses received by tissues other than the bone marrow have not yet been obtained.

48. The material and methodology of the ankylosing spondylitis study may be summarized as follows:

(*a*) Among studies of irradiated populations, the ankylosing spondylitis study has the second largest man-year experience next to that of the ABCC study;

(*b*) The results of the ankylosing spondylitis study are applicable only to adult, largely male, populations; the study gives no information on the risk of cancer induction among those exposed to radiation at ages under 15;

(*c*) In evaluating the excess risk of cancer in the ankylosing spondylitis series, it must be borne in mind that certain factors other than radiation (e.g., ankylosing spondylitis itself or other treatments of the disease) should be considered before the excess is simply attributed to the x-ray therapy;

(*d*) Since the ankylosing spondylitis study depends on mortality statistics, the results of the study do not provide information on less-fatal cancers (e.g., thyroid cancer) and cancers known to be unreliable on death notification (e.g., pancreas cancer).

2. *Leukæmia*

49. The major findings of the 1957 report of Court Brown and Doll (26) can be summarized as follows:

(*a*) The leukæmia incidence rises from 50 cases per million per year in the control population to 7,200 cases per million per year following a mean dose to the spinal marrow in excess of 2,250 rads (assuming one roentgen to correspond to about one rad);

(*b*) In the dose range between approximately 300 and 1,500 rads, the relationship between mean exposure to the spinal marrow and leukæmia incidence seems to be linear with a slope of about 0.5 case per million per year per rad;

(*c*) A significant excess of deaths occurs with all types of leukæmia, except chronic lymphocytic leukæmia (only one case of this type of leukæmia was observed);

(*d*) The leukæmia incidence rate increases with age from 1,100 per million for treatment at ages 14-24 to 5,600 per million at ages 55 and above. This age distribution (which is adjusted for the number of treatment courses) is consistent with the age distribution of the spontaneous leukæmia mortality of England and Wales (34).

50. Court Brown and Doll (28) briefly covered the further leukæmia-mortality experience of the ankylosing spondylitics in their 1965 report. In table 6, observed and expected numbers of deaths are presented for every three-year period after the beginning of the observation. Relative risks, the ratio of observed to expected deaths, reach a peak 3-5 years after the first observation and decline thereafter. At observation periods of 12 years and more, the relative risk is erratic because of large chance fluctuations. The extended (although incomplete) follow-up series probably gives more reliable relative risk figures by nearly doubling the man-years experience. At 12-14 years the relative risk is 9.2 (7 observed *versus* 0.76 expected) and at 15-27 years the relative risk is 1.9 (1 observed *versus* 0.54 expected). Because the observed numbers are so small, the above values of relative risk may not be very reliable. But they roughly indicate that the leukæmia risk, after a peak 3-5 years after irradiation, decreases with the passage of time, remaining still higher than that of the non-irradiated population at least up to 15 years after exposure.

51. The excess mortality of leukæmia from irradiation is about 50-55 cases per 15,000 patients (including some probable aleukæmic leukæmia cases mistaken as aplastic anæmia), or 3,000-4,000 cases per million exposed, over a follow-up period averaging 10-11 years from the date of the first observation. The excess mortality may naturally increase with extension of the follow-up period. However, because of the declining trend of the excess, and the already low yearly values, its over-all magnitude is not likely to be much higher than that already observed.

52. In the Committee's 1964 report, a possibility of error in evaluating the leukæmia risk by irradiation of the ankylosing spondylitis patients was pointed out, namely, a possible association between leukæmia and ankylosing spondylitis itself (109), and leukæmia and other therapeutic agents to which the spondylitics must have been exposed (8, 167). The 1964 report therefore stressed the necessity of determining the risk of leukæmia induction in ankylosing spondylitis patients without x-ray therapy.

53. In fact the number of ankylosing spondylitis patients who were not treated by irradiation is very limited. However, Doll (35) has indicated that in a series of nearly 1,000 patients with ankylosing spondylitis who were never treated by radio-therapy, only one case of leukæmia had thus far occurred. The case was one of chronic lymphatic leukæmia, which is known to be rarely, if ever, induced by ionizing radiation.

54. In view of (*a*) the clear dose-effect relationship; (*b*) the characteristic time trend; and (*c*) the specificity of leukæmia type, and also in view of the fact that leukæmia is known to be caused by ionizing radiation in a variety of populations, there can be no doubt today that the excess risk of leukæmia induction among the ankylosing spondylitis patients was largely caused by the x-ray treatment. Assuming that the irradiation involved, on the average, 30-50 per cent of the bone marrow, the slope of the dose-effect curve given in paragraph 49 would correspond to a risk estimate of 1-2 cases per million per year per rad between 300 and 1,500 rads.

D. Radiologist with occupational exposure

55. The results (40, 59, 94, 95, 114, 132, 160, 167, 170) of a number of studies on American radiologists, together with a study on British radiologists, were discussed in the 1962 and again in the 1964 report of the Committee. In addition, the study of Lewis (81) on American radiologists published in 1963 was reviewed in the 1964 report. According to this study, the average annual mortality from leukæmia among radiologists during the 14-year period 1948-1961 was 253 per million per year compared with an expected mortality (based on mortality rates in the United States general population) of 85 per million per year, so that the excess was 168 per million per year.

56. Seltser and Sartwell (134, 135) also studied the mortality experience of American radiologists. Their earlier paper gave the results of a pilot study that examined the practicability of assessing the effects of radiation on American radiologists. The subsequent study published in 1965 included 3,697 male members of the Radiological Society of North America who had entered the Society during the years 1915 through 1954.

57. Compared to the general population, this group of radiologists was certainly selected with regard to education, socio-economic status, etc., and may thus have had a different mortality experience. Therefore, comparison groups were chosen from among the various medical specialties rather than from the general population. The subjects of the comparison groups were: 7,052 male members of the American College of Physicians (ACP) and 6,059 male members of the American Academy of Ophthalmology and Otolaryngology (AAOO). The members of the AAOO were considered the group least exposed to irradiation, and were thus regarded as the group with the lowest risk. The members of the ACP were considered to have received exposures between those of the radiologists and those of the AAOO population. The study and comparison populations were traced successfully to the end of 1958, and the cause of death was secured for 99.3 per cent of those deceased. The number of deaths among the radiologists reached 944 for the years 1935-1958.

58. The mortality of the radiologists was examined in the four disease categories: cardiovascular-renal diseases, leukæmia, all other cancers, and all other causes. In considering all causes of deaths, radiologists in the age range 35-79 showed an excess of 228 deaths by comparison with members of the AAOO. Of this excess, 103.4 (nearly one half) were due to cardiovascular-renal diseases, 11.3 to leukæmia, 48.2 to "all cancers except leukæmia", and 65.3 to "all other causes". The relative risk of death in these age groups (the ratio of the observed number of deaths to the expected number) was the highest for leukæmia (2.5); the next highest were those for all cancers except leukæmia (1.6) and for all other causes (1.6); the lowest that for cardiovascular-renal disease (1.2).

59. Thus, the excess risk of leukæmia among the radiologists compared to the AAOO members was of the order of 200 cases per million per year; this is in accordance with the results of Lewis (81) who compared the mortality of radiologists with that in the general population. Since the radiation dose received by the radiologists over their entire occupational life could not be estimated, it was not possible to derive the risk of leukæmia induction per unit dose. The radiation exposure was obviously heavier in the earlier part of the radiologists' careers as a result of insufficient protection. No cell-specific analysis of leukæmia was reported.

E. Patients exposed to therapeutic irradiation in pelvic region

60. Three major cohort studies on patients exposed to therapeutic irradiation in the pelvic region have been reported since the 1964 report, and a summary of them is presented in table 7.

61. Doll and Smith (38) studied the mortality experience of patients with metropathia hæmorrhagica treated by x-irradiation. The irradiation was confined to the pelvic region and the doses employed, though considerably lower than those used in the treatment of uterine cancer, were sufficient to induce an artificial menopause. Most patients (97.2 per cent) were treated only once by short-term irradiation. The patients —2,068 women—were selected from three radiotherapy centres in the United Kingdom (Aberdeen, Dundee and Edinburgh) in the period 1940-1960 and were followed through 1963. The follow-up period ranged from 3 to 24 years, 13.6 years on average. The follow-up rate was as high as 99 per cent, and the cause of death was ascertained in each case. The observed number of deaths by cause was compared with the expected number computed by applying sex-age-period-specific rates for Scotland to the person-year experience of the study group. An agreement between the observed and expected number of deaths was found for the group of all causes of death (245 observed to 234.56 expected), as well as for several selected subcategories of causes.

62. For leukæmia, although the numbers were small, a significant excess of deaths was noted—6 observed to 1.31 expected ($P<0.01$)—yielding a relative risk of 4.6. The excess was found to occur in the period of five or more years after exposure, the largest excess showing in the 5-9-year range, with a slow decrease thereafter.

63. Among the 2,068 women studied, an excess of 4.69 cases of leukæmia was observed. Ninety-eight per cent of these women were estimated to have received mean doses of between 75 and 174 rads (average 136 rad) to the whole bone marrow. In this range, the risk of leukæmia induction per unit dose may be given as 1.2 cases per million per year per rad.

64. In 1960, Simon *et al.* (140) reported that the risk of leukæmia in a group of about 72,000 patients treated with radiation for carcinoma of the cervix was not increased in comparison with the British or American female population. However, because of

certain problems relating to the methodolgy of this study which were discussed in the 1964 report, Hutchison (63, 64) re-investigated the risk of leukæmia induction in another group of cervix cancer patients who had also received radiation therapy.

65. With the co-operation of 29 radio-therapy centres in nine countries, approximately 28,000 patients were followed by annual or semi-annual physical examinations, which included peripheral blood examinations. Hutchison's 1968 report (63) showed preliminary results of two to five years of observation subsequent to the inclusion of the patients in the study; 49 per cent of the patients were included within one year after radio-therapy, 26 per cent within 1-5 years, and the remaining 25 per cent more than five years after radio-therapy.

66. In 14 per cent of the patients, radio-therapy involved only intracavitary radium, in 8 per cent, only external radio-therapy, and in 69 per cent, external radio-therapy was combined with intracavitary radium. The remaining 9 per cent received no radio-therapy and served as a control group. In three fourths of the patients receiving external radio-therapy, the mean dose to the whole bone marrow was estimated to range from 300-1,500 rads. As with metropathia hæmorrhagica patients, the irradiation was restricted to the bone marrow in the pelvic region which constitutes one third of the total active (red) bone marrow. Therefore the mean dose to the pelvic bone marrow may be estimated to have been 900-4,500 rads. The course of radiation treatment usually ranged from four to eight weeks.

67. The person-year experience of the group of irradiated patients reached about 60,000 at the end of 1965, as against approximately 6,000 person-years in the non-irradiated group. Four leukæmia cases were identified in the irradiated group, with no significant deviation from the expected number of 5.1, computed by applying age-specific incidence rates of leukæmia in the general population to the person-year experience of the patients. The risk of leukæmia was also examined for three different time intervals after exposure (0-3, 4-8 and 9 or more years after exposure), but again no significant excess was observed in any of the three time periods. In the non-irradiated group, one leukæmia case was detected.

68. The continuation of this study (64) showed that, as of 1970, both the number of leukæmia cases and the person-year experience had approximately doubled, so that the incidence remained unchanged. The comparison of the observed number of deaths from leukæmia with the expected number, classified by type of treatment or time interval since irradiation, showed no significant difference. For the entire observation period, there were 10 observed deaths *versus* 10.6 expected in the irradiated group. In the non-irradiated group there were two observed to 0.6 expected deaths. An explanation of the failure to detect any excess risk of leukæmia was sought by the author in the apparent nature of the irradiation. In contrast to both the A-bomb survivors and the ankylosing spondylitis patients, the patients with cervix cancer received a very large dose in a relatively small volume of tissue. The author postulated that this heavy dose might have been more destructive to pelvic bone marrow than stimulative of leukæmogenesis.

69. Wagoner (164) investigated the effects of radiation on patients with benign and malignant gynæcological disorders. A first cohort, taken from Connecticut (U.S.A.), comprised 1,893 patients with benign gynæcological disorders—hyperplasia of the endometrium, fibrosis, etc.—who had been treated by either x rays (993 cases) or radium (900 cases) and 7,835 patients with uterine cancer treated by radio-therapy. A second cohort, taken from Massachusetts (U.S.A.), comprised 1,803 patients similarly treated by radio-therapy for their benign gynæcological disorders. The observation period ranged from 2 to 32 years and the numbers of observed and expected deaths were compared. The expected number of deaths was computed on the basis of incidence (in Connecticut) or mortality (in Massachusetts) rates in the general population, specific for age, sex and calendar year.

70. It is evident from table 7 that heavily-exposed patients had no increased risk of leukæmia. Among the patients who had uterine cancer and who were treated by radio-therapy (estimated mean pelvic-marrow dose of 900-4,500 rad) there was no increase of leukæmia occurrence—9 observed cases *versus* 8.6 expected. This finding is essentially the same as Hutchison's. The patients with benign gynæcological disorders who received relatively heavy radiation dose (300-900 rad) also showed no significant excess of leukæmia occurrence—3 observed cases *versus* 2.4 expected. In contrast to the patients who received a heavy dose of radiation, the lightly-irradiated patients did show an increased risk of developing leukæmia. In the patients with benign gynæcological disorders who received radiation doses of 159-503 rads (the Connecticut group) and 159-318 rads (the Massachusetts group), the relative risk was 3.2 in the former (9 observed to 2.8 expected), and 2.8 in the latter (10 observed to 3.5 expected).

71. Thus, the studies by Simon *et al*, Hutchison and Wagoner all demonstrate that patients with uterine malignancies who have received heavy doses from x-ray and/or radium therapy show no increased risk of developing leukæmia. In addition, the study on patients with benign gynæcological disorders treated by heavy irradiation also showed no increased risk of leukæmia induction.

72. As noted previously, this obvious absence of leukæmogenic effects of heavy irradiation can best be explained by the nature of the exposure. There were reasons to question the methodology of the study of Simon *et al.*, but the more carefully-conducted studies of Hutchison and Wagoner have yielded the same finding regarding leukæmia induction. While the evidence provided by Hutchison's earlier study could have been considered as inconclusive because of the short follow-up period, his newer data (in which the person-year experience had doubled) and the study of Wagoner with its long observation period (2-32 years) show that there is no excess risk of leukæmia. The fact that the irradiation area (that of the pelvic region only) was limited is not likely to account for the absence of effect since Doll's study of metropathia hæmorrhagica and Wagoner's study of patients with benign gynæcological disorders showed an evidently high rate of leukæmia induction although the patients had radio-therapy in the pelvic region only. It looks more likely that the cell-killing effect of high radiation doses far outweighs their leukæmogenic effect.

F. Patients treated with ^{131}I or ^{32}P

73. Pochin (119) investigated the long-term effects of ^{131}I therapy in a group of 215 patients with in-

operable thyroid carcinoma. The patients had been treated during the period 1949-1967, and, through periodic health examinations at intervals of approximately six months or less, the vast majority (96 per cent) were followed up to 1 January 1968.

74. The incidence and mortality from malignant neoplasms found to have occurred in this group of patients were recorded and compared with the expected incidence and mortality computed according to sex and age-group specific rates for the general population. For leukæmia, mortality equalled incidence: 4 observed deaths to 0.08 expected, showing a significant excess ($P<0.005$). Excluding leukæmia from the list of malignancies, the excess of the observed mortality over that expected vanishes. Based on incidence, however, a possible excess risk was noted for cancer of the breast: 4 cases observed to 0.94 expected.

75. To assess the risk of leukæmia, the radiation doses received by blood from circulating radio-iodide and organic radio-iodine and from iodine concentrated in tissues were estimated for each patient. On the assumption that the bone marrow received the same dose as blood, the excess risk of leukæmia was estimated as 14 cases per million per year per rad (total experience 2.7 10^5 man-year-rad). If the bone marrow received 80 per cent (51) or 44 per cent (80) of the blood dose, the estimated risk would need to be increased accordingly.

76. As indicated by the author, caution should be exercised in interpreting the results owing to uncertainties regarding the accuracy of the bone-marrow-dose estimate, the comparability of the patient group with the general population and the fact that the series had been selected for study specifically because of an increased incidence of leukæmia.

77. In its 1964 report, the Committee presented the results of two studies which investigated the risk of leukæmia in patients with thyrotoxicosis exposed to low-dose irradiation from radio-iodine. The study by Pochin (118) showed an observed incidence of 18 cases as opposed to an expected incidence of 21 in an estimated 59,000 patients with thyrotoxicosis treated by ^{131}I. In Werner's study (171), 10 cases of leukæmia were observed as opposed to 13.8 expected among the 32,000 patients with the same disease and receiving the same treatment. In both studies the general population served as the comparison group and as the basis for computing the expected figures.

78. Saenger *et al.* (128) have investigated the incidence of leukæmia in 36,000 patients with hyperthyroidism treated in 26 medical centres by low doses of ^{131}I from 1946 to 1964. The majority (96-97 per cent) of the patients were followed to mid-1967. In this study the risk of leukæmia in patients treated with radio-iodine was evaluated by comparison with those treated surgically.

79. The person-year experience of the ^{131}I group and the surgery group was similar—119,000 and 114,000, respectively; almost identical numbers of leukæmia cases were observed, 17 in the ^{113}I group and 16 in the surgery group. Each of these groups was further subcategorized by sex, type of leukæmia, and differing time intervals following treatment, in an effort to detect an increase in incidence relating with any of these factors. However, no such relationship was discernible among the subcategories.

80. Besides, the leukæmia cases found in this study were compared to the non-leukæmia patients on the basis of administered dosage of radio-active iodine; they were found not to have received radio-iodine in amounts greater than the non-leukæmia patients, i.e., 8.9 millicuries and 10.6 millicuries, respectively. The average bone-marrow dose was believed to have been in the range of 7-13 rads in the leukæmia patients and of 8-15 rads in the non-leukæmia patients.

81. The absence of excess risk of leukæmia in this study might well have been expected in view of the low dose of ^{131}I administered. In fact the rate of 0.7–2.0 per million per year per rad suggested by the studies of Japanese A-bomb survivors for low-LET radiation, when applied to approximately 1.2 10^6 patient-year-rads in the group treated with ^{131}I, leads to an expectation of at most three induced cases of leukæmia. This small excess lies within the range of chance variability, so that even if leukæmia induction had resulted from the exposure of these patients to ^{131}I, no significant excess would have been observed.

82. It is of interest to note that both the ^{131}I group and the surgery group had a higher rate of leukæmia mortality than the general population. This observation might indicate that hyperthyroidism itself may be associated with higher rates of leukæmia. In a group of patients treated with both radio-iodine and surgery, a significant excess of leukæmia cases ($P<0.05$) as compared to the groups treated with either radio-iodine or surgery alone was noted. No clear explanation of this finding was presented.

