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

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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

Introduction

1. The genetic effects of ionizing radiation were last reviewed comprehensively by the Committee in its 1966 report (575), whereas the particular problem of the induction of chromosome aberrations by irradiation of human somatic cells was reviewed in the Committee's 1969 report (576). The present review will consider the further experimental data that have been obtained since these reports. Of the recent advances in human genetics, those concerning the occurrence and transmission of translocations have particular relevance to the problem of estimating risks, and will be discussed in the last section of this review.

I. Effects in mammals

A. DOMINANT LETHALS

2. The 1966 report surveyed at some length the available data on the induction of dominant lethals in mammals, in *Drosophila* and in several other organisms and concluded that, with regard to this class of genetic damage (*a*) the sensitivity pattern of the various stages of gametogenesis is similar in widely different species; (*b*) among male germ cells, the highest frequencies are usually observed in spermatids and the lowest in spermatogonia; (*c*) among female germ cells, the highest frequencies are found in meta-

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 $= 100 - \left(\frac{\text{live embryos per female in the experimental group}}{\text{live embryos per female in controls}}\right) \times 100$

of 500 rads are lower in the guinea-pig and the rabbit than in the mouse; the pattern of relative sensitivity of the germ-cell stages, however, is similar in these three species, spermatids (sampled during the third week in the mouse but in the fourth and fifth weeks in the guinea-pig and in the rabbit) being more sensitive than mature 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 diœstrus 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^H (brachypody-Harwell), *fz* (fuzzy), *ln* (leaden), *pa* (pallid), *pe* (pearl).

bp^u 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^{-5} 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 ± 7.6) 10^{-4} to (9.1 ± 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 ± 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 ± 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 *In*) 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.

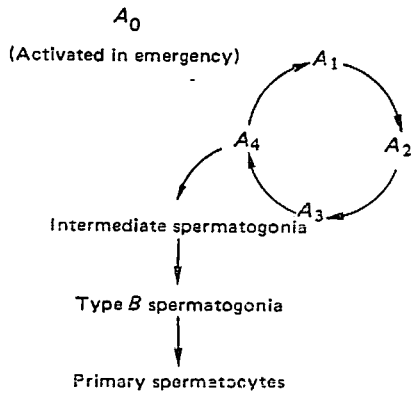
229. Attempts at measuring the over-all effects of induced mutations using several measurable end-points believed to be components of fitness have, in general, yielded negative results and suggest that the deleterious effects in heterozygotes are presumably much less severe than would be expected from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

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

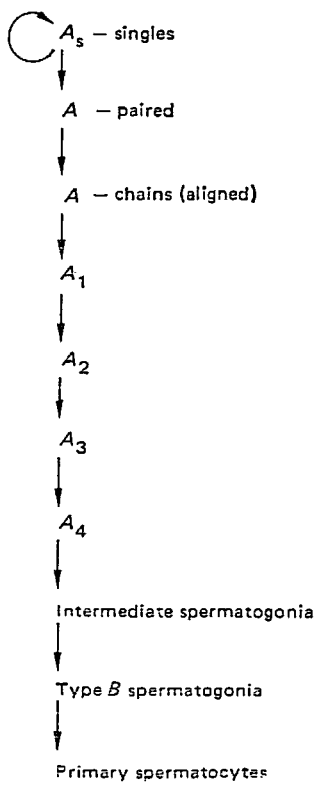
230. Description of the stages of the cycles of seminiferous epithelium has made possible the accurate identification of cells, the determination of cell lineages, the quantitation of cells and the elucidation of cell development times in spermatogenesis (233, 356). It became clear that the stem cell 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

irregularly-aligned spermatogonia which transform morphologically into the chains of A_1 spermatogonia. All A_1 cells divide into A_2 cells and A_2 cells into A_3 cells, etc. Division of the A_4 spermatogonia results only in the formation of cells of the intermediate type. There is no evidence that A spermatogonia of any type are formed from the A_4 cells.

