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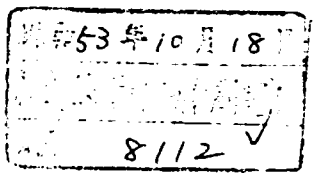
SOURCES AND EFFECTS OF IONIZING RADIATION

United Nations Scientific Committee
on the Effects of Atomic Radiation

1977 report to the General Assembly, with annexes



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ANNEX H

Genetic effects of radiation

CONTENTS

Introduction

1. The 1972 report of the Committee (589) presented a comprehensive review of the genetic effects of ionizing radiation. The present report will be devoted to (a) an updating of the 1972 report, especially those parts that require significant revision in the light of information that has accumulated during the last few years, with particular attention being paid to those results that bear on the problem of the evaluation of genetic radiation hazards in man, and (b) a comparison of the main conclusions reached by the Committee in 1972 with those of the Advisory Committee on the Biological Effects of Ionizing Radiation (BEIR) of the National Academy of Sciences of the United States of America (34).

I. RELEVANT HUMAN DATA

A. THE PREVALENCE OF NATURALLY OCCURRING HEREDITARY DEFECTS AND DISEASES IN HUMAN POPULATIONS

1. The results of the British Columbia Survey and comparison with other results

2. Trimble and Doughty (576) have published the results of a major study whose primary objective was to determine the frequency of live-born individuals affected by hereditary or partially hereditary defects and diseases in a large, geographically defined population, namely that in the Canadian province of British Columbia, with a current population in excess of two million people. Since these results have been obtained and interpreted using information on the inheritance of specific traits that has been gained during the last fifteen years or so, and since knowledge of the amount of hereditary diseases in human populations is crucial to an evaluation of genetic radiation hazards in man using the doubling-dose method, they will be discussed in some detail in the following paragraphs.

3. The data used for this study were derived from the records of the British Columbia Registry for Handicapped Children and Adults and pertain to a period of 21 years (1952-1972). These include cases actually registered with the Registry as well as those ascertained through the province-wide Surveillance System of Congenital Anomalies, both systems having multiple sources of ascertainment. Of the total case-load of 48 212 individuals recorded with the Registry by the end of 1972, only about 44 per cent (21 290) were born in the province during the period 1952-1972. In addition, 9313 children also born in the province during the period 1964-1972 were ascertained through the Congenital Anomalies Surveillance System. The data relate to this total of 30 603 affected children among 756 304 live births. Adequate precautions were taken to avoid duplicate entries from multiple sources of ascertainment. The details of the different kinds of diseases or disorders included in the analysis are given in tables 1 to 8. Diseases of unknown aetiology and environmentally caused disorders (representing a total of 4949 cases out of 30 603) are not listed in these tables.

4. An examination of the frequencies of children with one or another kind of genetic or partially genetic disease in the successive annual birth cohorts showed that, in general, these remained relatively constant up to the year 1963 (2.5-3.8 per cent), increased from 1964 to 1968 (5.1-6.0 per cent) but decreased in recent years (to 2.6 per cent in 1972 for example). The higher figures from 1964 onwards are due to individuals ascertained through the Surveillance System, which contains information only for children born since 1964. The decline in the more recent cohorts is only apparent, since there is a tendency for the affected children born in later years not to be registered until they reach school age. To arrive at the best estimates of the frequencies of single gene, chromosomal and multifactorial diseases, the authors used the data for the period 1964-1966; for congenital malformations, they used those from 1967-1969. The reason was that, for these birth years, there appeared to be nearly complete registration of conditions that manifest themselves during childhood or youth. The total thus estimated is about 6 per 100 live-born for all registered conditions, regardless of the particular aetiology of a condition (0.18 per cent, single gene diseases; 0.16 per cent, chromosomal anomalies; 3.58 per cent, congenital anomalies; 1.58 per cent, other multifactorial diseases; and 0.60 per cent, diseases of unknown aetiology).

5. To allow for biases due to delay in disease onset, migration and incompleteness of ascertainment, suitable correction factors were used to get "adjusted estimates" from "minimal estimates" given above and both these are presented in table 9 together with the earlier ones of Stevenson (566) for Northern Ireland, a division of the United Kingdom of Great Britain and Northern Ireland, which were revised subsequently by UNSCEAR (586, 587). Examination of table 9 will show that there are several differences between the estimated incidence rates in British Columbia and in Northern Ireland: (a) the total frequency of serious diseases or handicaps believed to be genetic is about 9 per cent in British Columbia as compared with about 6 per cent in Northern Ireland; (b) the incidence of diseases due to single dominant genes is lower by a factor of more than 10 in the present study; (c) the frequencies of single-gene autosomal recessives and of chromosomal anomalies are also lower (by a factor of 2) in British Columbia; and (d) the incidence of diseases that fall under the categories of congenital malformations and other multifactorial diseases is higher by a factor of more than 2 in the present work (9.0 per cent compared with 4.0 per cent).[1]

6. Nearly all (97 per cent) of the chromosomal anomalies recorded in the British Columbia Survey are due to Down's syndrome, whereas in the UNSCEAR estimates, other chromosomal anomalies were also included (see footnote c to table 9). The frequency of cases with Down's syndrome in British Columbia is roughly the same as that reported in the UNSCEAR reports. The low estimate of the total frequency of registered chromosomal anomalies (0.20 per cent versus 0.42 per cent) indicates that some of the individuals with other chromosomal disorders such as Klinefelter's

[1] The 9.0 per cent is from column 4 (4.28 + 4.73 = 9.01), and the 4.0 per cent is from column 2 (2.50 + 1.50 = 4.00)

or Turner's syndrome were not brought to the attention of the Registry, presumably because they were not regarded as handicapped during childhood or had the aetiology of their disorders incorrectly classified as not due to chromosomal aberrations. Many cases of sex-chromosomal abnormalities and structural anomalies of the autosomes would have been classified as congenital malformations or as diseases of unknown aetiology because the correct cause of the defect would not be readily recognized in the absence of routine cytogenetic analyses. While the precise extent of underestimation of these cases is difficult to determine, the authors surmise that accounting for these individuals could increase the estimate of the total frequency of chromosomal anomalies by as much as threefold (i.e., from 0.2 per cent to 0.6 per cent). The recent summary estimates on the incidence of chromosomal anomalies in new-born babies (table 11) in fact give a total frequency of 0.6 per cent.

7. The major discrepancies between the present data and the earlier ones based on the Northern Ireland survey relate to the very low incidence of diseases presumed to be caused by regular dominants (0.08 per cent compared with 0.95 per cent) and in the rather high incidence of conditions that fall under the categories of congenital anomalies and multifactorial diseases (9.0 per cent compared with 4.0 per cent). The discrepancy for regular dominants is in part due to a considerable underestimate of the incidence of dominant disorders with onset in adult life in the British Columbia survey, and in part, to the inclusion in the Northern Ireland survey of conditions which would not now be regarded as having a simple dominant mode of inheritance. This discrepancy is of major concern since regularly inherited dominant (and X-linked) conditions are expressed in the immediate descendants of exposed individuals and are expected to increase in direct proportion to an increase in mutation rate. They are therefore an important component of the genetic hazard from radiation exposure.

8. The 12 most frequent disorders in Stevenson's list of dominant diseases account for about 75 per cent of all his cases of dominants. A review of the inheritance of these 12 conditions[2] has shown that at least 7 of them would not now be regarded as being inherited as simple dominant disorders (360). This has been illustrated by Newcombe (360) for hydrocephaly, which accounts for one fifth of all the cases in Stevenson's dominant category. This condition is now known to be heterogeneous and the family patterns observed exclude simple dominant (or even simple recessive) inheritance in any substantial proportion of cases.