83. In its 1964 report the Committee also discussed the excess risk of developing leukæmia among patients with polycythæmia vera treated with ^{32}P and pointed out that polycythæmia vera itself might predispose to, or be closely associated with, leukæmia making it desirable to compare the risk of leukæmia in polycythæmics treated by ^{32}P with that in similar patients treated otherwise.

84. Since that report, Modan and Lilienfeld (104) have studied the risk of leukæmia in such patients and shown that it is much higher in ^{32}P-treated patients than in patients not treated by radiation. The authors selected from seven co-operating hospitals in the United States 1,222 patients treated between 1937 and 1955 who met certain diagnostic and demographic criteria. Of these patients, 228 were polycythæmia vera cases treated with ^{32}P only and 133 were cases with the same condition but with no radio-therapy. The majority (98.4 per cent) of the 1,222 cases were followed to 31 December 1961. The frequency of occurrence of acute leukæmia was found to be as high as 25 cases (11 per cent) in the 228 ^{32}P-treated group, in sharp contrast to only one case (0.8 per cent) in the 133 non-radiation-treated group. (Chronic leukæmia was not included because of possible diagnostic uncertainties.) Between zero and 30 millicuries, the incidence of acute leukæmia rose approximately in proportion to the dose of ^{32}P at the rate of about 1 per cent per millicurie of ^{32}P injected, with a mean follow-up time of about eight years. Using a conversion factor of 30 rads to the bone marrow per millicurie of ^{32}P injected (see annex B) this would correspond to a risk of about 40 cases per million per year per rad.

85. In addition to the ^{32}P treatment, several other factors were analysed by the authors in an effort to account for the observed difference in the incidence

of acute leukæmia occurring in the groups, but no plausible factors were identified. Although in this study the control group was adequately chosen from polycythæmia vera patients not treated by radiation, the very high risk of acute leukæmia after ^{32}P may, as the authors themselves pointed out, be the result of an unusually high sensitivity to radiation of the polycythæmic bone marrow.

86. Tubiana *et al.* (159) showed, in a series of 296 patients, that the amount of ^{32}P administered to polycythæmics was larger in those with high initial white counts and enlarged spleens, suggesting that the increased rate of leukæmia may at least in part be due to the biological factors that determine the treatment.

II. Thyroid neoplasms

A. A-BOMB SURVIVORS

87. In the Committee's 1964 report, it was stated that the two ABCC studies that had been published at that time (141, 176) suggested that the incidence of thyroid cancer among A-bomb survivors was inversely related to the distance from the hypocentre at the time of the bombing (ATB).

88. The recent report of Wood *et al.* (173) confirms the earlier findings. It now seems certain that thyroid cancer has increased among those A-bomb survivors who were proximally located to the hypocentre ATB.

89. Thyroid carcinoma is commonly a non-fatal disease. An intensive survey in one country found that the relative five-year survival rate[2] of thyroid cancer in females—thyroid cancer is predominantly a female disease—is 80 per cent for all ages combined and 96 per cent for those under 45 years of age (32). Because of the low fatality rate of thyroid cancer, it is appropriate for the Committee's purpose to measure the risk of the disease in terms of morbidity rather than of mortality.

90. Wood *et al.* (173) based their findings on the Adult Health Study Sample (morbidity sample) of about 20,000 subjects who had routine biennial health examinations from 1 December 1963 to 31 December 1965. In 1964-1965, the examination rate among all living subjects of the Adult Health Study Sample was about 80 per cent for those over 40 years of age and somewhat lower for those under 40 years. The authors believed that examination rates did not differ substantially with exposure status.

91. Among the more than 13,000 persons examined in 1964-1965, 39 thyroid cancer cases were found and histologically confirmed. In addition, 386 individuals showed other thyroid abnormalities, a majority of which (298) were non-toxic goitres. Although the report is not very clear, some of the 39 cancer cases were presumably diagnosed and treated sometime before the 1964-1965 examination. The distribution of these cases in relation to sex, age, and distance from the hypocentre is presented in table 8. Since little difference is noted between Hiroshima and Nagasaki, the data for both cities are combined.

92. From table 8, the following observations may be made:

(*a*) The proximally exposed subjects show much higher rates than those distally exposed. Among females, the difference between the rates in the different exposure categories is statistically significant ($P<0.01$). The number of male cases was too small for statistical tests to be performed. For females of all ages combined, the group exposed within 1,400 metres has a 2.5 times higher rate than the 1,400-1,999-metre group and a 3.9 times higher rate than the 3,000+-metre group. The corresponding figures for males are even higher than for females, i.e., 4.0 and 9.0, respectively;

(*b*) There are indications that thyroid cancer occurs among the survivors more frequently in females than in males. The sex ratio of females to males is 2.2 for the proximally exposed group (within 1,400 m group). However, this does not necessarily mean that females are relatively more sensitive to thyroid cancer induction than males, since the natural occurrence of the disease is known to be much higher in females than in males (37, 78, 102);

(*c*) The age variation in susceptibility to the induction of thyroid cancer by A-bomb irradiation is unclear. Among males within 1,400 metres, all thyroid cancer victims were less than 40 years of age at the time of examination. However, the number of cases is too small (only 5) to conclude that younger men are more susceptible than older men. For females, the cases of thyroid cancer do not cluster in younger subjects: for those within 1,400 metres, the rates are 10.7 for those <40 years of age, 4.4 for those 40-59 years, and 8.5 for those 60 and above.

93. Beside expressing the relation of thyroid cancer risk of induction by irradiation in terms of distance from the hypocentre as shown in table 8, Wood *et al.* also expressed this relation in terms of the new kerma estimates for survivors within 2,000 metres of the hypocentre. As the actual number of cases is not recorded, only the rates per 1,000 examined are shown in table 9. As seen in the table, the risk of thyroid cancer clearly increases with increasing kerma for both sexes. The rates of the 200+ rad group are 9.1 per thousand for females and 4.1 per thousand for males. The figure of 9.1 for females is 3.3 times that of the 0-49 rad group and 1.3 times that of the 50-199 rad group. The corresponding ratios for males are 3.7 and 1.6, respectively.

94. For the purpose of radiation protection, even a rough estimate of the risk of thyroid cancer induction per rad among the A-bomb survivors would be of value. However, because of the inclusion of cases which were detected at an undetermined time prior to the 1964-1965 examination and because of the long duration of thyroid cancer, the period of time during which the observed cases of thyroid cancer developed has been difficult to ascertain. Considering the time interval between exposure to radiation (1945) and examination (1964-1965), that period of time should be less than about 20 years; and because of the long duration of the disease, the time period is likely to be more than 10 years. If the time period ranges from 10 to 20 years and if the difference in the average dose is 100 rads between the 0-49 and the 50-199 rad groups and 200 rads between the 0-49 and 200+ rad groups, then the risk of induction of thyroid cancer in the range 25-200 rads is 1-2 cases per million per year per rad for males and 2-4 cases for females.

[2] The survival rates presented are the adjusted rates which the patients would have experienced had deaths been only from thyroid cancer.

These figures, of course, should be taken as highly tentative, particularly because of wide uncertainties about doses (no allowance having been made for the RBE of the neutron contribution) and about the duration of the observation period. More accurate estimates can only derive from further and more detailed data.

95. Sampson *et al.* (129) have reported on the prevalence of occult thyroid carcinoma in the autopsy series of the Life Span Study Sample. Under the ABCC autopsy programme, 3,067 autopsies were performed during 1957-1968 in Hiroshima and 1951-1967 in Nagasaki. The majority (89 per cent) of the autopsies were performed during 1961-1967, when the autopsy rate was 39 per cent with little bias relating to radiation exposure. Among the 3,067 subjects, 536 cases of thyroid carcinoma were found after histological examination of serial sections of thyroid glands. Almost all of the identified cases were clinically occult (97 per cent). Histologically, 98 per cent were papillary adenocarcinomas.

96. The prevalence of thyroid carcinoma in the autopsy series is shown in figure VIII. The authors

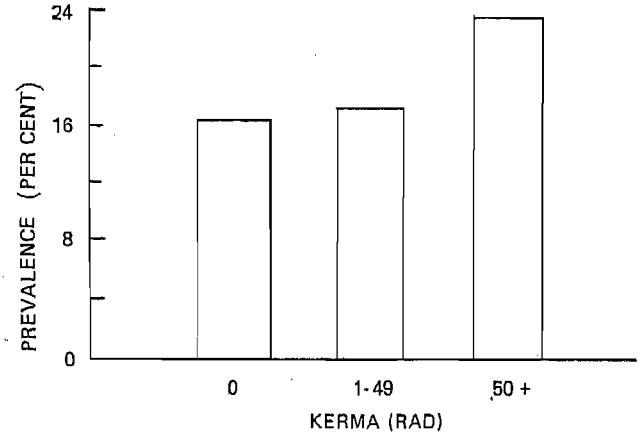

Figure VIII. Prevalence of thyroid carcinoma among autopsied cases of A-bomb survivors as a function of kerma (129)

indicate that the prevalence rate was significantly higher among those exposed to 50 rads or more compared to those exposed to less than 50 rads. The 50+ rad group had a 41 per cent excess, and the 1-49 rad group a 5 per cent excess over the non-exposed group.

97. In spite of the observed dose-effect relation, the meaning of this study is difficult to assess. The relation between clinically apparent thyroid carcinoma and occult thyroid carcinoma has not been clearly established. For occult carcinoma, ratios of males to females are 1.2 in this study and 1.0 in another similar study in Japan (155), whereas for clinically manifest thyroid carcinoma the sex ratios vary from 2 to 3 (36, 78, 102). The observed prevalence rates in this study—15.7 per cent for males and 19.4 per cent for females—are unusually high compared to the rates of clinically apparent thyroid carcinoma—0.8 per cent for males and 1.8 per cent for females (78). For occult thyroid carcinoma the observed rates of 10-20 per cent in the Japanese population in Japan (129, 155) and among Japanese descendants in Hawaii (U.S.A.) (49) are much higher than those reported (56, 105) in the American series (1-3 per cent), although the rates of clinically manifest thyroid carcinoma in Japan and the United States are similar (36, 37). Thus, in view of the unclear role of occult thyroid carcinoma in the development of clinically manifest thyroid carcinoma, the study of Sampson *et al.* is only suggestive of radiation-induced thyroid cancer among the A-bomb survivors.

B. Residents of the Marshall Islands exposed to radio-active fall-out in 1954

98. After the Committee's 1964 report, a substantial body of evidence has accumulated regarding increased risks of the induction of thyroid tumours among residents of the Marshall Islands accidentally exposed to radio-active fall-out in 1954 (23, 24). A comprehensive monograph of Conard *et al.* in 1970 (25) gave detailed information about thyroid-tumour induction in the residents exposed to fall-out.

99. The accidental exposure of these islanders occurred in March 1954 during hydrogen-bomb testing at Bikini Island. The inhabitants of the island of Rongelap were the most exposed, having received an estimated whole-body dose of 175 rads of gamma radiation and a dose contribution from the internal deposition of radio-nuclides such as ^{89}Sr and ^{131}I. The presence of a burden of radio-nuclides was detected by radio-chemical analyses of urine samples and was thought to have probably been brought about mostly by eating and drinking contaminated food and water and to a lesser extent by inhalation. The body levels of the radio-nuclides fell off rapidly, so that six months later radio-activity in the urine was barely detectable. Besides the Rongelap residents, the people of the islands of Ailingnae and Utirik were exposed to substantial internal and external doses.

100. Extensive medical examinations were performed on the exposed population immediately after the exposure, and annual health examinations have been carried out since. The relatives of the exposed individuals of Rongelap island who were away from the island at the time of the accident and who returned thereafter served as an adequate control population in evaluating the late effects of radio-active fall-out.

101. Only thyroid tumours are reported to have been induced in the exposed population. This is probably due to the fact that the thyroid gland was exposed to high doses of radiation from radio-iodine. No cases of leukæmia have been detected.

102. Table 10 shows the frequency of benign and malignant thyroid tumours in the 15-year period 1954-1969, together with estimated external gamma-ray doses and doses from internal deposits of radio-iodine in the thyroid gland. The estimate of the internal deposits was made on the basis of radio-chemical analysis of urine obtained several weeks after the exposure. In addition to ^{131}I, the isotopes ^{133}I, ^{135}I, and to a lesser extent ^{132}I contributed significantly to the thyroid dose. The main source of iodine ingestion was considered to be water, and since the water was being rationed at the time of the fall-out, it was assumed that the same amounts of iodine isotopes were absorbed by each person irrespective of sex and age. As shown in the table, the total estimated thyroid dose from the various iodine isotopes for the Rongelap people was 160 rads for adults and from 500 to 1,400 rads for children, taking into account the smaller size of the children's thyroid glands.

103. The first case with a thyroid lesion was detected in 1963. This case was found nine years after exposure, in the course of an annual health examination which disclosed an asymptomatic thyroid nodule that was later proved by histological examination to be a benign adenoma. Since then, increasing numbers of thyroid abnormalities have been detected in the exposed populations, particularly at Rongelap. As shown in table 10, the number of clinically apparent thyroid lesions reached 21 cases (19 cases of nodular gland and 2 cases of atrophic gland) in the Rongelap population in the 15-year period 1954-1969. Surgical exploration was carried out in 18 of the 19 nodular thyroid glands, and revealed malignant lesions in three of them and benign adenomatous lesions in the remainder.

104. The group of Rongelap children of ages <10 (the group exposed to the highest dose) showed a strikingly high frequency of thyroid lesions (89.5 per cent), in contrast to the absence of lesions in people of the same age in the less exposed and unexposed groups. It was clear that the more exposed group had a higher incidence of thyroid lesions. Three malignant lesions among the 53 Rongelap residents (5.7 per cent) were noted; the expected number on the basis of incidence statistics among the 17,000 Marshallese was 0.056, showing a significant difference at $P<0.01$.

105. Although it is probably impossible to make an accurate dose estimate, the risk of thyroid nodularity in the exposed Marshallese was estimated by the authors to be about 50 cases per million persons per year per rad in a dose range from 500 to 1,400 rads. Based on the one in six proportion of malignant cases revealed by surgical exploration, the risk of nodularity would correspond to a risk of carcinoma close to 10 cases per million per year per rad. This estimate is, of course, subject to the inaccuracy of numerous factors that affect the dose estimates, and therefore may be regarded as a tentative rough index of thyroid cancer induction by irradiation in the exposed Marshallese.

III. Breast cancer

A. A-BOMB SURVIVORS

106. Wanebo *et al.* (168) have investigated the risk of breast cancer among A-bomb survivors in Hiroshima and Nagasaki according to the new (T65D) kerma estimates. Because of the low early fatality of breast cancer (32) the authors assessed the risk in the morbidity sample (Adult Health Study Sample), on which biennial health examinations are performed. The study population comprised 10,357 women in 1958. Of these, approximately 98 per cent had been examined at least once by 1966. No remarkable difference was noted in the proportion of those examined in the different dose groups.

107. Beside the clinical data obtained at the biennial health examinations, the following sources gave additional information: autopsy diagnosis, surgical pathology diagnosis, death certificates, and local tumour registries. From these sources 25 cases of breast cancer were found among the women of the morbidity sample from 1958-1966. Of the 25 cases, 22 were confirmed by tissue examination, and the remaining three cases were designated as possible cases.

108. The distribution of the 22 cases in relation to kerma is shown in table 11. As noted, there is a clear increasing trend in the relative risk of breast cancer with increasing dose. Those who had received 200 rads or more have about twice the risk of those exposed to 40-89 rads and six times the risk of those exposed to 0-9 rads. This increase is statistically significant ($P<0.01$ between the groups of survivors over and under 90 rads). It is noteworthy that even low-dose groups (10-39 and 40-89 rad) show higher risks than the non-exposed population.

109. The excess number of breast cancer cases in the female A-bomb survivors exposed to 10 or more rads may be estimated as 11.5, or approximately 400 cases per million exposed per year. If the mean dose of these survivors is in the range from 100 to 200 rads, the excess risk would be of the order of 2-4 cases per million per year per rad.

110. The mean induction period for definite cases is 15.4 years and the mean age at onset of the disease is about 10 years less at high doses (50+ rad) than at low doses (<50 rad). No clear relation between dose and histologic type of breast cancer was observed. The majority of cases, 78 per cent, were infiltrating duct carcinomas.

111. Breast cancer is known to be associated with such factors as socio-economic status, lactation period, parity, and marital status. The recent international study of MacMahon *et al.* (92)—a case control study covering over 17,000 cases and controls in different countries—indicates that non-parous women have a higher risk relative to parous women. Among the Japanese the relative risk is 1.56.

112. Wanebo *et al.* (168) found that (although statistically non-significant) breast-cancer patients among A-bomb survivors did tend to be unmarried, less parous, and to have lactated for shorter periods. They did not record how such factors related to different exposure categories. Therefore, it is impossible to estimate to what extent the observed dose-effect relationship may be explained by the aforementioned factors. However, it is also obvious that the observed relationship could not be entirely explained by the confounding of extraneous variables. For example, even if all of the women in the 200+ rad group were non-parous and all non-exposed subjects were parous, the relative risk would then still be only 1.56 according to the data of MacMahon *et al.* (92), whereas the observed relative risk is about 6.0.

113. The uncertainty of the study of Wanebo *et al.* may lie in the fact that the observed number of cases is very small (only 9 cases in the 90+ rad group). This small number is likely to have been affected by large chance fluctuations and by the aforementioned variables, or even by the fact that the ascertainment rate of cancer may have been higher in the heavily exposed group if the subjects had appeared more frequently at medical examinations than had those in the less exposed group.

114. It may be concluded that the study of Wanebo *et al.* strongly suggests that the survivors heavily exposed to irradiation are at increased risk of breast cancer, but a definite conclusion will be obtained only after more data have accumulated.

115. In their mortality reports for 1950-1966, Beebe *et al.* (10, 11) dealt with the risk of breast cancer in the Life Span Study Sample. Sixty-seven deaths were ascribed to the disease. No statistically significant

dose-effect relation was observed for the whole 1950-1966 period, and none for any of the four-year periods betwen 1950 and 1966, except for the last one. In the 1962-1966 period, a statistically significant relation between breast-cancer mortality and dose was observed ($P \sim .05$). The authors concluded from the mortality data that the evidence regarding radiation effects on female breast cancer was merely suggestive.

116. The less clear evidence of radiation effects observed in this mortality study as compared to Wanebo's may be more apparent than real. Mortality data are expected to lag behind morbidity data because of the low fatality of the disease; the five-year survival rate of breast cancer is reported (32) to be 50 per cent, and only one third of the breast cancer cases in Wanebo's study was identified through death notification. Besides, radiation effects on breast cancer seem to have become apparent in recent years—the effects were only seen in the 1962-1966 period in the study of Beebe *et al.* Therefore, Wanebo's study covering more recent years (1958-1966) should show a stronger dose-effect relationship.