235. It is thus clear that the derivation of differentiating spermatogonia from a stem-cell population rather than from a recycling of more differentiated elements changes the base line—from the total A population as used in the past (357) to that of the A_s spermatogonia —for evaluating the relations between differential cell survival and genetic effects. Only A_s spermatogonia, being the true stem cells and also representing the most radiation-resistant cell type will, therefore, be responsible for the long-term genetic effects of radiation.

236. The experiments of Oakberg (362) that bear on the problem of sensitivity of spermatogonial cell types and on the interrelationships between cell survival and genetic effects can be briefly summarized as follows. In one experiment, 12-week-old male mice were given x-ray exposures of 100 roentgens and the spermatogonia surviving the irradiation were examined 72 hours later and classified as to cell type. These survival frequencies (relative to controls) were 58 per cent for A_s, 22 per cent for A_1 and 5 per cent for A_2-A_4 spermatogonia. These results are in agreement with the observations in rat that A_0 cells are the most resistant spermatogonial cell type.

237. The other experiment was designed to trace the progression of labelled A_s spermatogonia through two cycles of the seminiferous epithelium under irradiation, thereby throwing light on the possible effects of differential cell survival and/or cell synchronization on mutation frequency. Accordingly, a group of 12-week-old male mice were given intraperitoneal injections of 12.5 microcuries of ^{3}H-thymidine at five-hour intervals for a total of six injections, this régime being chosen on the basis of the duration of the cell cycle as determined by Monesi (307). Twenty-four hours after the last injection, the mice were given single x-ray exposures of 100, 500 and 1,000 roentgens and the first fraction of an equally divided 1,000-roentgen exposure; the second fraction was given 24 hours later. The mice were killed at intervals ranging from 12 hours to 17 days after irradiation and the testes were appropriately processed for autoradiographic examination. Suitable controls were maintained.

238. Data on the frequencies of labelled cells given in table 24 show fluctuations prior to eight days and a half which can be interpreted as arising from both division of surviving A_s spermatogonia and continued radiation-induced degeneration. After 8½ days, one cycle of the seminiferous epithelium had been completed and all cells had an opportunity to divide, thereby expressing lethal damage. At this time, it is observed that labelling is approximately 8 per cent for controls, 16 per cent for 100 and 500 roentgens, 2 per cent for the 1,000-roentgen single exposure and 39 per cent for the 1,000-roentgen fractionated exposure. This ranking was also observed at 17 days, with the percentage of labelling reduced by one order of magnitude. This suggests that the normal A_s kinetics in the irradiated groups had been re-established after 8½ days, and that cell behaviour in irradiated and control groups then was the same.

239. The relevance of the above observations to the observed mutation frequency cannot be definitely stated. It is remarkable, however, that the 100- and 500-roentgen exposures showed qualitatively the same labelling, the single 1,000-roentgen exposure showed a very low frequency of labelled cells, and the 500 + 500-roentgen exposure showed the highest amount of labelling. These differences are roughly comparable to those observed in Russell's data (table 14) and suggest that the population of cells labelled in Oakberg's experiment may be that in which mutations are preferentially induced. Though at first sight this may appear to be selection of cells with inherent differences in sensitivity, it is equally likely that selection operates by changing the frequency of cells which have the capacity for repairing pre-mutational damage.

240. The enhancement in the mutation frequency with a split 1,000-roentgen exposure (two 500 R fractions separated by 24 hours) observed in Russell's studies has been attributed to cell synchronization brought about by the first fraction of the exposure so that the cells are in a sensitive stage for mutation induction when the second fraction is delivered. Oakberg's earlier work (357) demonstrated that most A spermatogonia were in interphase 24 hours after 500 roentgens, consistent with the hypothesis of synchronization. It is now clear that this effect could also be explained by survival of A_s spermatogonia, most of which are normally in interphase. However, this does not deny the hypothesis of synchronization which would merely be limited to the A_s spermatogonia. The data in table 22 demonstrate that the second 500-roentgen exposure also has a selective effect in that proportionally more unlabelled cells are killed, resulting in an increase in labelling from the 16 per cent observed after 500 roentgens to 39 per cent after 500 + 500 roentgens. That differential division of labelled *versus* unlabelled cells is involved, appears unlikely in view of the maintenance of the relative effect at 17 days. Thus a selective action of the second 500-roentgen fraction could be a factor in the enhanced effectiveness of fractionated 1,000-roentgen exposure in mutation induction.