9. The British Columbia survey relates largely to diseases with onset in childhood and is based on a follow-up of individuals from birth to ages 1-21 years, and Sutton (555) has expressed the view that the study of Trimble and Doughty "clearly underestimates diseases of late onset such as Huntington's chorea". In the British Columbia survey, only 2 cases of Huntington's chorea were found, giving an incidence of 2.6 per million. However, *ad hoc* surveys such as that of Shokeir (534) indicate a prevalence in Saskatchevan and Manitoba of 8.7 per 100 000 which (since patients have the overt disease for only about 15 years) corresponds to a birth frequency of heterozygotes of about 1 in 2000.

10. Similar underestimates are likely to exist in the British Columbia data for conditions such as neurofibromatosis, the adult type of polycystic disease of the kidney, multiple polyposis of the colon and monogenic hypercholesterolaemia. The same limitations apply to Edwards' (145) low estimates of the incidence of dominant disease. Sutton (555) is of the opinion that "it would seem wise to use the figure of 1 per cent dominant disorders until additional reliable data indicate this not to be correct".

11. *Ad hoc* surveys of the prevalence of individual dominant conditions by observers skilled in the diagnosis of particular disorders and using all available means of ascertainment of cases, such as those quoted by Vogel and Rathenberg (599) in estimating human mutation rates and listed in table 10, with the addition of more recently recognized dominant disorders such as monogenic hypercholesterolaemia (82a) suggest that the total birth frequency of such disorders may well be 1 per cent. The total figure of 1 per cent dominants used in the 1972 report may therefore be retained, though the individual conditions contributing to it have been reappraised.

12. X-linked and autosomal recessive conditions are usually more severe and already expressed in childhood and therefore, the estimates of their incidence are more straightforward. Edwards' (145) estimates of 0.05 per cent for X-linked and 0.25 per cent for autosomal recessive conditions would appear to agree well with estimates from *ad hoc* prevalence studies (82a). However, some of the more common autosomal recessive disorders are probably maintained by heterozygous advantage and a figure of 0.1 per cent is a more realistic estimate for the incidence that is maintained by mutation.

13. Turning now to the incidence of diseases that falls under the category of congenital anomalies, multifactorial and irregularly inherited diseases,[3] as was

[2] Cataracts (senile and pre-senile), hydrocephaly (internal obstructive), alopecia areata, nystagmus (familial idiopathic), cystic disease of lungs, choroidal sclerosis, multiple exostoses, neurofibromatosis, colobomata, ataxia (dominant hereditary including Friedrich's), porphyria (dominant detectable) and cataracts (congenital).

[3] In contrast to simple dominant diseases where affected carriers transmit the disorder to half of their offspring on the average, the dominants of incomplete penetrance constitute mutationally-maintained conditions whose inheritance is not yet fully understood and which at present, may be incorrectly classified as disorders with a multifactorial aetiology. Incomplete penetrance may be due to environmental factors or due to genetic ones and in the latter case, the category of dominants of incomplete penetrance merges with the multifactorial category. It is difficult at present to estimate the birth frequency of these diseases due to dominant genes of incomplete penetrance or to give unequivocal examples. They may include some cases of common congenital malformations and other common disorders of late onset. However, the great majority of cases of common congenital malformations and constitutional and degenerative diseases are thought to be truly multifactorial in origin and are characterized by high frequency, a non-dominant pattern of inheritance but a familial pattern of incidence in twins, sibs, cousins and so forth that is indicative of multilocal transmission.

mentioned earlier, it is higher by a factor of 2 in British Colombia relative to Northern Ireland (9.0 per cent compared with 4.0 per cent). These diseases constitute a very substantial proportion of human disorders, but there are great uncertainties concerning the exact mechanisms involved in their aetiology. It is not really possible to make any realistic quantitative apportionment of their causation between genetic and environmental factors, both of which play varying roles. The 9 per cent of Trimble and Doughty may include an unknown proportion of incorrectly classified chromosomal anomalies, monogenic disorders and diseases of largely environmental aetiology. It is therefore likely to be somewhat of an overestimate. On the other hand, underestimations are likely to exist for constitutional disorders of adult onset, for example, schizophrenia or death in the fifth decade from coronary heart disease.

14. Insofar as this major group of disorders is genetically determined, the family pattern they show suggests that the genetic component is usually multilocal, i.e., depending on genetic variation in at least several gene loci (82b, 82c). Their high frequency, e.g., 1 in 250 to 1 in 2000 in different human populations for spina bifida cystica, 1 in 1000 for the commonest congenital heart defect (ventricular septal defect), and 1 in 100 for schizophrenia, indicates that selective mechanisms are responsible for their maintenance and that they are not mutation-dependent. Newcombe (361) has recently stressed this point.

15. On the other hand, it is known that there are relatively rare monogenic disorders already recognizable among this group of disorders, for example the dominant conditions cleft lip and mucus pits of the lower lip (among the large group of multifactorially determined facial cleft malformations). These are readily assigned to the dominant category. It is likely, however, that there are further unrecognized rare regular dominant and irregularly dominant conditions in this group which would be mutationally maintained. The size of this mutationally-maintained fraction is unknown. BEIR (34) conservatively assumed that this component[4] is not likely to be more than 50 per cent or less than 5 per cent. The upper limit of 50 per cent is perhaps implausibly high and a more realistic figure would be around 10 per cent. In this report, for purposes of hazard evaluation, we shall use the figure of 9 per cent for the incidence of the class of congenital, multifactorial and irregularly inherited diseases (based on the British Columbia data) and assume that the mutational component[4] is 5 per cent (best average estimate) (82).

2. Summary and conclusions

16. The rates of spontaneous incidence of different kinds of genetic disease in man available from the survey carried out in British Columbia represent a significant addition to our knowledge on the load of genetic ailments carried by the human population.

17. In this survey, the total frequency of these diseases has been estimated to be 9.44 per cent and can be broken down into 0.12 per cent autosomal dominant and sex-linked diseases, 0.11 per cent recessive and 0.20 per cent chromosomal ones, 4.28 per cent congenital malformations and 4.73 per cent other multifactorial diseases.

18. Discrepancies exist between these rates and those obtained earlier by Stevenson (which, with some revisions, are the ones that have thus far been used in hazard evaluations), particularly with respect to the incidence of dominant diseases and those included under the category of congenital malformations, multifactorial and irregularly inherited diseases. For the former, the present estimate is lower by about an order of magnitude (0.12 per cent compared with 1.0 per cent) and for the latter, higher by a factor of 2 (4.0 per cent compared with 9.0 per cent). In addition, the rate of incidence of chromosomal abnormalities in British Columbia is also lower by a factor of 3 relative to recent results of new-born surveys (0.20 per cent compared with 0.60 per cent).

19. The Committee reappraised the above figures, taking into account, among other things, the results from different *ad hoc* surveys for specific dominant conditions, those from new-born surveys for chromosomal anomalies and the uncertainties involved in the aetiology of diseases that fall under the category of congenital malformations, multifactorial diseases and irregularly inherited conditions. The following figures that were arrived at will be used in the context of hazard evaluations: 1.0 per cent dominant and X-linked diseases, 0.1 per cent recessive diseases, 0.4 per cent chromosomal diseases and 9.0 per cent congenital malformations, multifactorial and irregularly inherited conditions.

20. The magnitude of the mutational component for the last mentioned category of diseases is thought to be not more than 10 per cent (upper limit) in contrast to the upper limit figure of 50 per cent used by BEIR; for the purpose of calculations, the Committee will use a figure of 5 per cent as the best average estimate.