117. The mortality study of Jablon and Kato (73, 74) has added new mortality data for the period from 1967-1970. In table 5, the number of deaths from breast cancer in 1950-1970 amounted to 104, of which 80 were recorded in Hiroshima and 24 in Nagasaki. Compared to the expected deaths based on national rates, only the 100-199 rad group of Hiroshima, among the various individual dose groups, showed a significant excess ($P<0.05$). However, when all the survivors, except the virtually non-exposed belonging to the 0-9 rad group, are put together, both Hiroshima and Nagasaki show significant ($P<0.05$) excesses—29 *versus* 13.6 in Hiroshima and 12 *versus* 4.6 in Nagasaki. Assuming that the neutron RBE varies from 10 to 1 as in the case of leukæmia (see paragraph 36), the Hiroshima results would suggest tentative risk estimates for exposure to low-LET radiation of 0.3 and 1 case per million per year per rad at 60 and 400 rads, respectively. The lower risk for breast cancer obtained in this mortality study in comparison with Wanebo's morbidity study may be explained at least in part by the relatively low fatality of the disease.

B. Tuberculosis patients exposed to repeated fluoroscopic examinations

118. Mackenzie (87) reported in 1965 that tuberculosis patients exposed to repeated fluoroscopic examinations for pneumothorax treatment were at increased risk of developing breast cancer. This possibility was first suggested by the findings of an apparent radiation dermatitis of the skin over the right chest wall, breast and sternal region in a female patient in whom cancer of the breast had been diagnosed. Her past history revealed that, for the treatment of pneumothorax, she had undergone at least 200 fluoroscopies over a 46-month period some 14-15 years previously. Her radiation reaction was suggestive of an accumulated exposure of over 4,000 roentgens. The artificial pneumothorax was a world-wide common practice for the treatment of lung tuberculosis before the introduction of chemotherapy; in North America, this therapy was common from the 1920s to about 1950. Fluoroscopic examination was made each time (usually before and after) the pleural cavity was refilled with air.

119. The author then searched the Tumour Clinic files of Nova Scotia (Canada), which revealed 50 cases of breast cancer patients with a previous history of pulmonary tuberculosis. Of these, 40 were found to have had artificial pneumothorax therapy accompanied by fluoroscopic examination. In many cases, fluoroscopic examinations had been repeated quite frequently with consequent substantial radiation exposure of the patient: 16 patients had 100-200 fluoroscopies and 9 had more than 200.

120. An accurate estimate of the radiation dose received by the patients could not be made because of the inherent difficulties of dosimetry for fluoroscopic examinations. It is quite likely, however, that in many cases the doses to breast tissue were very high because of features related to the methods of the fluoroscopic examinations. These included orientation of the patients so that the x-ray beam entered anteriorly, the use of x-ray beams with low inherent filtration and little or no added filtration, and high screening currents to compensate for inadequate dark adaptation.

121. The patients tended to have cancer involvement in the breast on the same side as the treated lung. Among 24 cases having unilateral pneumothorax treatment, ipsilateral breast cancer was observed in 15 cases. The location of the tumour in these patients tended to occur in the central or inner half of the chest (72.8 per cent), the area most likely to have been included in the fluoroscopic field. This finding is in marked contrast to the usual distribution of malignant tumours within the breast, where the outer half is predominantly involved (53).

122. The age of onset of breast cancer was compared in two groups of patients classified on the basis of whether they had multiple fluoroscopic examinations or not. The exposed group showed a much younger age distribution than the non-exposed group, and the difference was statistically highly significant. However, this finding is difficult to interpret, since the pneumothorax treatment was introduced only after about 1920 and the number of individuals so treated must have been very limited among those that were at least 60 years old at the time of the report, in 1965.

123. The author also presented the results of a follow-up study comparing the occurrence of breast cancer between two groups of female tuberculosis patients in a sanatorium. Thirteen breast cancer cases were discovered in the pneumothorax-treated group of 271 cases, and only one case in the other group (without pneumothorax treatment) of 510. Although the difference between the occurrence rates of breast cancer in the two groups was impressive, this report lacked information about the extent to which the follow-up was complete, which is undoubtedly essential for the evaluation of the results. Therefore, in this report, even the relative rates of occurrence of breast cancer between the two groups was uncertain, and much more so for the absolute rates of breast cancer occurrence.

124. Myrden and Hiltz (107) studied tuberculosis patients traced up to 1966 who had been treated at the Nova Scotia Sanatorium—presumably the same sanatorium as that used as a source by Mackenzie—during the years 1940 to 1949 inclusive. The period of observation ranged for individual patients from 15 to 25 years after treatment. Among 867 female patients eligible for admission to the study, 783 were traced, with a follow-up rate of 90 per cent. Of those 783 patients, 300 received pneumothorax treatment accom-

panied by repeated fluoroscopies, while the remaining 483 were not so treated. Twenty-two cases of cancer of the breast were observed in the former (7.3 per cent) and only four in the latter (0.8 per cent). The annual incidence in the pneumothorax group was, on average, 0.36 per cent for the entire follow-up period—a strikingly higher rate compared to that of the general female population, which in Nova Scotia was 0.055 per cent in 1965.

125. The average time from the beginning of pneumothorax treatment to the development of breast cancer ranged from 8 to 24 years, or 17 years on the average.

126. There was a clear agreement between the side of the chest exposed to the fluoroscopies and that of cancer involvement of the breasts. In the 22 cases of breast cancer occurring in the treated group, pneumothorax treatment was restricted to one side in 17 of the patients; 14 of these patients were later found to have developed their breast cancer on the side of treatment.

127. When the 22 breast cancer cases with pneumothorax treatment were classified according to the number of fluoroscopies received, the majority of them (19 cases) were found to have undergone more than 75 examinations and, among these, 13 had had over 175 examinations.

128. The estimation of the doses received by these patients is difficult but Mackenzie (87) reported dose rates to the skin of the breast when the patient was facing the x-ray tube. The most probable conditions resulted in exposure rates of 22 roentgens per minute if a one-millimetre aluminum filter was used or 55 roentgens per minute without a filter, and assuming that a five milliampere screening current was used. A 10 second exposure was recommended to the physicians, but Mackenzie infers that higher currents and larger exposures were not uncommon. Assuming that the actual exposures were equivalent to a 10-20 second exposure, the total skin dose to the breasts of a patient examined for an average of 160 examinations would have been in the range 600-3,000 rads depending on the irradiation time and whether a filter was used or not (actual doses to some individuals may have been even up to 10-15,000 rad). The excess of 20 cases of breast cancer in 300 patients followed for an average period of 20 years reported by Myrden and Hiltz (107) would therefore correspond to 1-6 cases per million per rad per year in the dose range just discussed.

129. With regard to risk of cancer induction other than to the breasts, no information was given in the reports of either Mackenzie or Myrden and Hiltz, so that it is not clear whether the tuberculosis patients with repeated fluoroscopies had an excess risk of developing other cancers such as lung cancer, nor whether this possibility had been examined.

130. There have been several case reports (84, 96, 112) of cancer of the breast occurring in patients who had previously undergone radiation therapy. One such case was that of a male who developed breast cancer 35 years after radiation therapy for gynecomastia (84). However, these case reports of one or two patients are only suggestive of breast-cancer induction by irradiation.

131. Mettler *et al.* (100) followed up 606 women treated with x rays for acute post-partum mastitis. The follow-up period in most cases was from 10 to 25 years. Eighty-nine per cent of all patients were traced in a first survey in 1962 and 85 per cent in a subsequent survey in 1967.

132. The radiation treatments were mainly carried out with x rays generated in the 175-250 kVp range. Of the 606 patients, 183 received bilateral treatment. While the mean exposure to one breast field was about 350 roentgens, most of the exposures were in the 100-499-roentgen range but up to about 1,000 roentgens were given in some cases. The average exposure to all the breast tissue (expressing for all patients the mean of the exposures to both breasts even when the contralateral breast was not irradiated) was 211 roentgens.

133. Thirteen confirmed cases of breast cancer were observed in contrast to the expected number of cases, 5.86, the computation being based on the incidence of breast cancer in the female general population of comparable age. For cancer of all sites, this group of patients showed an excess of 6.35 cases (28 observed *versus* 21.65 expected), but the excess could be entirely accounted for by the excess of breast cancer.

134. Although the patient group apparently had a higher risk of developing breast cancer, its causation should not simply be attributed to the previous x-ray treatment. In this study the dose-effect relationship between radiation dose and the risk of breast cancer was not very clear. It is possible that acute post-partum mastitis itself might have been responsible for the high risk of developing breast cancer rather than the previous exposure to radiation. Some of the benign breast conditions are suspected of having had a positive association with breast cancer (83) and acute post-partum mastitis also might be so associated. This possibility could best have been evaluated had a comparison group been chosen from among the patients with acute post-partum mastitis treated by other than x-ray irradiation. However, the number of patients so treated is limited and such a study has not been reported thus far.

IV. Lung cancer

A. A-bomb survivors

135. The association of lung cancer with radiation exposure in the A-bomb survivors was first suspected by Beebe *et al.* (9) who observed in the 1961-1965 autopsy material of the Life Span Study Sample a significant excess of deaths from cancer of the lung (16 observed *versus* 9.8 expected, $P \sim 0.05$) among the survivors located at less than 1,400 metres from the hypocentre ATB, whereas such an excess could not be found in the less exposed groups at 1,400-1,999 metres and at 2,000+ metres.

136. Wanebo *et al.* (169) investigated the relation of lung cancer to irradiation by utilizing all available sources at the ABCC in both the Life Span Study Sample and the Adult Health Study Sample through 1966. The authors included among their sources the mortality and autopsy data of the Life Span Study Sample, the clinical data of the Adult Health Study Sample, the tumour registries of Hiroshima and Nagasaki, and surgical specimens sent to the ABCC by private practitioners.

137. During 1950-1966, 188 deaths occurring in the Life Span Study Sample (mortality sample) were

attributed to lung cancer, most of which (154) occurred in the latter half of the observation period. The risk of death from lung cancer appeared to increase with increasing kerma (T65D) and, when all the subjects were classified into either the 90+ rad or <90 rad group, the difference between the two groups was statistically significant ($P \sim 0.001$).

138. In the Adult Health Study Sample (morbidity sample), 66 cases of lung cancer were confirmed by pathologic examinations or thought to be probable cases on the basis of radiological and clinical findings. The distribution of the 66 cases, as with the mortality sample, showed the risk for lung cancer to be increasing with kerma. However, the difference between the 90+ rad and the <90 rad groups was barely significant ($P \sim 0.05$). Information regarding the distribution of lung cancer by histologic type is available for only 52 cases of which 40 per cent were classified as adenocarcinoma, 37 per cent as squamous carcinoma and 20 per cent as undifferentiated carcinoma. No relationship between radiation exposure and histologic type was observed in this small series of subjects.

139. Since smoking is known to be causative of lung cancer, an attempt was made to establish whether smoking was a confounding variable by determining its relationship to radiation dose and lung cancer in the adult health sample. The number of cases was too small to obtain conclusive results, but there was no evidence that the difference between exposure groups was due to different smoking habits in the two groups.

140. In their mortality analyses of various causes of death, Beebe *et al.* (10, 11) reviewed the deaths from lung cancer recorded in the Life Span Study Sample for 1950-1966. Except for minor differences, their results were essentially the same as those of the mortality part of the study of Wanebo *et al.*, because both studies covered the same study period and the same sample.

141. More recently, Jablon and Kato (73, 74) have reviewed the 1950-1970 mortality data from the Life Span Study Sample. The sample now includes 246 cases of lung cancer in Hiroshima and 71 in Nagasaki (table 5). When the deaths occurring in the practically non-exposed population (the NIC and the 0-9 rad groups) are excluded, the number of lung cancer deaths in the exposed survivors are reduced to 79 in Hiroshima and 22 in Nagasaki.

142. As stated earlier, the expected numbers of deaths calculated by three different methods give, in general, similar values for all causes. For lung cancers, however, the expected deaths based on the NIC or 0-9 rad groups are substantially and consistently higher than those, age- and sex-adjusted, based on national rates. The former may be preferred to the latter in comparing with the observed numbers, since the ABCC cohort belongs to an urban population, and the prevalence of lung cancer in urban areas is known to be higher than the country-wide average for Japan (137).

143. In the survivors exposed to more than 10 rads, a significant excess of deaths (observed minus expected) was noted for Hiroshima ($P \sim 0.01$), 79 observed against an expectation of 47.8 (0-9 rad) or 39.5 (national rates). The risk of lung-cancer death in Hiroshima clearly increases with kerma, the observed/expected ratios being 1.81 (10-49 rad), 1.97 (50-99 rad), 2.30 (100-199 rad), and 2.68 (200+ rad). The rate of increase of lung-cancer deaths with kerma, however, appears to decrease in higher exposure categories. Thus, in terms of kerma, the risk per million per year per rad varies according to exposure category as follows: 3.2[3], 1.5, 1.1 and .3.

144. Kerma is not the quantity in terms of which the risk of cancer of deep tissues, including lung, should be expressed, particularly if the risks need to be normalized to those of low-LET radiation, since the radiation incurred at Hiroshima had a substantial neutron component. Although the RBE for lung cancer induction and the depth of the tissue at risk are unknown, it is of some interest to indicate the relationship between effects and the doses that can be obtained on the basis of information on the attenuation of neutrons and gamma rays by body tissues (121).

145. The figures in table 12 give, for each kerma range (K), the mean total kerma ($\overline{K}_T$) and its neutron and gamma contributions ($\overline{K}_n$ and $\overline{K}_g$). From these are derived doses (D_n and D_g) at depth of approximately four centimetres in tissue. Neutron doses are multiplied by the arbitrary RBE values used earlier and added to the gamma doses to obtain the total dose (D_T) at a depth of four centimetres. The excess incidence (E) compared to the 0-9-rad group at Hiroshima is then combined with the dose to obtain risk estimates (R) in each exposure group. The same trend that was observed when excess incidences were related to kerma (paragraph 143) is observed here, although the actual risk estimates are somewhat different.

146. In contrast to Hiroshima, no significant excess of deaths is noted for Nagasaki, 22 observed in the exposed survivors as against 22.8 (0-9 rad) or 14.5 (national rates) expected. The reason for this discrepancy is unknown, although it may at least in part be accounted for by differences in radiation quality.

147. It now seems reasonable to assume that the A-bomb survivors at Hiroshima are at increased risk of dying from cancer of the lung. The risk estimates obtained at Hiroshima (2.3 and 0.6 cases per million per year per rad at 30 and 260 rad respectively), must of course be taken with the greatest caution, both because of the assumptions on which they are based (particularly about RBE values) and because of the negative evidence provided by the Nagasaki survivors. Taken at face value, they would indicate that at low doses (of the order of 30 rads) of low-LET radiation the risk of induction of lung cancer may be three times as high as the risk of leukæmia induction, whereas the opposite may be true at higher doses.

148. It must be noted, however, that, while we have reason to believe that the risk of occurrence of further cases of leukæmia among the survivors is now tapering off, we do not know whether new cases of radiation-induced lung cancer may not yet continue to be recorded and for how long, nor are we sure that estimates derived from the Hiroshima data would apply to a completely non-smoking population.

[3] Computed by dividing the excess deaths of observed over expected (based on the 0-9-rad group) by person-year-rad experience.

B. Ankylosing spondylitis patients treated by x-irradiation

149. In the study on ankylosing spondylitis patients treated by x-irradiation, Court Brown and Doll (28) observed a substantial excess of mortality from lung cancer over that expected in the general population in the United Kingdom. Relative risks and excess mortalities are presented in table 13 for each site of cancer within the x-ray beam. Among these 12 sites, the greatest excess was for lung cancer. The corresponding risk, in absolute terms, amounted to 252.4 cases per million per year and accounted for nearly half of the excess risk of all 12 cancers combined.

150. No estimates of the lung doses received by the spondylitics are available. However, Dolphin and Marley (39), on the basis of the average spinal-marrow dose being 880 rads, have estimated the average bronchial dose to be about 80 rads, which would correspond to a risk of some three cases per million per year per rad, if the excess incidence was all ascribable to irradiation, an estimate not too different from those that could be derived from the Hiroshima data at similar doses.

151. The data do not make it possible to ascertain the role of such factors as smoking habits, the disease itself that had required radio-therapy or the other forms of medication that the patients may have received.

C. Tuberculosis patients

152. Steinitz (148) has reported that tuberculosis patients in Israel were at increased risk of developing lung cancer compared to the general population. This finding was interpreted by some as evidence that diagnostic x-irradiation given to tuberculosis patients was causative of lung cancer.

153. In Israel, a cancer registry as well as a tuberculosis registry is maintained on a nation-wide basis. On the basis of tuberculosis registry, the author estimated the frequency (prevalence) of tuberculosis patients in the country specific for sex and age. The lung cancer cases newly reported to the cancer registry were searched to determine whether they had also been filed in the tuberculosis registry. Incidence rates of cancer of the lung were then estimated among the tuberculosis patients, and showed that the patients were at a 5-10 times greater risk of developing lung cancer than the general population.

154. The author also analysed the risk of lung cancer induction among tuberculosis patients on the basis of mortality records. The number of deaths that occurred in the registered tuberculosis patients were compared with those in the general population for "all causes", "all malignant neoplasms", and "lung cancer". It was noted that the tuberculosis patients had a much higher risk of dying from lung cancer than the general population.

155. Although little doubt remains that the tuberculosis patients in Israel were at increased risk of lung cancer induction, the extent to which irradiation is responsible for that increase is unclear. Information on the irradiation experience of the patients, essential with regard to radiation carcinogenesis, was lacking in the report, so that radiation effects could not be ascertained. The possibility that some people may be especially susceptible to lung diseases—that is, susceptible to both lung tuberculosis and lung cancer—cannot be ruled out. In addition, tuberculosis itself, rather than its treatment, may have facilitated the induction of lung cancer, e.g., scars of healed lesions of tuberculosis may predispose to cancer induction. It may be relevant to note that some other respiratory conditions (e.g., chronic bronchitis) are also suspected of having a causal association with lung cancer (14). Furthermore, since the clinical differentiation between lung tuberculosis and lung cancer is not always clear, some lung cancer patients might have been initially misdiagnosed as having had lung tuberculosis. Thus, further investigations are needed to assess the possible causative role of diagnostic irradiation in lung cancer induction in tuberculosis patients.

D. Workers exposed to high radon levels

156. Workers in certain underground mines, particularly uranium mines, are exposed to high levels of radon present in the mine's atmosphere. ^{222}Rn is a gaseous radio-nuclide that decays into radio-active daughters. These attach to aerosol particles present in the atmosphere. When inhaled, they can remain trapped in the bronchial tree where they deliver high-LET radiation to the respiratory epithelium.

157. Physiological and dosimetric details are considered in annex A of the present report. Here it will only be mentioned that the dosimetry of this situation presents considerable difficulties that have not all been solved. When known, the exposure of uranium miners is measured in "working levels", defined as any combination of short-lived radon-daughter products in one litre of air that will result in the ultimate emission of 1.3 10^5 MeV of potential alpha energy. Depending on the assumptions made on the cells at risk and the physiological and anatomical parameters involved, one working-level-month (WLM: exposure to one working level during 170 hours) corresponds to an alpha dose to the bronchial epithelium of 1-2 rads.