241. The most important point that emerges from Oakberg's study is that the spermatogonial types which previously had been shown to be the most sensitive to radiation-induced cell killing do not contribute to the stem-cell pool, and thus are of minor importance in the over-all estimate of genetic damage in spermatogonia. All long-term genetic effects will be based on A_s spermatogonia.

H. Mammalian cells in culture

242. Mammalian-cell-culture systems have been extensively used during the past several years for the study of chromosome aberrations resulting from radiation and other mutagenic treatments. The results of radiation studies on chromosome aberrations in human cells in culture were exhaustively reviewed in the 1969 report of the Committee (576). It has been hoped for some time that cell culture systems might be useful in experimental approaches to the problem of estimating mutation rates. There are now signs that such hopes are being fulfilled (145, 146).

243. The development of selective methods for genetic markers and the demonstration of mutation induction by chemicals in mammalian cells in cul-

ture paved the way for investigations into the dose-effect relationships and mechanisms of mutagenesis after the exposure of cells to radiation (78, 79, 199, 400, 539).

244. The mutations studied in somatic cells are biochemical and have been isolated in two ways: (*a*) by establishing cell cultures from animals having known hereditary variants which are also expressed at the cellular level; and (*b*) applying various selective techniques to *in vitro* cell populations and isolating clones of cells that have developed phenotypes different from those of the parental culture.

245. Until now, mutation-rate studies have been possible only with variants developed *in vitro* in established cell lines. Among these, the radiation-induction of forward mutations such as those to glycine auxotrophy and to resistance to 8-azaguanine (8-AG) have been intensively studied, mostly in Chinese hamster cells. Some preliminary information is available on the possible induction of 8-AG resistant mutations in cultured human fibroblasts.

246. Kao and Puck (200) showed that UV light and x rays (besides a number of chemical mutagens) can induce forward mutations to glycine auxotrophy ($gly^+ \rightarrow gly^-$) in a Chinese hamster cell line. The rate of induction by x rays was estimated to be $4\ 10^{-8}$ per locus per rad, the estimate being based on the average rate at four loci, mutation at any one of which can give rise to a gly^- phenotype (198).

247. Selection for resistance to 8-AG is based on the activity of hypoxanthine-guanine-phosphoribosyl transferase (HG-PRT) which is specified in humans by a gene on the X chromosome (495). Chu (76, 77) has advanced the hypothesis that, in Chinese hamsters and perhaps in other mammals as well, the gene controlling HG-PRT activity is also X linked.

248. Normal substrates for the enzyme are hypoxanthine and guanine which are converted to inosine 5′-monophosphate and guanosine 5′-monophosphate, respectively. Cells having HG-PRT activity can also convert purine analogues such as 8-AG and 6-mercaptopurine to their nucleotides, the incorporation of which results in inhibition or death, indicating that normal cells are sensitive to these metabolites. Cells with reduced HG-PRT activity have impaired ability to incorporate the abnormal purines and are relatively resistant to them. This is illustrated by fibroblast cultures from boys suffering of the Lesch-Nyhan syndrome, which show a marked deficiency of HG-PRT activity (6. 495).

249. Bridges, Huckle and Ashwood-Smith (52), Bridges and Huckle (51) and Chu (77) obtained evidence for the UV- and/or x-ray-induction of mutations to 8-AG resistance (azg^r) in an aneuploid cell line of Chinese hamster. A general observation made by all these investigators was that factors such as inoculum size, incubation time and the concentration of 8-AG profoundly influenced the mutation frequency, similar to what has already been known from chemical mutagenesis studies.

250. Furthermore, the work of van Zeeland *et al.* (580) shows that the selection of 8-AG-resistant mutants is largely influenced by a phenomenon known as metabolic co-operation which turns mutant cells into phenotypically wild-type cells (i.e. mutant cells are able to incorporate the substrate by co-cultivation with cells that have enzyme activity). As a consequence mutant cells cannot be selected above a certain cell density. The authors demonstrated that the underlying basis for metabolic co-operation is cell contact; when mutant and wild-type cells were separated by a fibrin layer, metabolic co-operation did not occur.