B. NUMERICAL AND STRUCTURAL CHROMOSOME ABNORMALITIES IN NEW-BORN INFANTS

1. Spontaneous rates of incidence

21. In its 1972 report, the Committee presented the results of surveys carried out in different parts of the world on the chromosomal constitution of live-born infants; the data available at that time showed that out of 21 996 babies, 114 (0.52 per cent) had an abnormal chromosome constitution. Additional data obtained since then in different laboratories (summarized by Jacobs *et al.* (250), Hamerton *et al.* (212), and Nielsen and Sillesen (363) and including the more recent work of Lin *et al.* (289) bring the total number of babies examined to 55 679, with 336 of them (0.60 per cent) having abnormal karyotypes. All these karyotypes (except those in the work of Lin *et al.*) were examined

[4] The mutational component of a disease is the proportion of its incidence that is directly proportional to the mutation rate.

with conventional techniques. The kinds and frequencies of the different abnormalities recorded in these surveys are given in table 11.

22. The data summarized in table 11 permit the following conclusions: (a) the total frequency varies from 0.47 per cent to 0.83 per cent in the different surveys; the overall rates of incidence (based on pooled results) are 0.22 per cent sex-chromosome anomalies, 0.14 per cent autosomal trisomies, 0.19 per cent euploid structural rearrangements, and 0.05 per cent aneuploid structural rearrangements (including supernumerary chromosomes);[5] (b) the frequencies of 47,XYY males range from 0 in Moscow to 0.03-0.05 per cent in Boston, Winnipeg and Århus, 0.13-0.14 per cent in Edinburgh and New Haven, to a high of 0.37 per cent in Ontario; (c) variations in the incidence rates of 47,XXY males is less (ranging from 0.07 per cent in Boston to 0.18 per cent in New Haven), and in general the rates are comparable; (d) the overall incidence of sex-chromosome anomalies in males is 0.26 per cent (93/34 872) and in females, 0.14 per cent (29/20 807); (e) the frequency of 45,X females is very low, being only 0.01 per cent (2/20 807); nearly all the other babies listed under "Other" (column 10) were mosaics, suggesting that the vast majority of XO conceptions do not survive to term and those which do are indeed mosaics; (f) the frequency of autosomal trisomies (trisomy-21; listed under +G) is 0.12 per cent (compare this frequency with that recorded in the British Columbia survey, 0.19 per cent, table 9); (g) the incidence rates of euploid structural rearrangements are reasonably consistent for six of the surveys (Edinburgh, Winnipeg, Hamilton, Boston, New Haven and Moscow: 0.14-0.21 per cent), while in Århus, the frequency is high (0.30 per cent) and in Ontario, low (0.05 per cent); the results also demonstrate that the frequency of Robertsonian translocations (D/D and D/G types) and that of detected balanced reciprocal translocations are roughly the same; and (h) aneuploid structural rearrangements are relatively uncommon, only 30 being observed in the whole sample.

2. Mutation rates

23. Jacobs *et al.* (250) used the data pertaining to 43 558 babies to estimate the "rates of mutation" for the different kinds of chromosomal abnormalities. For numerical errors of chromosomes which result in live-born children, this was $14.0\ 10^{-4}$ per gamete per generation (mosaic individuals and those with 46,X inv (Y) were excluded). The estimate based on 55 679 babies (table 11) is nearly the same, being $14.2\ 10^{-4}$ per gamete per generation (sex-chromosome anomalies: $7.5\ 10^{-4}$ per gamete per generation; autosomal anomalies: $6.7\ 10^{-4}$ per gamete per generation; mosaics and Y chromosome inversions likewise excluded).

24. For structural abnormalities of the autosomes, the estimates of Jacobs *et al.* were based on the incidence in the total sample of 43 558 and an analysis of the proportion which was non-familial (and thus new mutants) in a sample of 56 babies found to carry non-mosaic structural rearrangements; they found that 10 out of 50 euploid babies (20 per cent) and 2 out of 6 aneuploid ones (33 per cent) were new mutants. Multiplying the incidence rate of 0.19 per cent (euploid) by 0.2 and dividing by 2 (to correct for the possibility that the mutation could have arisen in either sex), the authors estimated that the rate for all euploid structural rearrangements (Robertsonian translocations, reciprocal translocations and inversions) and $1.9\ 10^{-4}$ per gamete per generation; for reciprocal translocations alone, the figure was $1.33\ 10^{-4}$ per gamete per generation (36/43 558 multiplied by 0.33 (proportion of new mutants: 8/24) and divided by 2). For aneuploid structural abnormalities (translocations, inversions and deletions), the rate was $0.30\ 10^{-4}$ per gamete per generation (8/43 558 multiplied by 0.33 and divided by 2).

25. Under the assumption that the proportions of new mutants found by Jacobs *et al.* are applicable to the present total sample of 55 679 babies, the following rates can be estimated using calculation procedures as above (para. 24): all euploid structural rearrangements, $0.0019 \times 0.2 \times 0.5 = 1.9\ 10^{-4}$ per gamete per generation; reciprocal translocations alone, $0.00086 \times 0.33 \times 0.5 = 1.4\ 10^{-4}$ per gamete per generation; aneuploid structural abnormalities, $0.00027 \times 0.33 \times 0.5 = 0.45\ 10^{-4}$ per gamete per generation. The above estimates are roughly similar to those made by Jacobs (249) from less extensive neo-natal data and to those of Jacobs, Frackiewicz and Law (249) who combined both neo-natal and post-natal data.

26. Jacobs *et al.* (247) have pointed out that the mutation rates for autosomal rearrangements must be underestimates of the true rates for at least two reasons: (a) only a fraction of all chromosome rearrangements in man is detectable in somatic cells by conventional staining techniques. Of the structural aberrations considered here, only Robertsonian translocations allow a detection rate approaching 100 per cent; and (b) many aberrations may be selected against before they give rise to live births; such selection is presumably almost completely restricted to unbalanced rearrangements. On the basis of unpublished results of Buckton[6] with irradiated human lymphocytes, Jacobs *et al.* (247) concluded that approximately 75 per cent of symmetrical exchanges (reciprocal translocations) may escape detection. However, human cytogeneticists who have used the new banding techniques (the latter, among other things, permit a precise identification of chromosomes or chromosome parts involved in translocations) are of the opinion that the efficiency of the new banding techniques with respect to the detection of reciprocal translocations is only slightly higher (by 10-20 per cent) than the conventional staining techniques; this means that the rate of $1.4\ 10^{-4}$ per gamete per generation estimated for reciprocal translocations is not likely to be strikingly lower than the true rate. Since there remains the possibility that, even with the banding techniques, small equal symmetrical exchanges may

[5] A person was considered to have a supernumerary chromosome if he had 47 chromosomes, 46 of which were apparently normal, the additional chromosome being equal to or smaller than a member of Group G and clearly different in morphology from a G-group chromosome (250).

[6] The paper has recently been published (66).

escape detection, it is perhaps wise at present to multiply the rate mentioned above by 2 to get an estimate of the probable true rate, i.e., we shall assume that the spontaneous rate of origin of reciprocal translocations in man is $2.8\,10^{-4}$ per gamete per generation.

C. CHROMOSOMAL ANOMALIES IN SPONTANEOUS ABORTIONS

27. The incidence of chromosomal anomalies in spontaneous abortions in man has been extensively reviewed by Carr (78, 79) and Boué and Boué (46) and has been considered to some extent in the Committee's earlier reports (587, 589). It is well established that chromosomal anomalies are frequent among spontaneous abortions with an incidence rate that is much higher than in live-born infants. The total frequency of these anomalies in the major abortion studies varies from 8 per cent in that of Stenchever *et al.* (565) to 64 per cent in that of Szulman (559); the wide discrepancies are most probably due to the operation of a variety of factors, such as geographical variation, maternal age, prevalence of unsuspected induced abortions in the population, variations in hospital admission practices and especially the procedure and scope (selected *versus* unselected) of the collection of abortuses. Boué and Boué (46) (and several others) have found that the phenotype and gestational age of the conceptus are both related to the incidence of chromosomal anomalies: in their large-scale study, they recorded one or another kind of chromosome abnormality in 66 per cent of the abortuses arrested in their development before 8 weeks of gestation, this falling to 23 per cent of zygotes with 8-12 weeks of development. (In 1097 out of 1205 observations, the developmental arrest occurred before the eighth week and these represent about 90 per cent of all abortions; developmental arrests before the third week are rare, since in these cases the pregnancy might be unrecognized and such specimens are seldom received for examination.)