158. Unusually high mortality due to so-called Bergsucht among underground miners in the Krušné Hory (Erzgebirge), in what are now Czechoslovakia on the southern side and the German Democratic Republic on the northern (Saxon) side, had been known for centuries, but it was not until 1876 on the Saxon side and 1926 on the Czechoslovak side that the disease was identified as lung cancer.

159. In 1933, lung cancer in miners was recognized as an occupational disease in Czechoslovakia. As a result, a high rate of autopsies was performed on miners and it became possible to obtain accurate mortality figures. Over a follow-up period of five years there were 53 deaths, 19 of which were due to lung carcinoma, among some 400 miners at risk, or a mortality rate of about 1 per cent per year. Of the 28 carcinomas in that series combined with an earlier one from the same population, 16 were oat-celled and 12 epidermoid (138). In a series of 55 lung-cancer cases in Czech miners collected after the second world war the proportion of oat-cell carcinomas was 70 per cent (61).

160. Increased lung-cancer mortality has also been reported among fluorspar miners in Canada (33), iron-ore miners in Britain (17, 41), tungsten, fluorspar and lithium miners in Czechoslovakia (113a) and, lastly, among underground workers in two Swedish mines (6). In all these reports the miners population

had been occupationally exposed to high levels of radon. By contrast, no increased mortality was detected in a sample of South African gold and uranium miners exposed to apparently much lower levels of radon (7).

161. In none of the instances mentioned above in which increased lung cancer mortality had been reported is it possible to study how the excess mortality is related to the exposure. This, however, can be done on a further group of underground miners, those working in the uranium-ore mines of the Colorado Plateau in the United States. This population had been considered by the Committee in its 1964 report but the data then available were inadequate to permit a full analysis. Much information has now been published on a group of 3,366 white and 780 non-white miners and has been reviewed in a detailed monograph (86).

162. Basically, the study attempted to establish accurately the exposure of the miners in WLM and to follow-up the subjects from the date of first examination to the cut-off date for mortality analysis. The numbers of deaths expected in the various exposure categories were obtained by applying to the groups at risk the rates, specific for age, race, calendar year and cause of death, derived from the vital statistics of the four states in which miners were examined, and were compared with the observed numbers.

163. The major uncertainty affecting the conclusions of this study lies in the assessment of the exposures. This for the most part is due to the fact that large numbers of very small mines had been operating at any one time. Thus, there were 450 mines employing an average of two underground workers in 1950, 850 with three workers in 1957, and 533 in 1966 with an average of five workers (43). In all, the study utilized 43,000 measurements made in 2,500 mines over 27 years. However, while the quality of the measurements was considered to be good, their frequency tended to be very unevenly distributed. Thus, in only five mines were more than five radon-daughter measurements made in 1950 as against 177 in 1962 and 110 in 1968. In many mines only one or two measurements were ever taken, and, the results of early (prior to 1950) measurements not being available for any mine, they had to be inferred from circumstantial evidence collected later and sometimes elsewhere. Likewise, where, as in most mines, uninterrupted series of measurements had not been made, the gaps were made up on the basis of the earlier and later measurements available. Since the amount of radon daughters in air depends on many variables, including ventilation, meteorological conditions and quality of the ore extracted, it is not possible to evaluate the errors that may have been involved in assessing the exposure nor determine whether they gave rise to a systematic bias.

164. The mortality experience of the white underground miners from 1950 to 1968 compared with that expected in the population of the four states shows an excess number of deaths (60 per cent above expectation) essentially due to larger than expected numbers of violent causes (by 145 per cent) and of lung cancers (by almost 500 per cent). The mortality experience of the much smaller group of non-white miners over the same period is insufficient (72 deaths in all) to be informative.

165. The distribution of observed lung cancer deaths among white underground miners according to exposure and the corresponding mortality rates in excess of those expected on the basis of the four-state mortality experience (uncorrected for smoking habits) are given in table 14. Taking the exposure estimates at face value, a simple regression analysis indicates that a straight line adequately fits the data. However, while the data suggest that the risk (excess number of cases per WLM) does not vary significantly over the range of exposures explored, it does not seem appropriate, in view of the uncertainties discussed in paragraph 163 to place much reliance on the exact shape of the curve or on the actual risk estimate (about two cases per million per year per WLM) that can be derived from it.

166. An additional difficulty in interpreting the results arises from the fact that most of the miners included in the study were cigarette smokers (85). The difficulty can to some extent be circumvented by comparing the mortality in the miners with that in the population of the four states adjusted according to smoking habits as well as according to the factors mentioned previously. While the excess mortality over the expected mortality adjusted for smoking was somewhat reduced compared to that given in table 14, the relation of the excess to the exposure remained basically unchanged.

167. The distribution of lung cancer by histologic type was studied by Saccomano *et al.* (127) among uranium miners (121 cases) and controls (138 cases) matched according to age and smoking habits. The relative frequency of small-cell and undifferentiated carcinomas rose from 35.7 per cent in the group exposed to 40-200 WLM (21.4 per cent in matched controls) to 76.7 per cent in the 2,501-9,700 WLM group (10.0 per cent in controls) with little or no increase at all in epidermoid or other types of tumours.

168. It is worth noting that the observations made on the uranium miners are difficult to reconcile with the results of the ABCC study discussed in paragraphs 135 to 148. According to the latter, the excess risk of lung cancer may be decreasing beyond doses around 100 rads, namely, at doses far lower than the cumulative doses likely to have been received by the miners. Likewise, the distribution of histological types of cancer observed among the survivors also differed from that observed among the miners. The conditions of irradiation, however, were quite different in the two groups: (*a*) the exposure of the Hiroshima and Nagasaki survivors was single at high dose rate whereas it was fractionated, protracted over years and at low dose rate in the miners; (*b*) the survivors were exposed to mixtures of gamma rays and neutrons while the miners were exposed predominantly to alpha particles. Not only is the quality of the radiations involved different, but the range of the alpha particles is so much shorter than that of neutrons and gamma rays that different cells might be at risk in either case; (*c*) miners are exposed to high levels of dusts and fumes; (*d*) the smoking habits of the two populations cannot be compared and are likely to be quite different.

V. Bone tumours

A. External irradiation

169. Information on the induction of bone sarcomas (osteo-, chondro-, and fibro-sarcomas) by external radiation is scanty. It consists of clinical case

reports of sarcomas observed after high local doses given for therapeutic purposes and of the results of the surveys of Hiroshima and Nagasaki survivors, and of ankylosing spondylitis patients.

170. Reports of clinical cases are scattered in a number of publications (4, 19, 22, 31, 48, 55, 106, 115, 126, 136, 142, 147). Most of the cases were attributed to radiation merely because the personal histories of the patients showed heavy exposures. No prospective or retrospective survey has been conducted that would give firm indications on the size of the exposed population in which the individual cases were observed.

171. Two important, if crude, pieces of information can, however, be derived from those cases. One is the order of magnitude of the local exposures received. In virtually all cases these were higher than 1,000 roentgens and frequently amounted to several thousand roentgens. Although it is possible that histories of high exposure were recorded more reliably than histories of low exposure, or that low exposures, even if recorded, were not related by the investigators to the observed sarcomas, it is difficult to exclude the possibility that radiation-induced osteosarcomas develop only after very high exposures of external therapeutic radiation.

172. The other point to be noted is that the time interval between irradiation and diagnosis of osteosarcoma is highly variable, with reported extremes 4 and 42 years, but that 73 per cent are less than 15 years and the average (based on 137 cases) is about 11 years. Here, again, a bias cannot be excluded that might weigh the data in favour of shorter time intervals.

173. The Hiroshima and Nagasaki survivors have so far provided negative evidence. In the fixed sample of the Life Span Study, one case of osteosarcoma came to autopsy and four were diagnosed but not seen at autopsy by 1965 (175). Of these five cases, two were not in the city at the time of bombing, two were within 1,400 metres from the hypocentre and one between 1,400 and 2,000 metres. The distribution of these cases by distance was reported to be random. More recently, the total number of bone cancer deaths in the Life Span Study from 1950 to 1970 has been reported (73, 74) as 23, or about the number expected from the Japanese vital statistics.

174. One may assume on the basis of the experience provided by the case reports discussed in paragraphs 169-172 that sarcomas that had been induced by radiation from the 1945 nuclear explosions would have developed clinically during the subsequent 25 years, unless the latency was much longer at the doses received by the survivors. The survival time of bone sarcomas—a few years—is short enough for most cases to have been recorded in the mortality study. It seems therefore clear that, at the doses received by the A-bomb survivors, the risk of induction is orders of magnitude lower than the risk of induction of leukæmia.

175. Among the ankylosing spondylitics (28) 5 deaths from bone tumour were reported, against 1.1 expected, a significant excess. Because local doses delivered in the course of the x-ray treatment were in some cases of the order of thousands of rads, it would be useful to know the dose category to which the cases of bone tumour belonged.

B. Internal irradiation

176. Carriers of radium burdens are among the groups of people exposed to radiation that have been most intensively studied for periods of several years. Of the three major surveys of radium-contaminated subjects, two concern carriers of long-lived ^{226}Ra (half-life 1,622 years), sometimes mixed with ^{228}Ra (half-life 6.7 years), and one involves subjects treated with injections of short-lived ^{224}Ra (half-life 3.64 days).

177. Carriers of ^{226}Ra consist of dial painters, radium chemists and patients that absorbed radium-containing drugs orally or intravenously for therapeutic purposes. The two major groups are known as the MIT group (42) and the ANL-ACRH group (46) and consist of some 500 and 300 subjects, respectively. Until recently these two groups were studied separately by different investigators but the two surveys have now been merged, and only the results of the joint survey (125) will be discussed here.

178. Mean cumulative doses to bone due to alpha radiation were estimated for all subjects included in the surveys. However, it must be underlined that the estimates, based on residual body burdens (themselves not always accurately known) were determined sometimes decades after the initial uptake of radium and are uncertain both because they involve assumptions on the metabolism of radium in bone and because—^{226}Ra and ^{228}Ra being alpha emitters—their dosimetry is very sensitive to the microscopic distribution of the nuclides in bone, which in turn depends on the amount of remodelling that has taken place.

179. Two types of tumour occur with increased frequency among radium carriers—bone sarcomas and antral carcinomas. The latter develop in paranasal sinuses and mastoidal cells. Table 15 and figure IX give the distribution of tumours in the joint survey according to cumulative bone dose averaged over the whole skeleton. It must be pointed out that, the cumulative dose being delivered at a diminishing rate over a period of several years, there is no way to determine which fraction of it is sarcomogenic and which is wasted. On the other hand, the effectiveness per rad might be higher for alpha particles than for x or gamma rays.

180. The most noticeable feature of the data is the apparent discontinuity in the incidence of both types of tumour at around 700 rads. Here also, the data suggest that no tumours are induced until such a dose has been delivered. However, the number of sarcomas expected among those exposed to less than 700 rads ($4.6\ 10^4$ man-rads altogether), based on 51 cases observed in about $1.3\ 10^3$ man-rads, would be 1.8 if proportionality between dose and incidence applied, against none observed. Similarly, 0.7 carcinomas would be expected in the group exposed to less than 700 rads. Much larger samples in the low-dose range would be necessary to make the negative results in these dose groups differ significantly from predictions based on proportionality between dose and incidence.

181. Another important observation is that the frequency of sarcomas and carcinomas does not, above mean doses of 1,000 rads and within a twenty-fold range of doses, increase monotonically with dose. As indicated in figure IX, the observed incidences

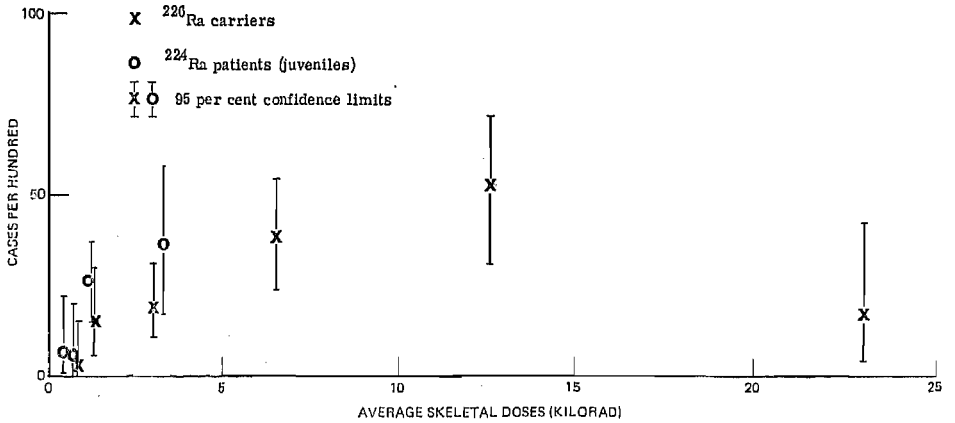

Figure IX. Incidence of bone sarcomas in carriers of ^{226}Ra burdens and in ^{224}Ra-treated patients against average skeletal dose (125, 144)

have a maximum near 12,000 rads and then fall to the same level that is observed near 1,000 rads.

182. Patients treated with ^{224}Ra constitute the third major source of information on the effects of radium exposure (143, 144). The treatment involved intravenous injections of "Peteosthor", a ^{224}Ra-containing preparation that had a period of vogue in Germany between 1944 and 1951, mostly in the treatment of bone tuberculosis and ankylosing spondylitis. Of the approximately 2,000 patients so treated, 802 were investigated and followed up for about 20 years.

183. The observed incidence of bone sarcomas is shown separately for juveniles and adults in table 16 and has been plotted in figure IX for juveniles alone. The main difference between people treated with ^{224}Ra and those with ^{226}Ra burdens lies in the appearance of bone sarcomas at doses about 10 times lower in the former than in the latter. It is as if ^{224}Ra was more effective than ^{226}Ra in inducing the tumours at low doses. At mean bone doses above 1,000 rads the rate appears to be the same in both groups, although it must be recalled that the ^{226}Ra carriers had been, on average, followed up for several more decades than the ^{224}Ra patients.

184. The higher effectiveness of ^{224}Ra at low doses has been attributed to the fact that, owing to its short half-life, ^{224}Ra decays before being incorporated into the bone matrix and therefore delivers to the cells (believed to be those at risk) that line bone surfaces a much higher dose than the same activity of ^{226}Ra, most of which finds its way into deeper bone layers. This explanation, if borne out by the results of continued follow-up of these subjects, would make the results of the study of "Peteosthor"-treated patients particularly valuable, since it would provide information of indirect relevance to the problem of plutonium contamination in man. This is because plutonium, a long-lived alpha emitter, owing to its chemical characteristics, tends to be fixed on bone surfaces and thus to irradiate bone in a manner similar to ^{224}Ra.

VI. Other cancers

A. A-BOMB SURVIVORS

1. *Mortality studies*

185. Table 4 from the study of Beebe *et al.* (11) in the Life Span Study Sample from 1950-1966 shows that, if all malignant neoplasms, except leukæmia, are put together, this combined group has a significant increase of mortality with increasing dose ($P \sim 0.05$). Among the variety of types of cancers included in this group, lung cancer and breast cancer have been discussed already. None of the remaining cancers selected by the authors for tabulation showed a statistically significant dose-effect relationship, although some increased risk in high-dose groups may be noted for stomach cancer, uterus cancer, and the group of other cancers (ICD No. 190-199).

186. The increased mortality from all malignant neoplasms (except leukæmia) with dose has been confirmed by the more recent study of Jablon and Kato (73, 74) which covers the 20-year period 1950-1970 (table 5). In the survivors of the Life Span Study Sample exposed to 10 or more rads, the observed deaths from malignant neoplasms other than leukæmia exceed the expected (national rates) by 144 in Hiroshima and 27 in Nagasaki and exceed the expected deaths from the 0-9 rad group by 113 in Hiroshima and 14 in Nagasaki. At Hiroshima, roughly half of the excess may be accounted for by that of lung and breast cancer.

187. At Hiroshima, the residual group (other cancers in table 5) shows an over-all, highly significant, excess of between 70 and 90 cases, depending on the expectation used. Within the individual exposure groups, the excess is significant only at the highest exposure but the rising trend of the mortality rates with Kerma is highly significant. This trend is not ascribable to any specific site. No significant excess is detectable and no clear-cut trend can be identified at Nagasaki.

188. As with lung cancer, risk estimates are difficult to obtain because the relevant doses are unknown. One may, however, proceed as in the case of lung cancer and use the same notional dose estimates and the same RBE values that were obtained in paragraph 145, for the purpose of showing the possible consequences of crude dosimetric assumptions. The resulting risk estimates then vary from 2 cases per million per year per rad at 30 rads of low-LET radiation to 2.5 cases per year per rad at about 260 rads. However, only the estimate for the group exposed to the highest dose is based on a statistically significant excess number of cases.

2. *Autopsy studies*

189. Several autopsy studies have investigated the role of radiation in the induction of cancer of different sites. Since the autopsy rate has been high (about 40 per cent in recent years in the Life Span Study Sample) and the material is not particularly biased in respect to radiation exposure, the autopsy data may be more reliable than the mortality data for those types of cancer that cannot be identified with sufficient accuracy from death certificates.

190. Schreiber *et al.* (130) have studied primary liver cancer in 2,437 autopsy cases performed from 1961 to 1967. Thirty-four cases were found, but there was no clear relationship between radiation and the disease. In the same autopsy material, Robertson *et al.* (124) found no detectable dose-effect relationship in 31 gall-bladder carcinomas, 14 bile-duct carcinomas and 3 ampullary carcinomas.

191. Yamamoto *et al.* (174) have reported 326 cases of gastric cancer in 2,908 autopsies performed from 1961 to 1968. Again, no clear relationship was observed between the rate of gastric cancer and radiation dose.

192. Nishiyama *et al.* (108) have investigated the relationship between radiation and both malignant lymphoma and multiple myeloma on the basis of a variety of ABCC records including autopsy, death certificate, leukæmia registry, etc. In the extended Life Span Study Sample, 45 cases (37 malignant lymphomas and 8 multiple myelomas) were identified from 1945 to 1965. For multiple myeloma, the number of cases was too small to warrant a study of their relation with kerma. For malignant lymphoma, 26 cases were observed in Hiroshima and 11 cases in Nagasaki. These cases were divided into three broad categories according to exposure and the risks of malignant lymphoma in the high-exposure categories were compared with the risk in the essentially non-exposed category (<1 rad). The relative risks were 0.7 in the 1-99 rad category and 8.0 in the over-100 rad category in Hiroshima; in Nagasaki, the respective figures were 0.7 and 0.6. Thus, only the over-100-rad category in Hiroshima showed an increased risk of malignant lymphoma, and more data appear to be needed to conclude that A-bomb survivors are at an increased risk of developing malignant lymphoma.