251. Bridges and Huckle (51) found that the UV-dose-response curve for the induction of azg^r mutations (7.5 μg ml^{-1} 8-AG) was linear in the 42-210 erg mm^{-2} dose range. With x-irradiation, however, the yield of mutants increased faster than linearly (dose range: 200 to 1,000 rad). The authors have estimated that at the dose of 450 rads, the mean mutation rate per rad is $9.2\ 10^{-7}$, a value obtained by averaging results of two experiments where the concentration of 8-AG used was 30 μg ml^{-1}.

252. The results of Chu (77) also show that the induction kinetics of azg^r mutants (30 μg ml^{-1} 8-AG) was non-linear in the exposure range from 100 to 1,200 roentgens (six levels). The rate of induction increased from $4.2\ 10^{-7}$ per roentgen after 200 roentgens to $1.8\ 10^{-6}$ after 1,200 roentgens.

253. Chu's data from reversion tests using specific chemical mutagens, limited as they are at present (72 randomly isolated azg^r mutants tested), suggest that both point mutations (nucleic acid base changes) and chromosome deletions (interstitial deletions or gross chromosomal changes encompassing the locus) may be induced by x rays.

254. The observations presented in paragraphs 250-253 would lead one to expect a dose-rate effect for the x-ray induction of azg^r mutations. There is some recent evidence showing that such an effect exists, the frequency of mutations being significantly lower at 20-30 rad min^{-1} than that at 100 rad min^{-1} (412).

255. Artlett and Potter (14) studied the induction of azg^r mutants (7.5 μg ml^{-1} 8-AG) by ^{60}Co gamma irradiation (100 rad min^{-1}) and found that the dose-response curve was non-linear (range: 200 to 1,200 rad); more mutants were induced at higher than at lower doses, an observation similar to that of Bridges and Huckle (51) and of Chu (77) (paragraphs 251, 252). Furthermore, the authors showed that while survival was higher after fractionated ($400 + 400$ rad) than after single exposure (800 rad) mutation frequencies were lower after fractionated than after single exposures. The effect of dose fractionation reached a maximum after an interval of 2.5 hours. The authors have concluded that the non-linear dose-response curve for mutation induction and the sparing effect of split dose régimes suggest the existence of repair mechanisms for premutational lesions. Artlett and Potter (14) also showed, using synchronized populations of cells, that responses for both survival and mutation induction were dependent on the cell cycle stage with G_2 phase cells being more mutable than G_1 or S phase cells.

256. Albertini and de Mars (6) have isolated two AG-resistant mutants from an experiment in which cultures of karyotypically normal human fibroblasts were irradiated at x-ray exposures of 150 roentgens. Although uncertainty exists as to whether the mutations were indeed induced, the authors believe that their mutants are the first biochemically-defined diploid mutants of human cells to be isolated *in vitro*. No esti-

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

duplication and the free IV (5.82 ± 0.20 per cent *versus* 1.95 ± 0.10 per cent). This loss-ratio is maintained for the first eight daily broods and probably corresponds to oöcyte stages 1-7. It should be pointed out that, despite this correlation, there is no strict correspondence with length since the length ratio is 10:1. Secondary factors are probably involved in reducing the over-all ratio from 10:1 to 3:1.

288. In a subsequent study using two kinds of females, one heterozygous and the other homozygous, for a multiply-inverted X chromosome, Day and Grell (104) obtained evidence indicating that neither structural heterozygosity of homologues nor exchange between homologues modifies the frequencies with which they are lost following irradiation of any oöcyte stage.

2. *Non-disjunction in* Drosophila

289. Kiriazis (219) failed to obtain evidence of induced non-disjunction of the X chromosome and of chromosome IV in stage-14 oöcytes with x-ray exposures ranging from 100 to 500 roentgens. The more recent results of Traut (562) on X-chromosome non-disjunction in the same germ-cell stage (400 R x rays) are entirely in line with the findings of Kiriazis.