28. Most of the chromosomal anomalies which have been described among abortuses are numerical such as monosomy-X, autosomal trisomy, triploidy and tetraploidy. Boué and Boué (45, 46) recently summarized the results of their continuing investigations and compared them with those obtained by some other workers whose sample sizes were adequate enough to permit some general inferences. These are given in table 12 in addition to the data of Kajii *et al.* (253) and of Creasy *et al.* (127) not covered in the above paper. Since the data of Boué and Boué are the most extensive, they can be used to establish a rank order of incidence of the different abnormalities to inquire the extent to which these results agree with those of others and to gain an insight into the kinds of aberrations that are more or less frequent in abortuses in contrast to live-borns. As will be evident from an inspection of table 13, the agreement between these results is good, with trisomy constituting the most predominant class of abnormalities (~50 per cent), followed by monosomy-X, triploidy, tetraploidy and others.

29. In trisomic abortuses, the extra chromosome is almost always an autosome (in contrast to monosomy in which the missing chromosome is a sex chromosome). Trisomies of all autosomal chromosome pairs are found, but their relative incidences are different, trisomy for C, D, E, and G being more, and those for A, B and F less, frequent (table 14). All the three possible kinds of triploidy (69,XXX, 69,XXY and 69,XYY) are seen although again their relative frequencies appear different (XXY > XXX > XYY) (46). The small number of triploid abortuses with the 69,XYY constitution does not necessarily demonstrate the rarity of occurrence of this class, since a similar result can be obtained if these karyotypes lead to very early developmental arrest. The virtual absence of autosomal monosomy and the rather low frequency of some kinds of trisomy (A, B and F) might likewise be due to their very early developmental arrest. This view is supported by the experimental work of Gropp (203) and of Ford and Evans (169), who studied the time of action of the lethal effects of zygotic aneuploidy in F_1 hybrids between the tobacco mouse and the laboratory mouse. It was found that autosomal zygotes could be observed only before implantation; the trisomic embryos however, died during the post-implantation period with a considerable reduction in numbers between the earlier gestation period (8-11 days) and the later period (12-15 days) and none was observed at birth.

30. An analysis of the relationship of maternal age and the different types of chromosomal anomalies (table 15) shows that the mean maternal age is higher for lethal trisomies relative to other abnormalities as well as to abortuses with normal karyotype. Close inspection of table 15 will reveal that it is primarily for the trisomies involving acrocentric chromosomes (D and G groups) that the influence of maternal age is marked; in lethal G-trisomies, the curve of maternal age distribution (45) shows bimodality, with 60 per cent of the observations in the age-dependent group, a pattern very similar to that noted by Penrose and Smith (406) for children with Down's syndrome. This suggests that whether a zygote with a trisomy G will lead to a spontaneous abortion or a delivery at term may not depend on the age of the mother.

31. It is instructive to compare the results from newborn surveys with those from the abortion studies (tables 11, 13 and 14). It will be evident that monosomy-X is one of the most frequently encountered class of abnormalities in spontaneous abortions whereas it is very rare in new-borns. About 40 per cent of all spontaneous abortions with chromosome anomalies (and about 80 per cent of all trisomy) are due to trisomy for D, E and G group chromosomes; in the new-born population, trisomy for these chromosomes accounts for 0.14 per cent of all new-born children (or about 23 per cent of the abnormalities recorded in these). Structural anomalies of chromosomes and mosaics constitute about 5 per cent of all abnormalities scored in the abortus material, whereas in new-borns, about 40 per cent of all abnormalities scored are structural and involve the autosomes (both euploid and aneuploid structural aberrations), 0.24 per cent of the children having structural abnormalities.

D. PARENTAL X IRRADIATION AND SPONTANEOUS ABORTIONS

32. Alberman *et al.* (5) made a comparison of the histories of medical irradiation received by parents of spontaneous abortuses of abnormal karyotypes with corresponding histories in cases of abortions of normal karyotype and with those in parents of live births. The overall finding was that the mothers of chromosomally abnormal foetuses had received the largest mean gonadal dose of irradiation for medical reasons compared with the other spontaneous abortions and the live-birth controls. As in their Down's syndrome study (6) the radiation doses tended to be rather small and the effects of radiation appearing to be cumulative and, seemingly, often maternal age-dependent. Irradiation appeared to be an especially important factor in the case of triploid abortuses: the mean gonadal dose of mothers of triploids (about 0.74 rad) exceeded that received by mothers of those with autosomal trisomy of one chromosome including trisomy of the G group (0.30 rad) or of the 45,X abortuses (0.18 rad). The authors stress the point that most of the chromosomally abnormal foetuses are not viable and are lost early in pregnancy.

E. PARENTAL X IRRADIATION AND DOWN'S SYNDROME

33. Even though several retrospective and prospective studies have been conducted to find out whether parental irradiation may increase the risk of producing Down's syndrome (trisomy for chromosome 21) in the progeny, the answer to the question remains equivocal. For instance, the retrospective studies of Uchida and Curtis (581), Sigler *et al.* (537) and Cohen and Lilienfield (115) have been interpreted as showing an association between maternal irradiation and Down's syndrome. The investigation of Alberman *et al.* (6) showed that the mothers of those with Down's syndrome had had more total x-ray examinations, both in number and in dose, before the conceptions (relative to those "control mothers") of children with a variety of other serious congenital handicaps which are not thought to be in any way associated with parental irradiation; the overall difference did not reach a significant level. However, when the mean doses for the different maternal age groups and conception histories were compared (i.e., radiation doses received less than 6 years before, 6-10 years before and more than 10 years before the conceptions), (a) the variation with age was more marked in the mothers of the Down's syndrome group (than in the control) and (b) the effect was greatest in the subgroup where x-ray exposures were administered more than 10 years before the conceptions.

34. In contrast, the retrospective studies of other investigators (83, 305, 324) failed to provide any evidence for an association between maternal irradiation and Down's syndrome in the progeny, but these reports were based on small numbers of cases.

35. The prospective studies of Schull and Neel (563) of children born to mothers exposed to the A-bomb radiation at Hiroshima likewise showed no association, but the doses involved were of a different order of magnitude from those used for medical purposes. The study of Stevenson *et al.* (567) also failed to support the hypothesis that x-ray diagnostic procedures before conception increase the frequency of Down's syndrome in children subsequently born. However, the prospective study of Uchida *et al.* (583) indicated that a significantly greater number of trisomic children were born after maternal radiation exposure.

36. In a recent paper, Kochupillai *et al.* (260), claimed that the incidence of Down's syndrome in the population living in an area of high background radioactivity in coastal Kerala in southern India was significantly higher relative to a control population and to the rates recorded in similar surveys by other investigators elsewhere in the world. The exposure risk in the study population was estimated as 1.5-3 rad per year (control, 0.1 rad per year) and the frequency of Down's syndrome observed was 12 in 12 918, giving a rate of 1 in 1076 (or 0.93 per 1000). No Down's syndrome cases were detected in the control (5938 individuals screened). In terms of maternal age, the distribution of Down's syndrome cases in the study population was as follows: 1 in 862 (age range 20-29), 1 in 81 (age range 30-39) and 1 in 266 (age range 40-49).