B. Cancer mortality among ankylosing spondylitis patients treated with x-irradiation

193. In their 1965 report (28), Court Brown and Doll showed that ankylosing spondylitis patients treated by radio-therapy are at an increased risk of developing a variety of malignancies. Based on the standard course of radio-therapy to the whole spine and to the sacro-iliac joints, all cancers (except leukæmia) were divided into two classes: those occurring inside the beam of radiation (heavily irradiated sites) and those occurring outside the beam (lightly irradiated sites). The lightly irradiated sites included brain and central nervous system, uterus, prostate, testes, kidneys and urinary bladder. All other sites except the colon were classified as heavily irradiated sites (the colon was excluded because of the possible relation of ankylosing spondylitis to ulcerative colitis and, consequently, to colon cancer).

194. As shown in table 17, cancers of heavily irradiated sites, when compared with the numbers expected on the basis of the national mortality rates, show an observed/expected ratio of 1.6 and an excess mortality of 512.9 per million per year during the observation period (5-25 years after the treatment). Cancers of lightly irradiated sites show a slight excess, which is not statistically significant.

195. Table 6 shows the occurrence time of the two categories of cancer in the complete follow-up of the patients to the end of 1960. The results of the incomplete follow-up to the end of 1963 are given in table 18. The excess number of observed deaths from both categories of cancer in the first three years after the first observation is likely to have been due to the inclusion of a small number of cancer patients mistakenly diagnosed as ankylosing spondylitics because of cancerous involvement of the spine.

196. While the leukæmia mortality decreased to nearly the natural rates after the peak in the 3-5-year period since first observation, cancers of heavily irradiated sites have shown no declining trend with the passage of time. In fact the observed/expected ratios increased constantly from 1.1 for the 3-5-year period to 2.3 for the 12-14-year period. For the 15-24-year period the ratio was 1.6 according to the complete follow-up, whereas the incomplete follow-up yields a ratio of 2.2 for the 15-27-year interval since first observation. In contrast to the time trend of cancers of heavily irradiated sites, no mortality increase with time is apparent either for cancers of lightly irradiated sites, or for all causes of death.

197. Among the 200 deaths from cancers of heavily irradiated sites that took place during the 6-27-year observation period, the excess over expectation is significant ($P>0.025$, one-tailed) for a variety of cancer sites: pharynx, stomach, pancreas, bronchi, bones, other lymphatic and hæmopoietic tissues, and others (table 13). The observed/expected ratios in the group as a whole during the 6-27-year observation period is 1.9 while the excess mortality is 561.2 cases per million per year for all cancers of heavily irradiated sites and nearly half of this excess is due to lung cancer.

198. The interpretation of the observed excess of cancers of heavily irradiated sites must be made with caution. As seen in table 17, causes of death (e.g., cerebrovascular disease) with no obvious relation to ankylosing spondylitis or irradiation show a significant excess which was discussed by the authors as follows:

(*a*) The broad disease groups may contain a small proportion of rare conditions which are, in fact, directly related to ankylosing spondylitis;

(*b*) The presence of certain complications may increase the risk of death from other, unrelated, causes;

(*c*) Some deaths related to ankylosing spondylitis are erroneously attributed on death certificates to other causes of death;

(*d*) Ionizing radiation may have non-specific deleterious effects;

(*e*) Other treatment may be harmful; and

(*f*) The computation of the expected number of deaths may be in error because of, for example, a difference of socio-economic class between the patients sample and the general population.

199. Whatever the true reasons, it is conceivable that the excess cancer deaths in these patients might

also be due to reasons other than radio-therapy. The only way to exclude such an explanation would be to determine whether the risk of cancer is higher among spondylitics treated with radio-therapy than among those treated otherwise. However, adequate control groups have yet to be studied. It would be much easier to interpret the excess risk of cancer if dose-effect relationships could be demonstrated as with leukæmia, but no dosimetry for tissues other than bone marrow is currently available. However, the fact that a significant excess of cancers is observed only in heavily irradiated sites and that only for cancers of heavily irradiated sites does the risk increase with time makes it beyond reasonable question that the excess cancer mortality among x-rayed spondylitics is largely due to the radiation treatment.

C. American radiologists

200. Seltser and Sartwell (135) have reported an increased risk of cancer among about 3,700 male American radiologists. During the period 1935-1958, 11.3 excess deaths from leukæmia and 48.2 excess deaths from all other cancers occurred among the radiologists in comparison with the group of ophthalmologists and otolaryngologists (this group was regarded as a virtually non-exposed population). In this study, only combined deaths from cancers of all types (except leukæmia) were presented, so that no analysis of cancer incidence at individual sites can be made.

201. All cancers other than leukæmia showed a relative risk of 1.6 and an excess mortality of about 1,000 per million per year (50 cases in 50,000 person-years). Because the radiation doses received by the radiologists are unknown, the risk per unit dose cannot be derived.

202. Although an apparent excess was noted for all cancers (except leukæmia) in the radiologists, it is not very clear whether the excess was caused by irradiation only. In this study, all deaths were classified into four groups: leukæmia, all other cancers, cardiovascular-renal diseases, and all other causes. Each of the four groups showed an apparent excess when compared to the ophthalmologists and otolaryngologists; e.g., excess deaths were 103.4 in cardiovascular-renal disease, 65.3 in the group of all other causes. If radiation alone were responsible for the observed excess, then it must be assumed that the radiation had deleterious biological effects of a non-specific sort on the American radiologists. Such non-disease-specific effects of radiation, however, have not been observed in a study on British radiologists (27). In addition, Beebe *et al.* (11) could find no such non-specific effects in Japanese A-bomb survivors.

203. A question may be raised as to the appropriateness of the comparison group used in the study of Seltser and Sartwell. In their study, the medical specialists chosen for comparison are obviously more closely related to radiologists than is the general population with respect to such factors as education or socio-economic status; but still the choice of radiology from among the various medical specialties is certainly not random, so that radiologists may indeed have a different mortality experience from the other medical specialists, regardless of their irradiation exposure.

204. Thus, the excess mortality from cancers other than leukæmia among the American radiologists observed by Seltser and Sartwell may not be totally ascribed to their occupational exposure, and definite conclusions should be made only after further data have been accumulated.

D. Patients exposed to therapeutic irradiation in the pelvic region

205. Wagoner (164) studied cancer morbidity in 1,893 patients with benign gynæcological disorders treated by either radium (900 cases) or x-irradiation (993 cases) during the period 1935-1966. Among the various cancers examined, leukæmia showed a significant increase and has been discussed in section I of this annex. The remaining individual cancers were: cancers of stomach, small intestine, large intestine, rectum, biliary passage and liver, pancreas, lung, breast, female genital organs, urinary organs, and lymphatic tissue.

206. Since the radium or x-ray therapy was limited to the pelvic region, most of the selected sites of cancer were outside the main irradiated area. Therefore, as expected, no significant deviation of observed numbers of cases from expected numbers was noted for the majority of cancer sites. The observed numbers were in excess only for cancers of the female genital organs—109 observed cases *versus* 54.87 expected ($P<0.01$)—and cancers of the urinary organs—17 observed cases *versus* 8.50 expected ($P<0.05$). In absolute terms, the excess mortality amounted to 1,532 cases per million per year for cancers of the female genital organs and to 241 cases per million per year for cancers of the urinary tract. The risk cannot be expressed per unit dose of radiation since no estimates of radiation dose received by the organs at risk were made, and it cannot be excluded that the benign gynæcological disorders that had prompted the radiotherapy are associated with an increased risk of cancer of the genital or urinary organs.

207. A study of patients with metropathia hæmorrhagica, was made by Doll and Smith (38) who examined the relationship between the x-ray therapy given to these patients and the ensuing excess mortality from malignancies. Observed and expected deaths (computed from the age-sex-period-specific mortality rates of the general population) in the six disease categories selected for analysis are compared in table 19. The deaths were divided into those that occurred within five years of the radiation treatment and those that occurred later. The former group was regarded as less reliable on the ground that the initial examination (at the time of diagnosis of metropathia hæmorrhagica) was likely to have revealed malignancies that would otherwise have been detected later.

208. As seen in table 19, no significant difference between observed and expected deaths was noted for coronary disease and for the group of other causes. The risk of leukæmia showed a large excess, but this is discussed in section I of this annex. There was a significant deficit in the observed deaths from breast cancer as compared to the expected numbers; this deficit could be explained by the available evidence (44) that artificial menopause tends to reduce the risk of breast cancer. Cancers outside the radiation beam (presented in the table as "other cancers") showed no significant increase of the observed/expected ratios.

209. On the other hand, cancers within the radiation beam (ovaries, large and small intestines, rectum, uterus, other pelvic organs and bladder) showed a significant excess. In the observation period of five or more years after radio-therapy, 31 deaths occurred in comparison with 18.40 expected ($P<0.002$) corresponding to an excess mortality of about 700 per million per year. The excess risk in the group of cancers of heavily irradiated sites was attributable to a variety of cancers (e.g., excess deaths were 5.16 for intestines, 2.66 for rectum, 1.54 for uterus etc.) but the excess cannot be expressed per unit dose of radiation for any of the types of cancer because estimates of the doses to the relevant tissues are not available. The observed/expected ratios were 1.6 in the 5-9-year period after treatment, 1.6 in the 10-14-year period, and 2.1 in the 15-year-or-more period. This time trend seems to indicate a tendency of the risk to increase after exposure.

210. As in the case of ankylosing spondylitis patients (28) and of patients with benign gynæcological disorders (164) the expected numbers of deaths were computed in this study from the mortality rates of the general population. It cannot be excluded that the excess, or part of the excess, observed in the group of cancers of heavily irradiated sites in this study might have been associated with metropathia hæmorrhagica rather than with radio-therapy.

VII. Malignancies in children

A. A-BOMB SURVIVORS

211. As already discussed in section I, the relative risk of leukæmia among the survivors exposed to radiation at ages 0-14 years ATB is known (66, 67) to be higher than that of the older group (ages 40 and over ATB).

212. The risk of cancer also seems to increase among the survivors exposed to radiation at young ages, particularly at ages 0-9 ATB (71, 75). Table 20 shows observed and expected deaths attributed to cancer (except leukæmia) for 1955-1966 among survivors aged 0-9 at the time of exposure. In the group of 20,415 subjects consisting of the survivors aged 0-9 ATB and their matched controls, 22 deaths were attributed to cancer during the period 1955-1969. Before 1955, only one death was reported.

213. In the non-exposed group (not-in-city or <10 rad), the observed number of deaths virtually equalled the expected number. Expected deaths were computed from the 1962 national rates. Eight deaths were observed in contrast to 0.98 expected among those who received more than 100 rads (T65D) or an unknown kerma (undoubtedly high but undetermined since the heavy shielding configuration made dosimetry impossible). Although the numbers are small, the difference is statistically significant. No deaths were observed in the 10-99 rad group, while 3.26 were expected.

214. No specific clustering as to site of origin of these cancers was observed: two stomach cancers, two osteogenic sarcomas, one pancreas cancer, one lymphosarcoma, one prostate sarcoma, one metastatic cancer of the liver. It may be concluded that because of the small number of observed deaths (8 in the exposed group), the evidence relating to increased risk of cancer in this group is still only suggestive and that, to obtain definite conclusions, this group of survivors must be followed for many more years to come.

B. CHILDREN IRRADIATED FOR THE TREATMENT OF *Tinea capitis*

215. *Tinea capitis* is one of the commonest fungal diseases of the scalp in children. For approximately half a century before 1960 epilation by x-irradiation was commonly practised as an effective treatment to free the scalp of fungal contamination. The number of patients so treated throughout the world was estimated (20) to have been 200,000 in the 50 years prior to 1960.

216. Albert *et al.* (1-3) and Schultz and Albert (131) made a follow-up study of *Tinea capitis* patients consisting of a study group treated by x-ray epilation (2,043 patients) and a control, non-irradiated, group (1,413 patients) who visited the New York University Hospital during the years 1940 to 1959. The x-ray therapy given to the patients was according to the Kienbock-Adamson procedure in which the scalp is irradiated in five different fields with 75-100-keV x rays at exposures of 300 to 400 roentgens for each field. After the irradiation, complete epilation followed in two to three weeks and lasted one to two months. On the basis of phantom experiments and theoretical computations, the radiation doses were estimated to have been 70-175 rads to the brain, 450-850 rads to the scalp, and 300-460 rads to the cranial bone marrow.

217. In the patients, males were predominant (86.1 per cent in the irradiated and 78.5 per cent in the non-irradiated groups) and the vast majority were white (about 75 per cent for each of the two groups). For both groups, the average age at the time of the treatment was seven years.

218. An attempt was made to trace the patients by a variety of follow-up methods in order to evaluate possible late effects, including cancer induction, by x-irradiation. During the average follow-up period of 15 years, 85 per cent of the irradiated and 79 per cent of the non-irradiated patients were traced. The patients thus traced were requested to answer a health questionnaire. In the case of tumours, diagnostic confirmation was secured from the treating hospitals or physicians.

219. In the non-irradiated group of about 1,400 patients, only one case of malignancy (Hodgkin's disease) was noted during the average observation period of 15 years. In contrast to this low occurrence, a much larger frequency of malignancies (14 cases) was observed in the irradiated population of about 2,000 patients, i.e., four leukæmias (two acute lymphocytic, one acute myeloblastic, and one chronic myelogenous), one fibrosarcoma of the mandible, two basal-cell carcinomas of the scalp, one submandibular lymphosarcoma, one Hodgkin's disease, one adenosarcoma of the rectum, one acinous-cell carcinoma of the parotid gland, and three brain tumours. Of these 14 cases, four died of leukæmia and one of brain tumour.

220. In view of the far higher occurrence of cancer in the irradiated group in comparison with the non-irradiated, and the fact that all but one of the 14 malignancies occurred in the tissue within the x-ray beam, the majority, if not all, of the observed cancers

can be attributed to the x-ray therapy. Although the number of cases is very limited, it may be of interest to speculate upon the risk of cancer induction per unit dose. For leukæmia, considering the average dose to the whole bone marrow to be of the order of 50 rads, the risk is of the order of three cases per million per year per rad. It is of the order of one case per million per year per rad for brain tumour. The meaningfulness of these estimates is limited by the smallness of the sample on which they are obtained and the fact that data at one dose level only can be used.

C. Children irradiated in the thymic area

221. In the past, thymus enlargement was thought to be a serious medical condition, and after the turn of the century, many children were subjected to x-irradiation for a supposedly enlarged thymus. This practice became less common with the passage of time as medical knowledge increased regarding the hazards of radiation and the non-harmful nature of thymus enlargement.

222. Since the Committee's 1964 report, two cohort studies have been updated (58, 116). The cohort study of Latourette *et al.* (79), already discussed in the 1964 report, was extended by Pifer *et al.* (116). The study population consisted of 958 individuals (59 per cent males and 41 per cent females) who received x-ray therapy for thymic enlargement at the University of Michigan (U.S.A.) mostly in the 1930s. The majority of patients (90 per cent) were treated during the first year of life. After the initial survey in 1958, late effects of x-irradiation were reinvestigated by a mail survey made on 786 persons whose follow-up data were available at the University in 1964-1965. When malignant conditions were encountered, the diagnoses were confirmed from the treating hospitals or physicians.

223. X-ray treatment was given to the anterior chest alone in virtually all subjects, with exposures of 100-199 roentgens in the majority (557) of cases. Thyroid glands were considered outside the main beam and received on the average a tissue dose of approximately 20 rads.

224. During the observation period of nearly 30 years, 9 malignant neoplasms were observed against 5.8 expected, a statistically non-significant excess ($P>0.05$). These nine malignancies were: one thyroid carcinoma, one leukæmia, one lymphosarcoma, two brain tumours, and four others. None of the observed cancers occurred in the tissue within the radiation beam. It may be of interest to note that no cancers of the breast were observed although the breast definitely had been irradiated.

225. In conclusion, the results of this study may be explained as providing no evidence of the induction of malignancies in children at the doses received (20 rad to the thyroid).

226. The authors found 7 cases of benign thyroid neoplasms in contrast to the expected number, 0.13-1.3; however, as the authors recognized, the validity of the expected number was dubious since no reliable data regarding the incidence rates of benign thyroid neoplasms in the general population were obtainable.

227. The Committee's 1964 report cited a follow-up study on children in upstate New York exposed to therapeutic x-irradiation for thymic enlargement (117, 157, 158). This study was updated (57, 58) to include the continuation of the follow-up of the same group of individuals. The study group consisted of 2,876 persons exposed to x-ray treatment for thymic enlargement and of their 5,006 non-irradiated siblings used as controls. The vast majority (90 per cent) was irradiated at less than six months of age. While more males (58 per cent) than females (42 per cent) were treated, the male-to-female ratio was approximately 1:1 in the controls.

228. The follow-up of the individuals was made by mail survey (the third survey), which traced 84 per cent of them. If tumours were recorded on the returned questionnaire, the diagnosis was confirmed by obtaining medical information from appropriate hospitals or physicians. The exposed subjects received x-ray therapy from 1926 to 1957 and, therefore, the observation period until 1963 ranged for individuals from 6 to 37 years.

229. Table 21 indicates the number of observed and expected cases of various malignancies in the treated and the control groups during the observation period. In the non-irradiated control group, the observed numbers were in good agreement with the expected numbers, computed from the incidence rates in the general population. In sharp contrast to the control group, the treated population showed a clear excess of observed malignancies as compared to expected. The most remarkable was the excess of thyroid carcinoma, 19 observed against 0.14 expected. A significant excess was also noted for leukæmia (6 observed to 2.02 expected), salivary gland tumour (4 *versus* 0.08), and all malignancies combined (33 *versus* 8.10). It is of interest to note that no breast cancer developed in spite of the fact that the breasts must have received substantial radiation doses. None of the 19 cases of thyroid carcinoma died from the disease.

230. The authors estimated that the risk of thyroid cancer induction was of the order of 2.5 cases per million per year per rad (50-600 rad); the estimate given in the 1964 report ($1.0\ 10^{-6}\ y^{-1}\ rad^{-1}$) was increased to reflect newly estimated tissue doses to the thyroid gland and the occurrence of further cases. The earlier value was computed according to exposures; as estimates of tissue dose were not available, it was then tentatively assumed that the thyroid glands were within the main beam. When doses to the thyroid glands were eventually estimated, it appeared that in many individuals the thyroid glands were outside the main beam and were exposed only to scattered x rays and so had received only a fraction of the exposure. It was not easy to decide retrospectively whether the thyroid glands were in the main beam since this depended on various factors such as port size, port placement, lead shielding, etc. It must be remembered, therefore, that considerable uncertainties exist in the estimated tissue dose of the thyroid glands and, consequently, in the risk estimate.