290. In the paper of Zimmering and Scott (616) the frequencies of chromosome losses and non-disjunction obtained with x-ray exposures of 750 roentgens to stage-14 oöcytes were pooled. The combined frequency dropped from nearly 8 per cent (first 6-hour sampling period) to 4 per cent (second 6 hours) and finally to 2.8 per cent (last 12 hours). Since x rays do not seem to induce non-disjunction in stage-14 oöcytes, the decline observed by Zimmering and Scott in the total frequency of non-disjunction and chromosome loss is most probably due to a reduction in the frequency of chromosome losses alone.

291. In another study, Traut (561) showed that stage-7 oöcytes are also refractory to induced X-chromosome non-disjunction but only up to an exposure of 1,000 roentgens. Beyond this exposure up to 1,800 roentgens, the frequency of non-disjunction increases approximately linearly with exposures.

292. In the study discussed in paragraph 281, Traut (563) also investigated the effects of exposure fractionation and lowering of exposure rate on the frequencies of X-chromosome non-disjunction in stage-7 oöcytes. The experimental design was identical to that used for measuring X losses. It was found that the induction of non-disjunction was not influenced by exposure-fractionation; however, at the lower exposure rate (10 R min^{-1}), the non-disjunction frequency was only one-quarter of that observed after irradiation at 850 R min^{-1}. These observations, together with those on X losses, suggest that true X losses and non-disjunction are produced by different mechanisms, a conclusion which is supported by other studies (556, 557, 561, 562, 564).

293. In contrast to the marked correlation that exists between chromosome size and induced frequency of losses (paragraph 287), non-disjunction has been found to be unrelated to chromosome length. With an x-ray exposure of 4,000 roentgens to newly eclosed females, Grell *et al.* (160) found that the average non-disjunction frequency of the two small extra chromosomes used (as measured in the first 12 daily broods) was 1.24 and 0.07 per cent, a value not significantly different from that of 1.19 and 0.15 per cent recorded for the large X chromosomes for the same period.

294. In another investigation, Day and Grell (104) showed that the frequency of induced non-disjunction of the X chromosomes was the same in females, irrespective of whether they were homozygous or heterozygous for inversions. This observation is similar to the one made with X-chromosome losses (paragraph 288).

295. Bateman (38) made a study of chromosome-II non-disjunction in female germ cells of *Drosophila*. Since ordinarily loss of any of the major autosomes would be lethal, the crossing scheme involved a special stock of males in which chromosomes II were present as isochromosomes with the two left arms attached to one centromere and the two right arms to another. The two isochromosomes behave as univalents passing independently to one of the two poles at first meiotic division so that aneuploid gametes carrying the left, the right, both or no isochromosomes are formed in equal numbers. On mating flies carrying normal chromosomes II to such a stock, all normal gametes will produce lethal zygotes, but disomic or nullisomic gametes will produce viable zygotes when combined with nullisomic and disomic gametes from the isochromosome stock.

296. The females were given x-ray exposures of 2,000-8,000 roentgens and mated to isochromosome males. Twelve successive one-day broods were raised. Among the progeny obtained, two thirds were from nullisomic eggs and the complementary class from disomic eggs constituted one tenth of the total progeny. The remainder was made up of a new unexpected class which carried one newly induced isochromosome and a paternal isochromosome.

297. The daily yield of progeny increased from the second to the seventh day (with a peak on day 6) and then fell in the next two days, stabilizing at a very low level during the next three days. Since post-DNA synthesis stages of the oöcytes are sampled during the first six days (72), Bateman concludes that both non-disjunction and isochromosomes can be induced in oöcytes in which DNA synthesis has been completed.

298. This elegant technique, while enabling the recovery of non-disjunctional progeny, does not permit an estimate of their relative frequency. Clark and Sobels (82) recently adapted Bateman's method for a quantitative study of radiation-induced autosomal non-disjunction, by using females with isochromosomes.