37. Sundaram (548) re-examined the data presented by Kochupillai *et al.*, and concluded that the author's claim could not be substantiated for the following main reasons: (a) only about 11 per cent of the females included in their study receive radiation doses in the range 1.1-2 rad per year, and 2.8 per cent receive exposures higher than 2 rad per year. This type of dosimetric profile is due to the non-homogeneous distribution of monazite-containing beach sands; (b) the age structure of the Kerala study population is quite different from those of others with which the data are compared, i.e., while in most surveys, only about 4 per cent of all births is contributed by females in the age group 40 years and older, in the Kerala survey, this figure is around 20 per cent and this age group is known to have the highest risk for children born with Down's syndrome; (c) when the data are re-calculated to estimate the incidence at birth and at the time of study (taking into account the family size, the number of females in each age group and the infant mortality rate), one arrives at 9-10 cases for the population sample studied, and the observed 12 cases therefore do not show any significant difference; (d) the observation of Kochupillai *et al.* that the risk in the maternal age group 40-49 is only one third of that in the age group 30-39 is puzzling and at variance with what is so far known about the relationship between maternal age and the incidence of Down's syndrome.

F. MORTALITY IN THE CHILDREN OF THE A-BOMB SURVIVORS IN HIROSHIMA AND NAGASAKI

38. Neel *et al.* (359) and Kato (254) have published the results of a continuing study of mortality rates among children born to survivors of the atomic bombings of Hiroshima and Nagasaki which updated those presented in an earlier report (255). Although the actual numbers of deaths since the previous review was

small, the present report was prompted more by the fact that significant revision of the dose estimates had become available.

39. The mortality experience pertains to (a) 18 946 children born live to parents both of whom were proximally exposed (i.e., either one or both parents less than 2000 m from the hypocentre and receiving jointly an estimated dose equivalent of 117 rem);[7] (b) 16 516 children born to distally exposed parents (i.e., neither parent less than 2000 m, but either or both less than 2500 m, from the hypocentre and receiving essentially no radiation) and (c) 17 263 children born to parents not in Hiroshima or Nagasaki at the time of the bombing. The average interval between birth and verification of death or survival was 17 years. Analysis of the data has shown that no significant effects of parental exposure on childhood mortality can be demonstrated.[8] However, the data permit an estimate of the lower limit of doubling dose[9] (for the type of damage[8] resulting in death during the first 17 years of life among live-born children conceived 0-13 years after parental exposure), and this is equal to a gamma-ray dose of 46 rad for fathers and 125 rad for mothers.[10] Neel *et al.* suggest that, on the basis of mouse data, the gametic doubling dose for chronic low-level radiation would be expected to be 3-4 times the value of 46 rad for males (i.e., at least 138 rad) and over 1000 rad for females.

G. CYTOGENETIC SURVEY OF THE OFFSPRING OF THE A-BOMB SURVIVORS IN HIROSHIMA AND NAGASAKI

40. A continuing survey of the offspring of the survivors of Hiroshima and Nagasaki bombings for structural and numerical anomalies of the chromosomes is in progress. The latest report published by Awa (15) shows that among 2885 children of A-bomb survivors[11] (1386 males and 1499 females), a total of 18 individuals (0.62 per cent) with chromosome abnormalities have been found (3 XXY, 3 XYY, 2 XXX and one X/XXX mosaic, together 0.31 per cent; 9 structural abnormalities, including 3 t(Dq Dq), 1 t(Dq Gq) and 5 balanced autosomal translocations, together 0.31 per cent). In the matched control[11] of 1090 subjects (510 males and 580 females), 3 individuals with chromosomal anomalies have been found (1 XXY and 2 balanced reciprocal translocations). Awa has pointed out that although the prevalence of sex-chromosome aneuploidy in the children of exposed parents is higher than in the controls (0.31 per cent compared with 0.09 per cent), the difference is not significant.

41. The above incidence rates can be compared with those obtained in surveys of new-born infants (paras. 21-22 and table 11):

	Hiroshima and Nagasaki data controls 1 090 subjects		*Children of exposed parents 2 885 subjects*		*Surveys of new-born infants 55 679 subjects*	
Anomaly	*Number*	*Percentage*	*Number*	*Percentage*	*Number*	*Percentage*
Sex-chromosome	1	0.09	9	0.31	122	0.22
Numerical autosome	–	–	–	–	76	0.14
Structural autosome	2	0.18	9	0.31	137	0.24
All	3	0.28	18	0.62	336	0.60

It can be seen that the frequencies in new-born surveys are similar to those in the children of exposed parents from Hiroshima and Nagasaki and that there are no significant differences. The control frequencies in the Hiroshima and Nagasaki data however are lower than in the other groups, but the differences are not significant.

H. SPONTANEOUS NON-DISJUNCTION IN THE HUMAN MALE

42. One of the important recent developments in mammalian cytogenetics has been the introduction of differential staining techniques that have permitted the identification of specific chromosomes in interphase nuclei. Thus, quinacrine staining of interphase cells has shown that the human Y chromosome can be visualized as an intensely fluorescent dot (404) and that it can be seen throughout the male germ-cell series (403) including the spermatozoa (26). New techniques have now been developed for differential staining of chromosomes 1 and 9 (37, 189, 401, 402). It has become clear that the specific staining reactions are confined to regions of constitutive heterochromatin composed of highly repetitive nucleotide sequences of DNA with varying base ratios dependent upon the chromosome concerned (251). The general category of differential staining involved here has been designated as C-banding.

43. These technical developments have been found useful to identify and measure the frequency of aneuploid spermatozoa (aneuploid with respect to chromosomes Y, 1 and 9) in ejaculates. Initial estimates for Y chromosome, i.e., for those spermatozoa containing two Y bodies (and not one) were 1.3 per cent

[7] The unit used in the paper of Neel *et al.* is the rem and was obtained by summing the dose equivalents of the gamma and neutron components of the irradiation and assuming that the quality factor for neutrons is 5.

[8] It is assumed that the childhood mortality as measured here is due to different kinds of genetic damage (point mutations, small deletions, unbalanced translocation and non-disjunction).

[9] The 97.5% lower confidence limit. The data exclude a doubling dose equivalent lower than 46 rem for males and 125 rem for females.

[10] The doubling dose is calculated from the relationship $d = xy/z$, where d is the doubling dose, x is the mortality due to spontaneous mutation in the preceding generation (included both gene and chromosome mutations), y is the contribution of a given sex to the mortality, assumed to be 0.5 for each sex, and z = the maximum slope of the dose-effect curve (calculated in a stepwise regression analysis) which can not be excluded by present data at the 5% confidence level. The numerical values used in the above equation are, for males, $x = 0.005$, $y = 0.5$ and $z = 0.000054$, and for females, $x = 0.005$, $y = 0.5$ and $z = 0.00002$.

[11] Subjects in the exposed group were those whose parents (either one or both) were exposed to a dose of more than 1 rad; the parents of the controls were either not exposed or received less than 1 rad.

(37) and 2 per cent (403). A later study (401) confirmed these frequencies for the Y chromosome and also suggested a similar frequency for chromosome 9. It should be pointed out here that the above frequencies refer only to those of hyperhaploid spermatozoa; if the reciprocal class of hypohaploids occurs at the same rates, the calculated figures have to be doubled. The implication of these findings is that, if non-disjunction of other chromosomes occurs at about the same rates, the proportion of aneuploid spermatozoa will be considerable and will lead to the production of many trisomic embryos, most of which will be spontaneously aborted.

44. The above data of Pearson and Bobrow (403), Bobrow *et al.* (37) and Pawlowitzki and Pearson (401) have been criticized on a number of points (141) as follows: (a) single Y chromosomes are known to have a bifid structure in a proportion of interphase cells and that extra dots in spermatozoa could be explained by single Y chromosomes being bifid; (b) the extra dots might represent adventitious spots either through staining artefacts or by areas on other chromosomes staining up with the same reaction; (c) such a high frequency of YY spermatozoa is not reflected in the number of XYY individuals found either in live-born or abortion series, and, since there is no evidence of aneuploid gametic selection in mice, there is likely to be little or no selection in man which could account for the differences found in the frequency of YY spermatozoa and XYY individuals.