231. Previously, types of treatment—AP (anterior and posterior) *versus* A (anterior) irradiations—were suspected to have influenced the risk of thyroid carcinoma, but the latest analysis indicates that this difference could be accounted for simply by the difference of radiation dose accompanying A and AP treatments, without requiring consideration of the possible tumourigenic role of the exposed pituitary gland in the case of AP treatment.

VIII. Malignancies in pre-natally exposed children

232. In the Committee's 1964 report, a number of studies (29, 47, 76, 77, 82, 88, 89, 90, 91, 120, 149, 150, 153, 154) relating to the risk of cancer induction in children exposed to radiation *in utero* were discussed. Most of these studies were of a "retrospective", or "case-control", type in which a study group of cancer cases was matched by sex and age with a control group of healthy children. In the two groups, the proportions of mothers exposed to diagnostic or therapeutic x-irradiation were compared and, on this basis, the risk of cancer induction in the irradiated children as compared with those non-irradiated was estimated. The estimated relative risks varied considerably, ranging from over 1.7 to almost 0.4. MacMahon and Hutchison (91) noted, however, that the studies reporting relative risks less than 1.0 tended to be based on small samples and to show large chance fluctuations, and that the confidence limits of the individual estimates overlapped considerably. The joint maximum likelihood estimate of the relative risk derived from the 10 major studies was 1.40 (1.21, 1.68 as 95 per cent confidence limits) and was within the 95 per cent confidence limits of each of the individual estimates of relative risks.

233. Since the 1964 report, the results of several further studies have been published (50, 52, 72, 151, 152). Graham *et al.* (50) in the United States made a case-control study investigating 319 leukæmia cases and 884 controls. An attempt was made to select all leukæmia cases at ages less than 15 years, based primarily on tumour registry records in upstate New York and the metropolitan and rural areas around Baltimore and Minneapolis-St. Paul. A control group of children in the same age range was also chosen by a stratified selection of households from the same geographical areas. The vast majority of the leukæmia cases and of the controls were interviewed to ascertain a number of demographic and medical risk factors, and the medical information thus obtained was carefully verified against the medical records of relevant hospitals and physicians.

234. The case group included more mothers who had experienced miscarriages or stillbirths prior to the birth of the subjects, the relative risk being 1.4-1.6. The radiation histories of the children before and after birth and of their parents (i.e., of preconception exposure of mothers and fathers) were recorded. Diagnostic radiation experience for mothers prior to conception differed significantly between the case and control groups. The case group included a higher proportion of mothers exposed to radiation (any site of the body) and the adjusted relative risks varied from 1.55 to 1.73 depending on which of the factors such as year of birth, age of mother, birth order, pregnancy order, miscarriage or still-births, were adjusted. From the report it is not clear how many mothers received irradiation to their reproductive organs. Neither dose-effect relationship nor the variation of risk with time interval before conception were well investigated because of small numbers and large chance fluctuations.

235. As to *in utero* exposure, it was found that the mothers of 27 out of 319 cases (8.6 per cent) and those of 54 out of 884 controls (6.3 per cent) had received only abdominal x-irradiation during pregnancy. The relative risk was 1.40 but was not statistically significant. Considering radiation to all sites, rather than to the abdomen alone, the proportions of mothers so irradiated in case and control groups were 29-30 per cent and 22-23 per cent, respectively. The relative risks ranged from 1.40 to 1.59 depending on the selection of adjusted risk factors such as year of birth, age of mother, birth order, and pregnancy order. These values of relative risks were close to the maximum likelihood estimate given by MacMahon and Hutchison (91).

236. As discussed in the 1964 report, Stewart (149) and Stewart *et al.* (153, 154) had reported that a higher proportion of mothers of children dying from leukæmia and other malignancies gave history of x-irradiation during pregnancy than did mothers of control children. Stewart and Kneale (152) confirmed this on the basis of a much larger sample of cases and controls and, in addition, asserted that a clear linear dose-effect relationship was observed between radiation dose and cancer induction.

237. The cases were 7,649 children born between 1943 and 1965 in England and Wales who had died from malignant diseases before 10 years of age. Of these, approximately one half had died of leukæmia. Equal number of controls, 7,649, were selected from live children on the basis of the local birth registers, and were matched with cases according to sex, date of birth, and region.

238. While 1,141 mothers (14.9 per cent) in the case group were found to have had abdominal x-ray examination during pregnancy, the corresponding number in the control group was only 774 (10.1 per cent), and this difference was statistically significant. The vast majority of them had x-ray examination in their third trimester of pregnancy. The x-rayed mothers were further classified according to number of films taken. Comparing the number of such classified mothers between cases and controls, the excess risk of cancer induction was estimated by film-number category. The excess risk appeared to increase linearly with the number of films taken, ranging from about 20 per cent for one film exposure to over 100 per cent for five or more film exposures.

239. The mean fœtal dose per single film exposure was estimated by Stewart and Kneale (152) as varying from 0.46 rad in 1943-1949 to 0.2 rad in 1960-1965. Utilizing these estimates the risk of cancer induction in children under the age of 10 was shown to be in the range 30-80 deaths per million children per year per rad with a mean of 57 deaths and a standard error of 13. Subsequently Stewart and Kneale (152a) showed that if values of 0.72 or 0.89 rad (as derived from the national radiation dose survey carried out in 1960 in the United Kingdom) were used, the estimates would be reduced to 36 or 29 deaths per million children per year per rad, respectively. There is obviously some uncertainty in the values of radiation dose to be used in such retrospective studies. A study of the British literature in the years concerned showed that estimates of fœtal doses were made by a number of authors (13, 21, 98, 99, 113, 145, 146) between 1946 and 1957. From their reports the following average values of fœtal dose per film were derived: 1.8 rads in 1943-1949, 1.0 rad from 1950 to 1954, 0.5 rad from 1955 to 1959, and 0.2 rad from 1960 to 1965. Using these values of dose in conjunction with the incidences reported by Stewart and Kneale, an estimate of 23 deaths per million children per year per rad (in a range 0.2-20 rad) over a 10-year period can be

deduced, to which leukæmias on the one hand and other malignancies on the other contribute in about equal proportions.

240. The results of a study by Jablon and Kato (72) of children whose mothers were pregnant at the time of the A-bomb explosions is difficult to reconcile with the estimates of risk per unit dose given by Stewart and Kneale, even if the revised risk estimate given at the end of the previous paragraph is accepted. Jablon and Kato attempted to interview the mothers of all the children whose births were recorded in Hiroshima and Nagasaki within approximately 10 months after the bombings. Ninety-seven per cent of the 7,720 eligible mothers were interviewed regarding exposure status to irradiation. A sample of 1,292 children was then selected including all 325 children whose mothers were within 1,500 metres of the hypocentres, and randomly sampled comparison groups in the location of 1,500-1,999 metres, 2,000-2,999 metres, and 3,000-3,999 metres from the hypocentres. The comparison groups were matched to the group within 1,500 metres by sex of child, month of birth, and city.

241. The selected children were followed successfully (more than 99 per cent) regarding their survival status and the cause of death was ascertained for those that died during the first 10 years of life. In the irradiated group of children (1,292), only one death from any form of cancer was observed. The case was a cancer of the liver in the group within 1,500 metres. The comparable number of deaths that may have been expected by applying Japanese national rates was 0.75. Thus, no material difference between observed and expected deaths was recorded.

242. The radiation dose received by the children while *in utero* was estimated assuming that the dose to the fœtus was not less than one half of the maternal dose. By taking 50 per cent as a conservative value, the authors estimated that this group of children comprised about 17,500 person-rads in 10 years of life which would have yielded 5.2-13.9 extra cancer deaths if the risk estimate of Stewart and Kneale had applied, or 3.9 on the basis of the revised estimate at the end of paragraph 239. The authors stated that their findings were inconsistent with the model of Stewart and Kneale, and estimated the upper limit of excess risk of leukæmia death consistent with their negative findings to be less than 20 cases per million per year per rad. The discrepancy might be even greater if it were possible to make allowance for the RBE of neutrons received by fœtuses at Hiroshima.

243. While the reason for the discrepancy between the two sets of data is still unknown it must be borne in mind that the excess risk of cancer in children from mothers x-rayed during pregnancy may not be entirely due to x-irradiation. The major form of x-ray examination during pregnancy is pelvimetry, which in most clinics is performed on about 5-10 per cent of pregnant women for such medical indications as poor obstetric history (e.g., prolonged or difficult labour), previous cæsarean section, pelvic abnormalities, fœto-pelvic disproportion, etc. The possibility cannot be excluded that these conditions, rather than radiation exposure, may be associated with the increased risk of developing leukæmia or other malignancies in children born of irradiated mothers. This possibility has been examined with some care by both Stewart and MacMahon who were unable to identify medical conditions that could be responsible for both an increased risk of cancer and prenatal irradiation. Conclusive evidence may come from studies in clinics where pelvimetry has been a routine procedure. The results of such a study were published by Griem *et al.* (52) but the number (1,008) of mothers who had undergone routine pelvimetry was too small to warrant a reliable conclusion.

244. It is well known that precise risk estimates must preferably be derived from cohort studies rather than from case-control studies. However, cancer risk in children of ages less than 10 is extremely rare (e.g., 10^{-4} or so in the United States), so that it is difficult to carry out a cohort study of sufficient size. Most of the studies of *in utero* exposure thus far reported are case-control studies.

245. Thus, although children born from mothers x-rayed while pregnant seem to have an increased risk of cancer after birth, a possibility still remains that the association, or at least part of it, is caused by factors other than radiation and further studies are needed to clarify this point.

IX. Summary and conclusions

246. The information on radiation carcinogenesis in man that has become available since the last report of the Committee and that has been reviewed in the foregoing pages modifies substantially some of the conclusions reached earlier by the Committee. Data currently available make it possible to single out additional tissues and organs, beside the thyroid and the bone marrow, that appear to be particularly at risk and for which tentative risk estimates can now be given. These new additions include lung tissues and the female breast.

247. The advances are mostly due to additional observations made on the two major samples of irradiated people, namely, those of the survivors of the atomic bombings and of ankylosing spondylitis patients treated by x-irradiation. At both Hiroshima and Nagasaki, mortality records in the Life Span Study Sample have been collected up to the end of 1970, 25 years after the bombings, and the British spondylitics have been followed up fully for 10-11 years on average and, in part only, for an average of 13 years. The results of the Life Span Study Sample apply to the general population (approximately 40 per cent males; 20 per cent less than 10 years old at the time of bombing), those of the spondylitics to a largely (84 per cent) male, adult population of patients affected by a specific disease. The conditions of irradiation were different in the two groups. A pulse of mixed radiation in the case of the survivors, with a much larger neutron component at Hiroshima, and fractionated x-irradiation over long periods of time in the case of the spondylitic patients.

248. Revised estimates of the kermas (see paragraphs 16-19) received by the survivors and of their gamma and neutron components are now available both for Hiroshima and for Nagasaki. However, accurate dose estimates have not yet been made and the Committee had to base its assessment of dose-effect relationships on a number of assumptions. These are particularly critical with respect to the RBE values to be applied to the neutron component of the doses. Since the appropriate values are unknown, the Committee was forced to choose arbitrary ones and decided

to use values varying from 10 at low doses to 1 at high doses. The Committee's analyses, however, show that, while the introduction of any RBE value affects the form of the dose-effect relationship, the risk estimates obtained within the dose range that can be explored do not vary by more than a factor of three.

249. While it is hoped that work now in progress on the dosimetry of the survivors may in the future yield more reliable estimates of the doses received, it will not overcome the basic uncertainties concerning the relative effectiveness of neutron and gamma rays. Because the radiations received at Hiroshima and Nagasaki were of different qualities, continued and prolonged observations of the survivors in the two cities may eventually provide realistic indications of the actual RBE values to be used. At present, numerical values as are given in this annex must be taken for what they are, crude estimates that no amount of statistical or mathematical sophistication will protect from the basic uncertainties of the data from which they stem and the simplifying assumption used in deriving them.

A. Leukæmia

250. Among the survivors of Hiroshima and Nagasaki, incidences rise with kerma in each city at the same rate, whether they are based on mortality or on morbidity data. The rise is steeper at Hiroshima, presumably because of the larger neutron contribution. Assuming RBE values varying from 1 to 10 at high and low doses, respectively, risk estimates of 0.7 (at 60 rads) and 2 cases (at 400 rads) per million per year per rad of low-LET radiation can be obtained (see paragraph 36). The estimates derived from the ankylosing spondylitis patients with bone marrow partially exposed to 300-1,500 rads fall within this range. Since both studies indicate that the risk after 20 years is close to that in the non-irradiated population, the estimates correspond to an over-all risk of between 14 and 40 cases per million per rad.

251. A significant excess of leukæmias is seen at Hiroshima after a mean kerma as low as 22 rads of mixed radiation, corresponding to perhaps 50 rads of low-LET radiation. No excess is seen at comparable doses in Nagasaki, possibly because the sample size in that city is about three times smaller, making the expectations liable to wider chance fluctuations, but more probably because of the lower neutron contribution and therefore the much lower doses received.

252. Other studies reviewed in this annex confirm Marinelli's (97) observation that the risk of leukæmia remains within the limits given above, regardless of distribution of dose in space and time over a wide range of doses. At doses of the order of 1,000 rads, however, there is evidence indicating that the leukæmogenic effect of radiation is overshadowed by its call-killing effect, so that the yield of leukæmia per rad decreases.

B. Thyroid cancer

253. Because of its long times of survival, thyroid cancer must preferably be studied through morbidity surveys. One such survey has been carried out among the atomic bomb survivors of both cities and, in the 20-200 rad range, suggests estimates of between one and two cases per million per year per rad in males and twice as many in females (see paragraph 94), values rather higher than those suggested by the Committee in its earlier report from information derived at higher doses. These provisional estimates would correspond to 20-40 and 40-80 cases per million per rad in males and females, respectively, over a period of 20 years. As for all malignancies other than leukæmia, it is not known when the number of induced cases will start to decline.

C. Breast cancer

254. Breast cancer also has relatively long survival times. It is therefore best studied by means of morbidity surveys. The results of such an investigation among the survivors of the atomic bombing show a significant excess among the irradiated but the numbers are too small to obtain meaningful risk estimates. The mortality study (1950-1970) recorded significant excesses in both cities.

255. At Hiroshima, assuming a varying RBE as for leukæmia, the excess mortality of breast cancer among women is about 0.3 case per million per year per rad at 60 rads of low-LET radiation and about 1 case per million per year per rad at 400 rads (see paragraph 117). Because they are based on mortality statistics, these values are probably underestimates of the risk of induction. A survey based on morbidity reports on patients receiving high breast doses of x rays in the course of pneumothorax therapy, suggests that at average doses in the range of 600-3,000 rads the risk may be 1-6 cases per million per year per rad for about 20 years (paragraph 128).

256. While the rates from which the risks have been calculated have not been adjusted for factors such as parity and lactation history that appear to play a role in the occurrence of breast cancer, it does not seem likely that the risk estimates have been significantly distorted as a result. It is not known whether the increased risk will continue in the future or will soon taper off. Based on 20 years of observation at Hiroshima, the excess mortality per rad for the first 25 years after exposure appears to be from 6 to 20 cases per million per rad, depending on the dose. The morbidity survey of women treated by pneumothorax therapy would suggest a rate of induction up to five times higher. Since no breast cancer appears to be induced in males the figures should be halved to apply them to the general population.

D. Cancer of the respiratory tract

257. Significantly increased lung cancer mortality has been reported from Hiroshima (although not from Nagasaki) in the 1950-1970 period at a total mean kerma of 22 rads. Because of the differential absorption of neutrons and gammas by body tissues, this may be fairly close to the tissue dose, even allowing for a neutron efficiency in inducing lung cancer 10 times higher than for gamma rays. The dose-effect curve for the induction of lung cancers at Hiroshima appears to rise with kerma and reach a plateau somewhere between 150 and 450 rads. If crude allowance is made for depth distribution of the doses and the higher efficiency of neutrons, the resulting risk estimates vary from about 2 cases at 30 rads of low-LET radiation to 0.6 case per million per year per rad at 260 rads (see paragraph 147).

258. No dose-effect relationship for lung cancer is obtainable from the surveys of ankylosing spondylitis patients in which lung cancer is the malignancy whose

incidence contributes the largest part of the excess of malignancies of heavily irradiated sites over the incidence of tumours of the same sites in the general population. If, however, following Dolphin and Marley (39), the dose received on average by the bronchi of the patients is assumed to be some 80 rads, the risk of lung cancer is around three cases per million per year per rad (see paragraph 150), not very different from the estimate for low-LET radiation given above. Considering the uncertainties of the data, however, the agreement could well be fortuitous.

259. There is no way to determine for how long the recent rise in the annual incidence of lung cancers among the Hiroshima survivors and the spondylitics will last. The over-all risk (as based on observations from 5 to 25 years after exposure) can only be stated for the first 25-year period after the exposure as being about 40 cases per million per rad (at 30 rad of low-LET radiation) and possibly 12 cases per million per rad (at 260 rad). The estimates, however, are based on very crude assumptions, particularly concerning RBE. Neither among the survivors nor among the spondylitis patients are the data adequate to exclude the possibility that at least part of the radiation risk might be due to confounding of dose with smoking habits or to a synergistic effect of radiation and smoking.

E. Mortality from other malignancies

260. Increases in the mortality from malignancies other than leukæmia, lung cancer and breast cancer have been observed among both the survivors and the spondylitics. At present, only in the survivors can one attempt to study this excess residual mortality according to dose. The over-all excess is not significant at Nagasaki, presumably as a result of the sample being smaller and the average tissue doses (largely from gamma rays) lower than at Hiroshima where the neutron component was substantial. At Hiroshima only at the highest doses (260 rad) is a significant excess of these malignancies to be observed, corresponding to a risk of about 2.5 cases per million per year per rad of low-LET radiation (see paragraph 188).

261. The types of cancer that contribute significantly to this excess cannot yet be identified in the results obtained with the survivors, nor can it be ascertained whether the excess is to be expected in the future and for how long but some clue is provided by the surveys of the spondylitis patients which indicate that pharynx, pancreas, stomach, bone and lymphatic and hæmapoietic tissues might be particularly at risk. Inferences from the observations made in the spondylitics must, however, be made with caution. On the one hand, without knowledge of the tissue doses involved it is extremely difficult to ascertain the extent to which the observed excess mortality are the result of high doses rather than of high tissue sensitivity. Thus, on present information, it is likely that no excess of bone sarcomas will be seen in the survivors since few induced ones are likely to occur 25 years after exposure, whereas the slight excess of bone tumours observed among the spondylitics may have been due to the very high doses received by the spine during treatment, much higher than the highest doses received by the survivors. On the other hand, increases in cancer frequencies at certain sites that are seen in the spondylitics may reflect the effect of factors other than radiation and the possibility that this might be so must be left open until the observations in the spondylitics are borne out by similar ones among the survivors. A case in point is that of gastric cancer which does not seem to increase among the atom bomb survivors and may have done so among the spondylitics as a direct result of the medication that these patients must have received in large amounts for long periods of time, or of a synergistic effect between radiation and medication.