299. In females, isochromosomes normally disjoin regularly and non-disjunction occurs at very low frequencies. Following x-irradiation of isochromosome-carrying females (stage-7 oöcytes), Clark and Sobels (82) were able to demonstrate the induction of non-disjunction at exposures of 1,000 roentgens and lower. This finding is in contrast to that of Traut (561) which indicates an apparent threshold exposure of 1,000 roentgens, below which no X-chromosomal non-disjunction seems to be induced (paragraph 291).

3. *Summary and conclusions*

300. The evidence presented in the preceding paragraphs demonstrates that in *Drosophila* most of the X-chromosome losses induced in mature spermatozoa are partial. There is no oxygen enhancement effect for

the loss of ring-X chromosomes, although such an effect is known to exist for normal rod-X chromosomes. Loss of ring-X chromosomes from irradiated spermatozoa is influenced by the genotype of the females with which the irradiated males are mated.

301. In stage-7 oöcytes, the frequencies of X-chromosome losses increase linearly with exposure between 100 and 500 roentgens. Beyond 500 roentgens the increase is non-linear. In stage-14 oöcytes, the increase is non-linear between 100 and 500 roentgens.

302. Nearly 39 per cent of the X-chromosome losses observed in stage-14 oöcytes after 400 roentgens are partial. This proportion of partial losses is enough to account for the two-track component of the dose-response curve, as observed in genetic experiments. In contrast, after 3,500 roentgens to stage-7 oöcytes, only 7-23 per cent of the losses are partial, a proportion which cannot entirely account for the two-track component of the dose-response curve for this germ-cell stage.

303. In stage-7 oöcytes there is a positive correlation between the x-ray induction of complete X-chromosome loss and chromosome-IV non-disjunction.

304. Stage-14 oöcytes are refractory to the induction of X- or IV-chromosome non-disjunction. In stage-7 oöcytes, there seems to be a threshold exposure of 1,000 roentgens below which non-disjunction is not induced. Beyond this exposure, up to 1,800 roentgens, the frequency of non-disjunction increases linearly; at exposures higher than 1,800 roentgens, the dose-effect curve first flattens and then rises again. In stage-7 oöcytes, fractionation of x-ray exposures and lowering of the exposure rate lead to marked reduction in the frequencies of X-chromosome losses; in contrast, only the latter procedure causes a reduction in the frequencies of X-chromosome non-disjunction.

305. Techniques are available in *Drosophila* to study non-disjunction of the autosomes. In contrast to the observations on X-chromosomal non-disjunction, data indicate that chromosome-II non-disjunction can be induced by exposures of 1,000 roentgens and lower.

B. Isochromosomes

306. In a study primarily designed to measure non-disjunction of chromosomes II in *Drosophila* females (paragraphs 295-297) Bateman (38) found that nearly 25 per cent of the viable progeny carried newly induced isochromosomes. By x-irradiating females which carried isochromosomes, Bateman also showed that normal chromosomes II can be reconstituted from isochromosomes. The frequencies of induction, however, cannot be determined (paragraph 298).

307. Sobels (524) and Leigh and Sobels (242, 243) have extended the study of the induction of isochromosomes to male germ-cell stages of *Drosophila*. X-irradiated males were crossed to females carrying isochromosomes for chromosomes II or III, and successive stages of germ-cell development were sampled using a brood-pattern scheme.

308. All regular zygotes were inviable. Most of the viable progeny carried newly induced isochromosomes or were triploid. The new isochromosomes were either heterozygous or homozygous for the paternal markers. The former (heteroisochromosomes) were only induced in diploid cells and the latter (homoisochromosomes) were induced in all of the germ-cell stages which were tested. The rates of induction could not be measured directly but were estimated from the results of calibration tests which showed that spermatocytes and spermatogonia are about 30 times more sensitive than spermatozoa and mature spermatids.

309. The unexpected finding is that homoisochromosomes can be recovered from irradiated spermatozoa and late spermatids. Several models have been proposed to explain the induction of isochromosomes. Leigh and Sobels (243) favour the hypothesis that isochromosomes originate from a chromatid-type exchange when two breaks, one on each side of, and close to, the centromere, are induced. In post-meiotic germ cells, the isochromosomes can be formed only at the post-zygotic chromosome replication since in *Drosophila* male and female pronuclei undergo one mitotic division and at late anaphase of this division, there is a double fusion to produce two diploid daughter nuclei (529).