45. In a subsequent paper, Pearson *et al.* (405) obtained results that confirmed their earlier ones and were compatible with their expectations (rates of "non-disjunction" for chromosomes 1, 9 and Y being respectively 3.5, 5 and 2 per cent), but have not been able to rule out the criticisms mentioned above. It is perhaps worth remembering that the specific staining reactions used in these studies are confined to regions of constitutive heterochromatin and that chromosomes 1, 9, Y (and 16) have well defined and prominent blocks of these at the centromeric region (except in the Y where it is localized in the distal portion of the long arm) (14). To what extent heterochromatin *per se* may play a role in non-disjunction and whether one can generalize from the behaviour of those chromosomes with large blocks of heterochromatin to others which do not have these, are questions that have to be satisfactorily answered before the results discussed above can be used in the context of estimating total non-disjunction frequencies (for all chromosomes).

I. MITOTIC NON-DISJUNCTION IN LYMPHOCYTES OF YOUNG ADULTS

46. Uchida *et al.* (584) conducted experiments to see whether low-dose irradiation of human lymphocytes would lead to non-disjunction of chromosomes. It was realized that a positive finding in a study of this type does not necessarily imply that these results can be extrapolated to meiotic segregational errors; rather, it was thought that such a finding may indicate the usefulness of somatic cell systems as models for the study of meiotic non-disjunction.

47. Peripheral blood from 28 subjects aged 19-29 years was used for lymphocyte cultures; 7 of these were fathers and 7 were mothers of children with trisomy-21; the remaining 14 were age- and sex-matched controls with no known history of Down's syndrome among close relatives. After irradiation (50 R of ^{137}Cs gamma rays at 29 R/min), the irradiation and control samples were appropriately processed for chromosome studies. (After 69 h in culture, colcemid was added; harvesting was at 72 h. The latter time period was used to recover cells containing non-disjunctional products in preference to chromosome breakage products.)

48. The results were: (a) the total frequency of hypermodal cells in the irradiated sample was 0.1 per cent (29/28 000), which was higher than that in the controls (0.025 per cent); (b) in the unirradiated lymphocytes exposed to irradiated serum, there was also an increase in the frequency of hypermodal cells (28/28 000); (c) more hyperdiploid cells were found in the irradiated samples from control males than from fathers of progeny with trisomy-21; 2 of the control males had a total of 12 hyperdiploid cells, suggesting an increased susceptibility to non-disjunction; (d) of the total of 57 hyperdiploid cells, 1 was tetrasomic for chromosome 21, 1 had double trisomy and 1, triple trisomy. The extra chromosome could be accurately identified in 38 cases; there appeared to be a non-random non-disjunction of chromosomes, this being higher for the G-group chromosomes (10 cases) and for the X (C-group, 11 cases). All identifiable G-group chromosomes were No. 21. These results therefore suggest that the X and chromosome 21 may be particularly susceptible to somatic non-disjunction.

J. SUMMARY AND CONCLUSIONS

49. Since the publication of the 1972 report, additional data have become available on the incidence of chromosomal anomalies in new-born babies. All these results considered together show that the total incidence is 0.60 per cent of which roughly one third (0.22 per cent) attributed to sex-chromosome anomalies, one quarter (0.14 per cent) to autosomal numerical anomalies, one third (0.19 per cent) to autosomal euploid structural anomalies and one twelfth (0.05 per cent) to autosomal aneuploid structural anomalies.

50. The total frequency of chromosome anomalies recorded in the children of the A-bomb survivors of Hiroshima and Nagasaki (0.62 per cent) is very similar to that found in new-born surveys (0.60 per cent) although, in the control group for the former, this figure is only 0.28 per cent. The differences however, are not significant.

51. On the basis of the total data from new-born surveys and the family history of some of the abnormalities studied, spontaneous mutation rates can be estimated. These are $14.2\ 10^{-4}$ per gamete per generation for all numerical errors of chromosomes which result in live-born children, $7.5\ 10^{-4}$ for sex-chromosome anomalies alone and $6.7\ 10^{-4}$ for autosomal numerical anomalies. The corresponding figures for all euploid structural rearrangements of the

autosomes is $1.9\,10^{-4}$ and for balanced autosomal reciprocal translocations alone, $1.40\,10^{-4}$. Considering the fact that the efficiency with which the latter type of aberration can be scored in somatic cells may be lower than 100 per cent, it is thought that the true rate may be $2.80\,10^{-4}$ per gamete per generation, for those gametes which give rise to live-born children.

52. Chromosome anomalies are frequent among spontaneous abortions, with an incidence rate that is much higher than in live-born infants and most of the former are numerical anomalies. It has been found that both the phenotype and gestational age of the conceptus are related to the incidence of chromosome anomalies in spontaneous abortions. In over 90 per cent of observations, the developmental arrest occurred before the eighth week of gestation.

53. In spontaneous abortions, trisomy for one or another autosome is the predominant type accounting for about 50 per cent of all the abnormalities, followed by monosomy-X, triploidy, tetraplody and others. In contrast, in new-borns, autosomal trisomies (especially of D, E and G) accounts for only one quarter of all abnormalities. A second difference lies in the high frequency of monosomy-X in spontaneous abortions (about 20 per cent of all anomalies) and its rather low frequency (0.6 per cent of all anomalies) in new-borns. Thirdly, structural anomalies of chromosomes and mosaics account for about 5 per cent of the abnormalities scored in the abortus material, whereas in new-borns, more than one third of the abnormalities are of this type.

54. There seems to be an association between maternal radiation history and spontaneous abortions in the sense that in the work reported, mothers of chromosomally abnormal foetuses have received higher mean gonadal doses relative to comparable controls.

55. The results of both retrospective and prospective studies on the relationship between maternal irradiation and the incidence of Down's syndrome are conflicting. Recent work in India showing that the frequency of Down's syndrome in a population living in an area of high natural background radioactivity is significantly higher than in controls and than those recorded elsewhere has not stood the test of a critical re-examination which took into account the family size, the number of females in each age group and the mortality rate.

56. Recent results of a continuing study of mortality rates among children born to the A-bomb survivors of Hiroshima and Nagasaki show that no significant effects of parental exposure can be demonstrated. However, the data permit the estimation of doubling doses, lower than which can be excluded. These are 46 rad for males and 125 rad for females under the radiation conditions during the bombings. For chronic low-level exposures, based on mouse data, it has been estimated that the gametic doubling dose for males is about 138 rad and for females, over 1000 rad.

57. Techniques are now available for specifically staining human chromosomes Y, 1 and 9 and studying non-disjunction in sperm samples. However, no radiation studies have thus far been reported.

58. Data have become available which show that non-disjunction can be induced by irradiation of human lymphocytes and that the X chromosome and chromosome 21 may be particularly susceptible to somatic non-disjunction.

II. EFFECTS IN EXPERIMENTAL MAMMALS

A. DOMINANT LETHALS

1. Introduction

59. The 1972 report of the Committee considered in some detail the induction of dominant lethals in mice and in other experimental mammals. In most of the experiments reviewed in the report, dominant lethality had been measured by the pre-natal method, i.e., by examination of the uterine contents of females at suitable stages of pregnancy, counting the numbers of corpora lutea, living and dead implanted embryos and assessing the amount of mortality occurring before and after implantation.

2. Dominant lethals in male mice

60. Although no substantially new information has accumulated since the publication of the 1972 report, at least as far as male mammals are concerned, the paper of Searle and Beechey (510a) has helped to throw light on the best choice of an index dominant lethality where the mutagenic treatment also induces germ-cell killing.