262. Comparison of the complete with the incomplete follow-up of the spondylitics suggests that the excess risk of tumours of heavily irradiated sites may have increased during the additional observation period. Therefore the estimates that can currently be derived from the survivors and from the spondylitics will have to be periodically reviewed. Since it is not possible now to indicate which trends in over-all incidence are to be expected or which specific tumours are likely to contribute to future increases and to what extent, it is imperative that the long-term investigations that have been carried out so far be pursued for several more decades and their results published in detail at suitable intervals, and that no efforts be spared to obtain adequate estimate of tissue doses.

F. Effects of age at irradiation

263. The surveys of the atomic bomb survivors indicate that subjects irradiated before 40 years of age have a higher relative risk of leukæmia than those irradiated later in life. The survivors that were irradiated in childhood (before 10 years of age) have recently (since 1960) shown a sudden increase in tumour incidence. There did not seem to be any specific pattern in the distribution of the types of tumours observed, although it might be of significance that only one pulmonary carcinoma was reported.

264. The observation of this sudden increase in the incidence of malignancies among subjects irradiated in their childhood is not unexpected. Development of malignancies with long latencies have been and are still being observed in a number of surveys of patients having received head or neck irradiation in their childhood. The continued follow-up of the survivors within the ABCC samples, however, is likely to provide in the long run information on the variation of risk with age that would be difficult to obtain reliably by other means, except if the differences were extreme.

G. Tissue irradiation by alpha particles

265. Because of their very short range, alpha particles emitted by nuclides deposited in body tissues give rise to highly inhomogeneous distributions of dose. This, coupled with the particles' high LET and their low rate of emission makes the few cases of alpha irradiation particularly difficult to investigate since their interpretation is seldom assisted by knowledge of the effects of spatially more uniform, short-term, irradiation. The major groups of alpha-irradiated people are miners whose lungs are exposed to high levels of radon and its daughters, and subjects carrying substantial burdens of radium (^{226}Ra, ^{228}Ra) acquired for medical or occupational reasons or treated by injections of ^{224}Ra-containing drugs.

266. Underground uranium miners provide the largest and best studied group of people exposed to high radon levels. The inhaled radon decays while in the res-

piratory tract and its radio-active daughters trapped on the bronchial epithelium, irradiate it and the tissue layers immediately underneath. Lung cancer has been known for a long time to occur with high frequency among these workers and is considered an occupational disease. The incidence appears to rise linearly with cumulative exposure but so many uncertainties attach to the estimates of the exposure that little reliance can be placed on the shape of the curve, except in so far as it fails to bear out the decrease in risk of lung cancer at high doses that the survey of Hiroshima survivors suggests. Because of differences in quality and in time and space distribution of the radiation, and because of the intervention of extraneous factors such as protracted inhalation of fumes and dusts by the miners and of possible differences in smoking habits between the two populations, close agreement between the observations would have been surprising.

267. People with substantial body burdens of long-lived radium (mostly ^{226}Ra) are few but have been followed for long periods of time (40 years on average) and have received on average much higher cumulative mean bone doses (in rads) than the people included in any of the surveys mentioned before. Only a fraction of the cumulative dose received must have been effective in inducing tumours, but its size cannot be ascertained. At cumulative doses above 1,000 rads they show a much higher incidence of bone sarcomas than the general population and a less pronounced excess of antral carcinomas. The incidence of bone sarcomas appears to reach a peak at around 14,000 rads. The size of the sample is too small to exclude that doses lower than 1,000 rads may in fact give rise to bone tumours of the type reported at higher doses.

268. A larger sample of people treated with short-lived ^{224}Ra, but followed up for shorter periods of time, have also shown increased incidence of bone sarcomas. Sarcomas are seen in groups exposed to significantly lower cumulative mean skeletal doses (about 300 rad) than from ^{226}Ra. The apparent higher sensitivity to radiation from ^{224}Ra may be due to the fact that this nuclide decays before it is embedded in the bone matrix so that substantially higher doses of radiation are delivered to the cells at risk.

H. Effects of pre-natal irradiation

269. The 1964 report of the Committee reviewed the results of a number of surveys of malignancies in children irradiated pre-natally for medical reasons which indicated that these children stand a 40 per cent greater chance than non-irradiated children to die of a malignancy within 10 years of birth. More information has now accumulated which is consistent with the earlier results. Estimates of the doses received by the fœtus during pelvimetry and other radiological procedures suggest that the risk of malignancies (50 per cent of them leukæmias) induced by pre-natal irradiation may be of about 20 cases per million per year per rad over a 10-year period (in the range of 0.2-20 rads).

270. This estimate is not borne out by a survey of survivors of *in utero* irradiation at Hiroshima and Nagasaki which did not show the increased cancer mortality to be expected on the basis of the estimate. It is conceivable that at least part of the increased risk seen among children irradiated *in utero* for medical reasons may be associated with the reasons that had prompted the exposure. It may also be that, owing to inaccurate dosimetry, the risk mentioned above is an over-estimate. It is important that further studies be undertaken aimed at securing reliable dosimetric information on a sufficiently large number of children, and at separating unequivocably the several contributions of the various factors that may affect the risk estimates.

I. Conclusions

271. This annex has reviewed in detail the evidence available on the induction of malignancies by ionizing radiation in man, and derived risk estimates for a few of them. These are summarized in table 22. The Committee wishes to re-emphasize that all the estimates apply to short-term exposures at high dose rates and, as discussed in annex G, are likely to be over-estimates of the risks per unit dose that may result from protracted irradiation at low dose rates of low-LET radiation. The estimates given in this annex are all subject to revision, both because the total risk of any malignancy can only be assessed by observing a cohort of irradiated people until extinction, and in no case has there been an opportunity for such prolonged observation yet, but also because of the basic uncertainties of the data.

272. These reflect a still inadequate knowledge of the tissue doses received by all groups of irradiated people, but even more our ignorance of the RBE values that must be applied in obtaining risk estimates from these groups that were exposed to mixed neutron and gamma radiation and that have so far provided the largest amount of information on the induction of malignancies in man.

Table 1. ABCC-JNIH Life Span Study Sample by sex, exposure category, and city (12)

			Exposure category (distance from A-bomb hypocentre)							
	Total		*0-1,999 metres*		*2,000-2,499 metres*		*2,500-9,999 metres*		*10,000+ metres or not-in-city*	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
Hiroshima	30,691	43,665	8,828	12,501	4,775	6,749	8,798	12,477	8,290	11,938
Nagasaki	11,005	14,032	3,059	3,742	2,053	3,091	3,024	3,718	2,869	3,481
Both cities	41,696	57,697	11,887	16,243	6,828	9,840	11,822	16,195	11,159	15,419

TABLE 2. COMPARISON OF T57D AND T65D KERMA ESTIMATES AT 500-METRE INTERVALS FROM A-BOMB HYPOCENTRE (101)

Ground distance (metre)	Gamma rays (rad)			Neutrons (rad)			Total (rad)[a]		
	T57D	T65D	T65D / T57D	T57D	T65D	T65D / T57D	T57D	T65D	T65D / T57D
				Hiroshima					
0	12,000	10,300	0.86	18,000	14,200	0.79	30,000	24,500	0.82
500	4,030	2,790	0.69	4,390	3,160	0.72	8,420	5,950	0.71
1,000	572	256	0.45	321	192	0.60	893	448	0.50
1,500	80.0	21.6	0.27	20.9	10.1	0.48	100	31.7	0.32
2,000	12.1	1.9	0.16	1.4	0.5	0.36	13.5	2.4	0.18
				Nagasaki					
0	27,000	25,100	0.93	5,500	3,910	0.71	32,500	29,000	0.89
500	7,230	7,090	0.98	1,030	703	0.68	8,260	7,790	0.94
1,000	865	889	1.03	61.0	35.9	0.59	926	925	1.00
1,500	113	119	1.05	3.6	1.7	0.47	117	121	1.03
2,000	16.5	17.8	1.08	0.2	0.1	0.50	16.7	17.9	1.07

[a] Gamma and neutron components added without weighting.

TABLE 3. INCIDENCE OF LEUKÆMIA AMONG A-BOMB SURVIVORS IN THE MASTER SAMPLE, BY SPECIFIC TYPE OF LEUKÆMIA, TOTAL KERMA, AND CITY, OCTOBER 1950-SEPT. 1966 (67)

Type	T65D total kerma (rad)							
	Total		100+		5-99		Under 5	
	Cases	Rate[a]	Cases	Rate[a]	Cases	Rate[a]	Cases	Rate[a]
				Hiroshima				
Acute granulocytic	25	2.0	11	24.3	3	1.1	11	1.2
Acute lymphocytic	13	1.1	5	11.1	4	1.5	4	0.44
Acute (other types)	20	1.6	6	13.3	6	2.3	8	0.87
Chronic granulocytic	29	2.4	10	22.1	15	5.7	4	0.44
Chronic lymphocytic	0	0	0	0	0	0	0	0
Chronic (other types) ...	1	0.08	0	0	1	0.38	0	0
TOTAL	88	7.2	32	70.8	29	11.1	27	3.0
Person-years at risk ..... (*thousands*)	1,221.7		45.2		261.4		915.1	
				Nagasaki				
Acute granulocytic	13	3.0	6	16.4	1	0.96	6	2.0
Acute lymphocytic	7	1.6	6	16.4	0	0	1	0.34
Acute (other types)	4	0.91	1	2.7	0	0	3	1.0
Chronic granulocytic	4	0.91	2	5.5	1	0.96	1	0.34
Chronic lymphocytic	1	0.23	0	0	0	0	1	0.34
Chronic (other types) ...	0	0	0	0	0	0	0	0
TOTAL	29	6.6	15	41.1	2	1.9	12	4.0
Person-years at risk ..... (*thousands*)	437.6		36.5		103.9		297.2	

[a] Cases 10^{-5} y^{-1}.

TABLE 4. OBSERVED AND EXPECTED MORTALITY AMONG A-BOMB SURVIVORS OF ABCC LIFE SPAN STUDY SAMPLE (1950-1966), SEXES AND CITIES COMBINED (10)

Cause of death		*Total T65D kerma (rad)*					
		Total	*0-9*	*10-39*	*40-179*	*180+*	*Unknown*
Leukæmia[a]	Observed	116	35	13	24	35	9
	R.R.[b]	—	1.00	1.62	4.98	17.49	3.85
	Excess number[c]	63.9	—	5.0	19.2	33.0	6.7
	M.R.[d]	95.6	43.1	68.9	214.3	764.3	164.0
All malignant neoplasms except leukæmia[a]	Observed	2,276	1,489	365	233	88	101
	R.R.	—	1.00	1.03	1.13	1.27	1.19
	Excess number	72.2	—	10.6	26.8	18.7	16.1
	M.R.	1,875.0	1,832.8	1,993.4	2,080.4	1,921.7	1,840.4
Cancer of stomach	Observed	959	628	153	99	32	47
	R.R.	—	1.00	1.05	1.17	1.11	1.31
	M.R.	790.0	773.0	810.5	883.9	698.8	856.4
Cancer of large bowel	Observed	129	89	22	11	2	5
	R.R.	—	1.00	1.05	0.92	0.54	1.08
	M.R.	106.3	109.6	116.5	98.2	43.7	91.1
Cancer of liver and biliary tract	Observed	249	176	30	25	13	5
	R.R.	—	1.00	0.70	0.98	1.35	0.41
	M.R.	205.1	216.6	158.9	223.2	283.9	91.1
Cancer of pancreas	Observed	61	47	8	3	2	1
	R.R.	—	1.00	0.74	0.46	0.89	0.36
	M.R.	50.3	57.9	42.4	26.8	43.7	18.2
Cancer of bronchus, trachea, and lung	Observed	145	83	25	22	8	7
	R.R.	—	1.00	1.28	1.89	1.98	1.40
	M.R.	119.5	102.2	132.4	196.4	174.7	127.6
Other cancers[e] (ICD 190-199)	Observed	126	80	19	13	5	9
	R.R.	—	1.00	1.01	1.23	1.44	2.05
	M.R.	103.8	98.5	100.6	116.1	109.2	164.0
Cancer of female breast	Observed	67	41	11	9	2	4
	R.R.	—	1.00	1.05	1.54	1.07	2.07
	M.R.	55.2	50.5	58.3	80.4	43.7	72.9
Cancer of uterus	Observed	194	119	39	19	5	12
	R.R.	—	1.00	1.29	1.14	.98	2.27
	M.R.	159.8	146.5	206.6	169.6	109.2	218.7

[a] Significant ($P < 0.05$) linear increase of excess number of cases with dose.

[b] R.R.: Sex and age-adjusted risk relative to that of 0-9-rad dose category. In the 0-9-rad group median dose is zero.

[c] Observed $\times$ (R.R. $-$ 1)/R.R.

[d] M.R.: Average annual mortality rate (crude rate) per million.

[e] Other cancers, ICD No. 190-199: 191 skin, 193 brain and nervous system, 194 thyroid, 195 bone; 199 others and unspecified, etc.

TABLE 5. OBSERVED AND EXPECTED DEATHS FROM SELECTED TYPES OF CANCER 1950-1970 ACCORDING TO T65D TOTAL DOSE. SEXES ARE COMBINED, EXCEPT FOR BREAST CANCER (74).

A. *Hiroshima*

		NIC[a]	*0-9*	*10-49*	*50-99*	*100-199*	*200+*	*Unknown*	*10+*[b]	*Total*
Mean kerma (rad)		0	1	22	70	139	463	—	—	—
Person-years at risk	Both sexes	342,955	795,669	195,448	48,275	30,223	26,708	29,829	330,483	1,469,107
	Females	202,563	472,722	122,761	30,614	17,810	15,223	17,856	204,264	879,549
Leukæmia	Obs.[c]	10	34	17	7	10	27	5	66	110
	(C.L.)[d]	4.8-18.4	23.6-47.5	9.9-27.2	2.8-14.4	4.8-18.4	17.8-39.3	1.6-11.7	51.1-83.9	89-131
	Exp.1[e]	10.1	22.3	5.5	1.4	0.9	0.8	0.8	9.4	41.8
	Exp. 2[f]	—	—	8.4	2.1	1.3	1.1	1.3	14.2	—
	Exp. 3[g]	—	—	7.6	1.9	1.2	1.0	1.2	12.9	—
	Rate[h]	29	43	87	145	331	1,011	168	200	75
Bronchus, trachea, and lung cancer	Obs.	52	115	42	12	9	8	8	79	246
	(C.L.)	38.8-68.2	96-138	56.7-30.3	6.2-21.0	4.1-17.1	3.5-15.8	3.5-15.8	62.6-98.5	215-277
	Exp. 1	41.2	90.4	23.2	6.1	3.9	3.0	3.3	39.5	171.1
	Exp. 2	—	—	28.2	7.0	4.4	3.9	4.3	47.8	—
	Exp. 3	—	—	28.7	7.1	4.4	3.9	4.4	48.5	—
	Rate	152	145	215	249	298	300	268	239	167
Breast cancer	Obs.	11	40	13	4	6	3	3	29	80
	(C.L.)	5.5-19.7	28.6-54.5	6.9-22.2	1.1-10.2	2.2-13.1	0.6-8.8	0.6-8.8	19.4-41.7	63.5-99.6
	Exp. 1	13.6	31.6	8.5	2.2	1.2	1.0	0.9	13.6	58.8
	Exp. 2	—	—	10.4	2.6	1.5	1.3	1.5	17.3	—
	Exp. 3	—	—	9.3	2.3	1.3	1.2	1.3	15.4	—
	Rate	54	85	106	131	337	197	168	142	91
Other cancers	Obs.	587	1,517	388	110	66	73	62	699	2,803
	(C.L.)	540-634	1,441-1,593	349-427	89-131	51.1-83.9	57.3-92.1	47.5-79.6	647-751	2,699-2,907
	Exp. 1	598.4	1,418.1	366.5	95.6	57.3	44.7	45.4	609.5	2,626.0
	Exp. 2	—	—	373.0	91.8	57.6	51.0	56.9	630.3	—
	Exp. 3	—	—	360.5	89.0	55.9	49.4	55.2	610.0	—
	Rate	1,712	1,907	1,985	2,279	2,184	2,733	2,079	2,115	1,908

B. *Nagasaki*

		NIC[a]	*0-9*	*10-49*	*50-99*	*100-199*	*200+*	*Unknown*	*10+*[b]	*Total*
Mean kerma (rad)		0	2	21	71	146	402	—	—	—
Person-years at risk	Both sexes	118,309	209,872	67,649	22,914	23,017	24,349	27,025	164,954	493,135
	Females	65,723	121,986	39,725	13,202	12,677	13,078	14,093	92,775	280,484
Leukæmia	Obs.	3	11	2	0	3	15	3	23	37
	(C.L.)	0.6-6.8	5.5-19.7	0.2-7.2	0-3.7	0.6-8.8	8.4-24.7	0.6-8.8	14.6-34.5	26.1-51.1
	Exp. 1	3.2	5.6	1.8	0.6	0.6	0.7	0.7	4.5	13.3
	Exp. 2	—	—	3.5	1.2	1.2	1.3	1.4	8.6	—
	Exp. 3	—	—	2.9	1.0	1.0	1.0	1.2	7.0	—
	Rate	25	52	30	—	130	616	111	139	75
Bronchus, trachea, and lung cancer	Obs.	20	29	7	1	4	5	5	22	71
	(C.L.)	12.2-30.9	19.4-41.7	2.8-14.4	0-5.6	1.1-10.2	1.6-11.7	1.6-11.7	13.8-33.3	55.5-89.6
	Exp. 1	10.5	18.1	6.2	2.1	1.9	2.0	2.3	14.5	43.1
	Exp. 2	—	—	9.3	3.2	3.2	3.4	3.7	22.8	—
	Exp. 3	—	—	10.1	3.4	3.4	3.6	4.0	24.5	—
	Rate	169	138	103	44	174	205	185	133	144
Breast cancer	Obs.	4	8	6	2	2	1	1	12	24
	(C.L.)	1.1-10.2	3.5-15.8	2.2-13.1	0.2-7.2	0.2-7.2	0-5.6	0-5.6	6.2-21.0	15.4-35.7
	Exp. 1	3.3	6.4	2.2	0.7	0.6	0.6	0.5	4.6	14.4
	Exp. 2	—	—	2.6	0.9	0.8	0.9	0.9	6.1	—
	Exp. 3	—	—	2.5	0.8	0.8	0.8	0.9	5.8	—
	Rate	61	66	151	151	158	76	71	129	86
Other cancers	Obs.	165	281	105	37	19	37	32	230	676
	(C.L.)	140-190	248-314	85-125	26.1-50.9	11.4-29.7	26.1-50.9	21.9-45.2	200-260	626-729
	Exp. 1	154.5	280.3	98	32.1	28.6	28.3	31.2	218.2	653.0
	Exp. 2	—	—	90.6	30.7	30.8	32.6	36.2	220.9	—
	Exp. 3	—	—	91.9	31.1	31.3	33.1	36.7	224 1	—
	Rate	1,395	1,339	1,552	1,615	825	1,520	1,184	1,394	1,361

[a] Not in city at the time of bombing.
[b] Including unknown dose group.
[c] Observed number of deaths.
[d] 95 per cent confidence limits.
[e] Expected number of deaths based on national rates.
[f] Expected number of deaths based on 0-9-rad group.
[g] Expected number of deaths based on NIC + 0-9-rad group.
[h] Mortality rate, per million per year.