310. The two daughter nuclei will normally be identical, but in the situation where isochromosomes have been induced by the irradiation of a post-meiotic germ cell, they will each receive a different newly-formed isochromosome. One of these will contain a balanced chromosome complement and the other will be aneuploid (243). The isochromosome carried by the female pronucleus will determine which of the two fusion nuclei is balanced. The fact that individuals carrying isochromosomes have been recovered indicates that one of the first two cleavage nuclei is competent to produce an adult fly.

311. The two-break model described earlier predicts that chromatid-type exchange can result from chromosome breakage. When isochromosomes are not formed but another type of exchange occurs such that both fusion nuclei receive viable genetic complements, it should be possible to obtain mosaics for chromosome rearrangements after irradiation of haploid male germ cells. Such mosaics have indeed been observed by Leigh (239), Lee *et al.* (235), Abrahamson *et al.* (4) and Sobels and Leigh (527).

C. Differential sensitivity of germ-cell stages

312. The existence of radio-sensitivity differences among germ cells of the insect species investigated in this respect is well documented (383, 386, 522, 545, 547, 583, 591). The subject was exhaustively reviewed in the 1966 report of the Committee. Since then new data have become available. Most of the new information bearing on this problem will be discussed in the following paragraphs while some of it is considered in other sections of this review because, although the problems investigated directly or indirectly were based on differential radio-sensitivity of the germ-cell stages, the scope, emphasis and design of the experiments were such that it was considered appropriate to include the material in other sections.

1. *Male germ cells*

(a) *X- and neutron-irradiation*

(i) *Drosophila*

313. Shiomi (515) subjected *Drosophila* males to x-ray exposures of 1,000 to 4,000 roentgens in air or in a nitrogen atmosphere and compared the frequencies

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_{50} 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 radiosensitivities 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 radiosensitive 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.

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

[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.

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 *B*3 and also with those of Neary *et al.* (350) with the DNA of phage *T*7. 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 *T*7 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 semiconservative 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^{-5} 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 nondisjunction (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[e]	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 ...[a]	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]	$2.9\ 10^{-4}$[e, 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 × 10⁵*[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* $R \times 10^{7}$[c]	*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* $\times 10^{6}$	*95 per cent confidence limits of mutation rate* $\times 10^{6}$
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 $R \times 10^7$ [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	sc	Df(d se)	Spermatogonia d	Spermatogonia se	Spermatogonia Df(d se)	Post-spermatogonial stages d	Post-spermatogonial stages se	Post-spermatogonial stages Df(d se)	Oöcytes d	Oöcytes se	Oöcytes 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	Cross-over length >2 map units[b]: 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		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	— 29[b]	<0, 29 <0, 65	274 275
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	— 0.9[d]	<0, <0 0.4, 1.5	510 275
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 (i) birth (ii) 1 day (iii) 21 days (iv) 69 days	Recessive lethals	 0.0±0.6 1.0±0.8 1.2±1.1 1.6±1.3		541
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 (i) birth (ii) 1 day (iii) 21 days (iv) 69 days	Recessive lethals	 0.5 1.6 1.5 1.4	 <0, 2.7 <0, 4.1 <0, 5.0 <0, 5.7	173

[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 "high" population (see experiment 9)	Random non-sib	4,494 R (14 generations) 4,847 R (15 generations)	(i) Mean body weight of male offspring declined at a rate of 6.8 grammes and of the female offspring at about 2.0 grammes per	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 ($azgr^{-7.5}$)[c]	200–1,000	$4.1\ 10^{-4}$–$2.1\ 10^{-3}$	51
5 ..	Chinese hamster	Somatic cells in culture (from lung); aneuploid cell line	($azgr^{-30}$)[d]	450	$9.2\ 10^{-7}$	51
6 ..	Chinese hamster	Somatic cells in culture (from lung); aneuploid cell line	($azgr^{-30}$)[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			X rays		RBE	Reference
			Mean energy	Dose[a]	Dose rate[b]	Dose or exposure[c]	Dose or exposure rate[d]		
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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