61. The reasoning of Searle and Beechey is based on their study involving exposure of adult male mice to 200 rad of acute x irradiation and mating them at intervals to females over the next 9 weeks. Testis weights fell to 44 per cent of normal during the 5th week and were still subnormal when observations were terminated in the 10th week. Epididymal sperm counts fell in the 4th week (after a peak in the 3rd) and were very low in the 6th and 7th weeks, though still above zero. This reduction was correlated with high frequencies of pre-implantation loss in females mated to these males and dissected on the 12th day of gestation (table 16). Examination of eggs 1-2 days after ovulation showed that there was a sharp decrease (from the normal level of almost 100 per cent) in the fertilization index; it was therefore concluded that much of the pre-implantation loss really stemmed from non-fertilization rather than zygotic death.

62. It has long been suggested that, if a particular mutagen causes intense cell killing with resultant spermatozoal depletion, then what appears to be pre-implantation death may really be the expression of a lowered fertilization rate (29, 451, 560). The work of Searle and Beechey provides experimental proof for that assertion, and they propose that, in these circumstances, an index of dominant lethality based on post-implantation survival should be used (e.g., the ratio live

embryos/total implants) rather than the ratio live embryos/corpora lutea or the number of live embryos per pregnant female. They show that use of the last two indices can sometimes lead to a very substantial overestimate of the true rate of dominant lethality. However, the index of post-implantation mortality underestimates the dominant lethal frequencies in spermatozoa and spermatids. Therefore, for a precise determination of the dominant lethal frequency, it is necessary to establish the fertilization rate of the ova.

63. In experiments designed mainly to study the induction of translocations in mouse spermatozoa, Searle *et al.* (520) estimated the amount of dominant lethality from the rate of decrease of mean litter size with increasing dose. It was found that the relationship between mean litter size and dose was roughly exponential and gave a satisfactory fit to the equation $y_D = y_0 e^{-kD}$, where y_D is the litter size at dose D, y_0 is the litter size in controls and k is the rate of induction of dominant lethals. The estimated value of y_0 was 9.44 ± 0.35 and that of k was $(1.28 \pm 0.07)\ 10^{-3}$ rad^{-1}. The authors stress that the rate of dominant lethals as estimated above can only be approximate, based as it is on new-born litters.

64. Léonard *et al.* (285) studied the genetic radiosensitivity of different mouse strains (RF, BALB/c, C3H, CBA, CO13, AKR/T1Ald, C57BL and AKR) using the rate of induction of dominant lethals in spermatozoa as the criterion (germ cells were sampled during the first week of mating after irradiation). An x-ray exposure of 400 R was used and both pre- and post-implantation mortality were scored. The results showed that (a) the frequency of fertile matings in most of the strains was slightly lowered by the radiation exposure; in the RF and CO13 strains, this was enhanced and significantly so in the latter; (b) there were no significant differences between the numbers of corpora lutea in the control and irradiated series of each strain; (c) the magnitude of radiation-induced pre-implantation losses varied widely in the different strains from a low 13.4 per cent in the BALB/c strain to a high 52.7 per cent in the AKR/T1Ald strain; likewise, the amount of post-implantation losses also varied from 13.3 per cent in the AKR/T1Ald to 39.3 per cent in the BALB/c; and (d) the total amount of dominant lethality (both pre- and post-implantation losses) estimated from the expression $100 \log_e (1-P)^{-1}$ where

$$P = 1 - \frac{\text{Number of live embryos/ number of corpora lutea in the irradiated series}}{\text{Number of live embryos/ number of corpora lutea in the controls}}$$

also varied from about 13 in the RF strain to 43.5 in the C57BL strain. In most of the strains, the increased pre-natal loss is the result of an increase in both pre- and post-implantation death. A comparison of these data with those available for rats, guinea-pigs and rabbits (most of the latter were reviewed in the 1972 report: see paragraphs 22-23, Annex E (1)) would indicate that the variation between different strains of mice with respect to radiation-induced dominant lethals is as large as that between the mouse and the other species.

3. Dominant lethals in female mice

65. The 1972 report of the Committee considered the early data of L. B. Russell and W. L. Russell (458) and those of Edwards and Searle (146) on the sensitivity of mouse oocytes to dominant lethal damage. In addition, the results of Lyon and Smith (313) in guinea-pigs and golden hamsters were dealt with. It was concluded that, as after irradiation of males, most of the induced embryonic death occurs after implantation. Recently, Searle and Beechey (513) have published some data on the induction of dominant lethality in mouse females after x and neutron irradiation. In the x-ray series, mature females received acute x-ray doses of 100-400 rad and were mated to males either 1 day after irradiation or 2 weeks later with daily examination for vaginal plugs; in the neutron series, the females were irradiated with doses of 50-200 rad of fission (0.7-MeV) neutrons and were mated 1 day later. Pregnant females were killed on or around the 14th day of gestation for examination of the uterine contents. Appropriate controls were maintained. In contemporaneous experiments, meiotic preparations of oocytes were made to assess the amount of cytological damage.

66. The x-ray data are given in table 17. It can be seen that the survival of implanted embryos to the time of examination in late pregnancy (as measured by the live embryo/total implant ratio) declines with increasing radiation dose. Unexpectedly, this decline is much more rapid when these embryos were conceived in the 3rd week after irradiation. The increasing dominant lethality correlated well with the amount of chromosomal damage found in the meiotic preparations of the oocytes. The dose-effect relationship for post-implantation dominant lethality is roughly linear in both the 1st- and 3rd-week groups, but in the latter, its level is twice as high over the 200-400 rad range. In the 3rd week the amount of post-implantation loss rose considerably at higher doses, this rise being associated with increasing non-fertilization of ova. Therefore, the live embryo/corpus luteum ratio is not a satisfactory index of total dominant lethality for the 3rd-week group (133). In the 1st-week group, the dominant lethality seemed to reach a plateau at about 200 rad.

67. In the fission-neutron experiments, post-implantation dominant lethality is negligible at 50 and 100 rad but high at higher doses (table 18). For total dominant lethality, the dose-effect relationship seems curvilinear.

68. Since the females exposed to neutrons were mated 1 day after irradiation, Searle and Beechey compared their neutron results with those of the 1st-week x-ray data to estimate the efficiency of neutrons relative to x rays in inducing dominant lethals. Because of the lack of clear-cut dose response in the post-implantation mortality data with neutrons, they do not lend themselves to a ready calculation of the RBE; likewise the "total dominant lethality" data with x rays also do not permit an easy comparison with neutron results. The finding that at a dose of 100 rad (neutrons or x rays) the total dominant lethal frequencies are roughly the same may suggest that at low doses the RBE may be about 1, but more data are needed to verify this suggestion.

4. Species differences

69. In the 1972 report, the work of Lyon (307) comparing the x-ray induction of dominant lethals in post-meiotic male germ-cell stages of the mouse, guinea-pig, golden hamster and rabbit (scored by the pre-natal method) were discussed. The x-ray doses used were: 100 and 500 rad (mouse), 500 rad (guinea-pig and rabbit) and 100-300 rad (golden hamster). It was found that (a) the pattern of relative sensitivity of the germ cells in mouse, guinea-pig and rabbit was similar, with spermatids being more sensitive than mature spermatozoa; in hamsters, spermatids and mature spermatozoa were about equally sensitive to dominant lethal induction; (b) in the guinea-pig and rabbit, the frequency of dominant lethals at 500 rad were lower than in the mouse; after 200 rad to hamsters, the yield of dominant lethals in mature spermatozoa was nearly as high as after 500 rad to the mouse; for weeks 2-4, however, the yield in hamsters after 200 rad was considerably lower than in mice; (c) in the mouse, guinea-pig and hamster, a large proportion of deaths occurred after implantation, whereas in the rabbit, they occurred before implantation.