TABLE 6. NUMBERS OF DEATHS OBSERVED AND EXPECTED AMONG ANKYLOSING SPONDYLITIS PATIENTS, BY CAUSE AND PERIOD AFTER FIRST OBSERVATION[a] (28)

Cause of death	No. of deaths	Years after first observation 0-2	3-5	6-8	9-11	12-14	15-24	All periods
Leukæmia	Observed	7	19	14	6	5	1	52
	Expected	1.10	1.49	1.32	0.86	0.45	0.27	5.48
	Obs./Exp.	6.4	12.8	10.6	7.0	11.1	3.7	9.5
Cancer of heavily irradiated sites	Observed	33	36	46	46	27	12	200
	Expected	22.48	33.25	31.32	21.16	11.54	7.52	127.27
	Obs./Exp.	1.5	1.1	1.5	2.2	2.3	1.6	1.6
Cancer of lightly irradiated sites	Observed	13	15	13	12	2	5	60
	Expected	10.27	14.09	12.64	8.27	4.28	2.88	52.42
	Obs./Exp.	1.3	1.1	1.0	1.5	0.5	1.7	1.1
All other causes	Observed	234	336	290	191	113	66	1,230
	Expected	139.07	178.56	155.45	102.30	54.22	35.93	665.56
	Obs./Exp.	1.7	1.9	1.9	1.9	2.1	1.8	1.8
Person-years at risk (*thousands*)		35.5	40.7	31.9	19.2	9.6	4.9	141.8

[a] Followed to 1 January 1960.

TABLE 7. RISK OF LEUKÆMIA IN PATIENTS EXPOSED TO THERAPEUTIC IRRADIATION IN THE PELVIC REGION

Disease	Number treated	Follow-up in years	Treatment	Dose[a] (rad)	Leukæmia Observed	Leukæmia Expected	R.R.[b]
Metropathia hæmorrhagica (33)	2,068	3-24	X rays	222-522	6	1.3	4.6
Benign gynæcological disorders (164)							
A. Connecticut	900	2-32	Radium	159-503	9	2.8	3.2
B. Massachusetts	1,803	2-32	Radium	159-318	10	3.5	2.8
C. Connecticut	993	2-32	X rays	300-900	3	2.4	1.3
Uterine cancer (164)	7,835	2-32	Radium / X rays	900-4,500	9	8.6	1.1
Cervix cancer (64)	28,171	2-5	Radium[c] / X rays	900-4,500	4	5.1	0.8

[a] Mean pelvic marrow dose.
[b] Relative risk: observed cases/expected cases.
[c] 14 per cent treated by radium only, receiving 150-450 rads.

TABLE 8. NUMBER OF CASES OF THYROID CARCINOMA AND RATE PER 1,000 EXAMINED BY AGE AT EXAMINATION, SEX, AND DISTANCE IN METRES FROM HYPOCENTRE (173)

Age (year)	Male <1,400 No.	Male <1,400 Rate	Male 1,400-1,999 No.	Male 1,400-1,999 Rate	Male 3,000+[a] No.	Male 3,000+[a] Rate	Male All distance groups No.	Male All distance groups Rate	Female <1,400 No.	Female <1,400 Rate	Female 1,400-1,999 No.	Female 1,400-1,999 Rate	Female 3,000+[a] No.	Female 3,000+[a] Rate	All distance groups No.	All distance groups Rate
<40	5	9.7	0	0	1	1.1	6	3.3	10	10.7	2	3.1	1	0.7	13	4.3
40-59	0	0	0	0	0	0	0	0	4	4.4	3	3.7	6	3.7	13	3.9
60+	0	0	1	3.3	0	0	1	0.7	4	8.5	1	2.2	1	1.1	6	3.2
All ages	5	3.6	1	0.9	1	0.4	7	1.4	18	7.8	6	3.1	8	2.0	32	3.9

[a] Includes not-in-city at the time of bombing.

TABLE 9. FREQUENCY OF THYROID CARCINOMA PER 1,000 SURVIVORS EXAMINED BY AGE AT EXAMINATION, SEX, AND KERMA (173)

Age (year)	Male				Female			
	0-49	50-199	200+ (rad)	Total	0-49	50-199 (rad)	200+	Total
< 40	0	6.8	9.8	5.3	5.5	4.0	12.8	7.5
40-59	0	0	0	0	2.5	3.8	7.7	4.1
60+	3.5	0	0	1.4	0	16.4	0	5.3
Total	1.1	2.5	4.1	2.4	2.8	6.8	9.1	5.7
Examined .	928	789	740	2,457	1,806	1,332	1,100	4,238

TABLE 10. THYROID NODULES (PLUS HYPOTHYROIDISM) AND MALIGNANCIES FROM 1954 TO 1969 IN RESIDENTS OF MARSHALL ISLANDS EXPOSED TO FALL-OUT (25)

Island	Age at exposure (year)	Estimated thyroid dose[a] (rad)	Percentage of thyroid lesions[b]	Percentage of malignancies[b]
Rongelap (175 rad)[c]	< 10	500-1,400	89.5 (17/19)	5.3 (1/19)
	> 10	160[d]	8.8 (3/34)	5.9 (2/34)
	all		39.6 (21/53)	5.7 (3/53)
Ailingnae (69 rad)	< 10	275-550	0.0 (0/6)	—
	> 10	55	12.5 (1/8)	—
	all	—	7.1 (1/14)	—
Utirik (14 rad)	< 10	55-110	0.0 (0/40)	—
	> 10	14	5.1 (3/59)	1.7 (1/59)
	all	—	3.0 (3/99)	1.0 (1/99)
Rongelap unexposed	< 10	—	0.0 (0/61)	—
	> 10	—	2.3 (3/133)	—
	all	—	1.5 (3/194)	—

[a] Dose from ^{131}, ^{132}, ^{133}, ^{135}I.
[b] Based on present population.
[c] Dose from gamma radiation.
[d] Children 10 to 20 years of age at exposure received up to about 500 rads.

TABLE 11. OBSERVED AND EXPECTED BREAST CANCER IN WOMEN EXAMINED IN THE ABCC-JNIH ADULT HEALTH STUDY 1958-1966 BY ESTIMATED TOTAL KERMA (168)

Total kerma (rad)	Number examined	Breast cancer			
		Observed	Expected[a]	Relative risk[b]	Excess rate per 1,000[c]
NIC	2,458	2			
0-9	3,082	3			
10-39	1,262	4	1.14	3.5	2.3
40-89	857	2	0.77	2.6	1.4
90-199	802	4	0.72	5.6	4.1
200+	841	5	0.76	6.6	5.0
Unknown	840	2	0.76	2.6	1.5

[a] Based on rate in NIC and 0-9-rad groups combined.
[b] Risk relative to that of NIC and 0-9-rad dose category.
[c] (Observed-expected)/examined.

TABLE 12. RISK ESTIMATES OF LUNG CANCER INDUCTION BASED ON THE HIROSHIMA DATA

	Kerma range (rad)			
	10-49	*50-99*	*100-199*	*200+*
$\overline{K_T}$	22	70	139	462
$\overline{K_g}$	18	57	109	332
$\overline{K_n}$	4	13	30	130
$\overline{D_g}$	10	35	64	195
$\overline{D_n}$	2	7	15	65
RBE	10	5	2.5	1
$\overline{D_T}$[a]	30	70	102	260
E[b]	70	104	152	154
R[c]	2.3	1.5	1.5	0.6

[a] $\overline{D_T} = \overline{D_g} + \text{RBE} \times \overline{D_n}$.
[b] Excess number of cases per million per year by comparison with 0-9-rad group.
[c] Absolute risk: $R = E/\overline{D_T}$.

TABLE 13. NUMBERS OF DEATHS OBSERVED AND EXPECTED FROM CANCER OF HEAVILY IRRADIATED SITES SIX OR MORE YEARS AFTER FIRST OBSERVATION[a] AMONG ANKYLOSING SPONDYLITIS PATIENTS (28)

Primary site (death certification)	*Deaths*			*Relative risk*	*Excess mortality per million per year*
	Observed	*Expected*	*Difference*		
Pharynx	5	1.05	3.95	4.8[b]	23.8
Œsophagus	3	3.37	−0.37	0.9	−2.2
Stomach	38	23.62	14.38	1.6[b]	86.8
Pancreas	12	5.71	6.29	2.1[b]	38.0
Larynx	2	1.81	0.19	1.1	1.2
Bronchi	96	54.20	41.80	1.8[b]	252.4
Ovaries	4	2.16	1.84	1.9	11.1
Skin	0	1.37	−1.37	0.0	−8.3
Bones	5	1.11	3.89	4.5[b]	23.5
Hodgkin's disease	1	2.47	−1.47	0.4	−8.9
Other lymphatic and hæmopoietic tissues	10	3.39	6.61	2.9[b]	39.9
Others	24	6.78	17.22	3.5[b]	104.0
All heavily irradiated sites	200	107.04	92.96	1.9[b]	561.2

[a] Followed to 1 January 1963.
[b] Statistically significant: $P < 0.025$ on a one-tailed test.

TABLE 14. LUNG CANCER MORTALITY AMONG URANIUM MINERS (86)

WLM	*Estimated cumulative WLM*					
	< 120	*120-359*	*360-839*	*840-1,799*	*1,800-3,719*	*>3,720*
Observed number of deaths	1	12	14	12	21	10
Expected number of deaths[a]	1.81	2.57	2.95	2.52	1.43	0.42
Excess		9.43	11.05	11.48	19.57	9.58
Man-years at risk	8,516	9,365	9,045	6,607	3,455	978
Excess mortality rate $\times 10^3$		1.0	1.2	1.7	5.7	9.8

[a] Based on mortality in the population of the four states in which miners were examined. Not adjusted for smoking habits.

TABLE 15. BONE SARCOMAS AND ANTRUM CARCINOMAS IN CARRIERS OF ^{220}Ra (125)

Median dose (rad)	*Sample size*	*Bone sarcomas*	*Antrum carcinomas*	*Total tumours*
23,300	18	3	4	7
12,600	23	12	1	13
6,590	39	15	4	19
2,980	72	14	7	21
1,280	42	6	4	10
756	44	1	0	1
284	80	0	0	0
139	83	0	0	0
65	88	0	0	0
31	139	0	0	0
15	73	0	0	0
5.2	73	0	0	0
0.45	6	0	0	0
TOTAL	780	51	20	71

TABLE 16. BONE SARCOMA IN PATIENTS EXPOSED TO ^{224}Ra (144)

Juveniles (<20 y)			*Adults*		
Mean dose	*Sample size*	*Bone sarcomas*	*Mean dose*	*Sample size*	*Bone sarcomas*
47	5	0	53	210	0
146	7	0	139	229	3
363	35	2	306	214	4
727	76	4	650	55	3
1,345	72	19			
3,329	22	8			

TABLE 17. OBSERVED AND EXPECTED NUMBER OF DEATHS BY CAUSE[a] AMONG ANKYLOSING SPONDYLITIS PATIENTS (28)

Cause of death	*Deaths*				*Excess mortality per million per year*
	Observed	*Expected*	*Excess*	*Obs./Exp.*	
Leukæmia	52	5.48	46.52	9.5	328.1
Cancer of heavily irradiated sites[b]	200	127.27	72.73	1.6	512.9
Cancer of lightly irradiated sites[c]	60	52.42	7.58	1.1	53.5
Causes with no obvious relation to ankylosing spondylitis[d]	752	555.41	196.59	1.4	1,386.4

[a] Followed to 1 January 1960.

[b] Cancer of pharynx, œsophagus, stomach, pancreas, larynx, bronchi, ovaries, skin, bones, Hodgkin's disease, and cancer of other lymphatic and hæmopoietic tissues except leukæmia.

[c] Cancer of brain and central nervous system, mouth, liver and gall bladder, rectum, breast, uterus, prostate, testes, kidneys, and urinary bladder.

[d] Such as peptic ulcer, cerebro-vascular disease, bronchitis, violence, etc.

TABLE 18. NUMBERS OF DEATHS OBSERVED AND EXPECTED BY CAUSE AND PERIOD AFTER FIRST OBSERVATION[a] AMONG ANKYLOSING SPONDYLITIS PATIENTS (28)

Cause of death	Number of deaths	Years after first observation 0-2	3-5	6-8	9-11	12-14	15-24	All periods
Leukæmia[b]	Observed	7	19	16	10	7	1	60
	Expected	1.10	1.49	1.59	1.27	0.76	0.54	6.75
	Obs./Exp.	6.4	12.8	10.1	7.9	9.2	1.9	8.9
Aplastic anæmia[b]	Observed	3	7	5	1	0	0	16
	Expected	0.11	0.14	0.14	0.11	0.06	0.05	0.61
	Obs./Exp.	27.3	50.0	35.7	9.1	0.0	0.0	26.2
Cancer of heavily irradiated sites	Observed	33	36	52	67	46	35	269
	Expected	22.48	33.25	38.55	32.52	20.29	15.67	162.76
	Obs./Exp.	1.5	1.1	1.3	2.1	2.3	2.2	1.7
Person-years at risk (thousands)		35.5	40.7	37.4	27.1	15.2	9.8	165.6

[a] Incomplete follow-up to 1 January 1963.
[b] Although all patients were not followed individually until 1 January 1963, the total number of deaths is probably known, as the names of the untraced patients had been checked against a nominal roll of persons dying of these conditions.

TABLE 19. NUMBERS OF DEATHS BY CAUSES AND TIME SINCE FIRST TREATMENT IN PATIENTS TREATED BY X-IRRADIATION FOR METROPATHIA HÆMORRHAGICA (38)

Cause of death	Within 5 years of first treatment Observed	Expected	Observed/Expected	5 years or more after Observed	Expected	Observed/Expected	P[a]
Leukæmia	0	0.36	0	6	0.95	6.32	< 0.0005
Cancer of heavily irradiated sites	2	6.99	0.29	31	18.40	1.68	< 0.002
Cancer of breast	4	4.42	0.90	5	10.54	0.47	< 0.05
Other cancers	7	6.66	1.05	25	21.65	1.15	—
Coronary disease	1	3.92	0.26	27	28.22	0.96	—
Other causes	48	33.56	1.49	87	98.77	0.88	—
All causes	64	55.94	1.14	181	178.62	1.01	—

[a] One-tailed test.

TABLE 20. OBSERVED AND EXPECTED DEATHS ATTRIBUTED TO CANCER EXCEPT LEUKÆMIA, 1955-1969, AGES 0-9 YEARS AT EXPOSURE (71)

Dose	Survivors, January 1960	Estimated person-years 1955-1969	Deaths Observed	Deaths Expected[a]	Observed/Expected	P
Not-in-city or < 10 rad	15,667	235,005	14	13.98	1.00	
10-99 rad	3,650	54,750	0	3.26	0.0	—
100+	799	11,985	6	0.713	8.40	~ 0.0001
Unknown	299	4,485	2	0.267	7.48	~ 0.03
TOTAL	20,415	306,225	22	18.22		

[a] Computed with Japanese national rates of 1962.

TABLE 21. OBSERVED AND EXPECTED MALIGNANCIES IN INDIVIDUALS TREATED BY X-IRRADIATION FOR THYMIC ENLARGEMENT AND THEIR SIBLING CONTROLS (58)

Type	Exposed subjects Observed	Exposed subjects Expected	Sibling controls Observed	Sibling controls Expected
All malignancies	33	8.10	14	14.56
Thyroid carcinoma	19	0.14	0	0.31
Leukæmia	6	2.02	2	3.21
Hodgkin's disease	0	0.47	1	0.80
Salivary gland tumour	4	0.08	1	0.15
Breast carcinoma	0	0.11	0	0.46
Brain tumour	1	1.23	2	2.48
Others	3	——	8	——

TABLE 22. SUMMARY OF RISK ESTIMATES

Irradiated population[a]	Radiation quality[b]	Mean dose or dose range (rad)	Observation period[c]	Type of data[d]	Sex	Age at exposure[e]	Risk per 10^6 y rad[f]	Paragraph
			Leukæmia					
H	GN	60	5-25	Mt	MF	AC	0.7	36
H	GN	400	5-25	Mt	MF	AC	2.0	36
N	G	10-400	5-21	Mb	MF	AC	1.6	24
S	X	300-1,500	(5.5)	Mt	M	A	1.2	54
P_1	X	0.2-20	0-10	Mt	MF	F	10	239
P_2	GN[g]	25	0-10	Mt	MF	F	NE	241
			Thyroid cancer					
HN	GN[g]	25-200	5-20	Mb	M	AC	1-2	94
HN	GN	25-200	5-20	Mb	F	AC	2-4	94
I	X	50-600	(16)	Mb	MF	C	2.5	230
			Breast cancer					
HN	GN[g]	150	13-21	Mb	F	AC	2-4	109
H	GN	60	5-25	Mt	F	AC	0.3	117
H	GN	400	5-25	Mt	F	AC	1.0	117
N	G	20-400	5-25	Mt	F	AC	0.7[h]	117
T	X	600-3,000	(17.5)	Mb	F	AC	1-6	128
			Lung cancer					
H	GN	30	5-25	Mt	MF	AC	2.3	147
H	GN	260	5-25	Mt	MF	AC	0.6	147
N	G	20-400	5-25	Mt	MF	AC	NE	146
S	X	80	(10.5)	Mt	M	A	3	150
			Other types of cancer					
H	GN	30	5-25	Mt	MF	AC	NE	188
H	GN	260	5-25	Mt	MF	AC	2.5	188
N	G	20-400	5-25	Mt	MF	AC	NE	187
P_1	X	0.2-20	0-10	Mt	MF	F	10	239
P_2	GN[g]	25	0-10	Mt	MF	F	NE	241

[a] H = Hiroshima survivors; N = Nagasaki survivors; S = ankylosing spondylitis patients; P_1 = children irradiated pre-natally for medical reasons; P_2 = children exposed while *in utero* to A-bomb radiation; I = infants irradiated in the cervical region; T = tuberculosis patients.

[b] G = gamma rays; N = neutrons; X = x rays; GN = mixed radiation.

[c] Years elapsed between exposure and beginning and end of follow-up period or, in brackets, average duration (years) of follow-up.

[d] Mt = mortality; Mb = morbidity.

[e] A = adults; C = children; F = fœtuses.

[f] NE = no excess or no statistically significant excess.

[g] No neutron RBE applied to calculate dose.

[h] Based on the over-all excess among those exposed to known doses > 10 rad (average 113 rad).

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