70. In experiments aimed at studying the x-ray induction of heritable translocations in post-meiotic male germ cells of guinea-pigs, rabbits and golden hamsters, Cox and Lyon (122) have collected data on litter-size reduction (a rough index of dominant lethality) which corroborate the earlier results of Lyon (307) discussed above, in terms of pattern of relative sensitivity of the different germ-cell stages and the amount of damage observed in the successive "mating weeks" after irradiation (1-4 in hamsters and rabbits and 1-5 in guinea-pigs). The x-ray doses used in the present study were: 200 and 600 rad (hamster), 600 rad (guinea-pig, rabbit). The results demonstrate that (a) in all the species studied, irradiation of post-meiotic cells result in reduction in litter size; (b) after 600 rad, the pattern of litter-size reduction and the pattern of sterility for the matings in the successive weeks are similar for golden hamster, guinea-pig and rabbit; for matings utilizing irradiated spermatocytes, i.e., week 4 for the hamster and possibly week 5 for the guinea-pig, a high incidence of infertility is not accompanied by a high reduction in litter size, suggesting that the latter infertility is only partly due to the induction of dominant lethals; such infertility could also stem from reduced fertilizations; (c) in the hamster, the dominant lethal yields (as measured by litter-size reduction) in weeks 1-4 after 200 rad and in week 1 after 600 rad are similar to those of the mouse, but the yields for both these species are above those obtained for the rabbit and guinea-pig; (d) it can be concluded that the golden hamster is more sensitive than both the rabbit and the guinea-pig for the induction of dominant lethals by x rays in post-meiotic male germ cells.

71. Cox and Lyon (121) have completed a comparative study of x-ray-induced dominant lethality in guinea-pig and golden hamster females. The choice of these two species was dictated in part, by the following considerations: (a) in all female mammals, the bulk of the oocytes are in small follicles (primordial follicles), and the response of these stages more accurately reflects the reproductive performance of the irradiated females; in the guinea-pig, the immature oocytes in primordial follicles are more resistant than those of the mouse with respect to cell killing by irradiation, although more sensitive than those of human oocytes (guinea-pig, LD_{50} = 500 R; man, $LD_{50} \cong$ 2000 R (25, 376)); (b) in contrast, the oocytes in the maturing follicles in the guinea-pig are more sensitive than those of the mouse: an exposure of 200 R virtually destroys the larger follicles in the guinea pig whereas this does not happen in the mouse; (c) on the evidence available (408) the golden hamster appears to be intermediate in sensitivity between the mouse and guinea-pig; (d) it thus appears that there is no relationship between the nuclear stage of the "arrested" oocyte, cell killing, and dominant lethal induction; for the dictyate oocyte of mouse and hamster is sensitive, and the condensed diplotene oocyte of the guinea-pig is resistant, to cell killing, yet all three show a low frequency of dominant lethality; (e) in the mouse, dominant lethal yields are higher in the oocytes sampled in the third post-irradiation week than in the first (513), and it would be instructive to inquire whether the pattern observed in guinea-pigs and golden hamsters resembles that of the mouse and whether any correlation between sensitivity to killing and genetic radiosensitivity can be made.

72. Guinea-pigs aged 3-5 months and golden hamsters aged 2.5-3.5 months were irradiated during middle dioestrus (day 6-12 of the 17-day cycle in the guinea-pig and day 2 and 3 of the 4-day cycle in the golden hamster) with 100, 200 and 400 rad of x irradiation at a high dose rate. Three post-irradiation matings were employed for the guinea-pig 1st oestrus, 2nd oestrus and 3 months) and this number was 4 in the golden hamster (1st oestrus, 2nd oestrus, 1 month and 4 months). In addition, the effect of maternal age on dominant lethal yield in the golden hamster was determined in parallel experiments involving irradiation of animals 9-11 months old and the study of dominant lethality at the 1st oestrus mating after irradiation. The pregnant females were killed at appropriate times after matings (3-4 weeks for the guinea-pigs and 10-12 days for hamsters) and the amount of dominant lethality was ascertained by the pre-natal method.

73. Table 19 shows that irradiation did not cause any sterility in the guinea-pig, except at the 1st-oestrus matings (after 400 rad) suggesting that at this dose the mature oocytes are more sensitive than the immature (3-month matings) to killing by radiation. In the golden hamster, there is a marked effect: a large number of females irradiated with 400 rad and mated 3 months after exposure were sterile (10 out of 13) and had ovaries that were approximately one third of the normal size. Histological sections of these ovaries revealed no corpora lutea or oocytes, suggesting that the sterility was due to killing by the radiation of all oocytes which were in either primary or growing follicles at the time of treatment. Sterility in one month matings after 400 rad may have similar causes although histological evidence on this was not obtained. All the other groups of females, both control and irradiated, showed a small amount of infertility and this might have been, in part, due to the seasonal breeding habit of this species. These results thus indicate that the less mature oocytes, i.e., 1- and 3-month matings are more sensitive to killing than are the mature oocytes.

74. The data given in table 19 also show that irradiation of mature oocytes of the guinea-pig with 400 rad lead to superovulation, i.e., a 30 per cent increase in the number of corpora lutea per female. Although this difference was not statistically significant, the amount of increase was very similar to that seen when mature oocytes of the mouse were treated with the same dose of x rays (455). No superovulatory effects could be seen for the 2nd-oestrus and/or the 3-month matings. In hamsters, irradiation with 400 rad produced a significant increase in the number of corpora lutea per female for the 2nd-oestrus but not for the 1st-oestrus matings.

75. The details of the intra-uterine mortality for both species are presented in table 20. It can be seen that in the guinea-pig, in general, the incidence of dominant lethals increases with dose and that the yields for mature oocytes were above those for the immature oocytes at each level. In the golden hamster, the situation is nearly the same.

76. An examination of the components of dominant lethality will show that, in the guinea-pig, both components increased with dose for the 1st-oestrus matings; for the 1st- and 2nd-oestrus matings, pre-implantation death exceeded port-implantation mortality in contrast to the 3-month matings, where the magnitude of both of these mortalities was relatively low. In the golden hamster, mortality before and after implantation increased with dose for the 1st- and 2nd-oestrus matings and in general, the amount of death before implantation was somewhat higher than that after implantation. In both species, some of the pre-implantation death could have been due to the shedding of dead or dying ova, rather than to true zygotic death.

77. The experiments on the effects of maternal age on dominant lethality in the 1st-oestrus matings in the golden hamster showed that the yields of dominant lethals were higher from older females than from young females at 100 and 400 rad levels, but not at 200 rad; however, the differences were not significant.

78. Statistical comparison of the dose-effect relationships for dominant lethals in both species showed that these were consistent with linearity with no significant differences between the slopes (model: $M = M_0 + bD$; $b_{\text{guinea-pig}} = (1.19 \pm 0.25)\,10^{-3}$ and $b_{\text{golden hamster}} = (1.59 \pm 0.18)\,10^{-3}$ (M is the mortality, M_0, the control mortality)). Although statistically a difference between the two species cannot be demonstrated, examination of the raw data suggests that the mature oocytes of the golden hamster may be slightly more sensitive to dominant lethal induction than those of the guinea-pig; in addition, the higher dominant lethal yields for the 2nd-oestrus matings in the golden hamster are indicative of a similar situation.

79. As may be recalled, in the mouse, the work of Searle and Beechey (513) demonstrated that the yields of dominant lethals for the 3rd week were generally higher than those for the 1st week; this pattern is in contrast to that seen in the two species studied by Cox and Lyon (121), where the yields for the 2nd-oestrus matings were in general lower than those for the 1st-oestrus matings. A second difference between the mouse and the other two species is that, in the 1st-week matings of the former, the total frequency of dominant lethals seemed to reach a plateau at about 200 rad, whereas in the latter, the data were consistent with a linear increase with dose. Since the x-ray data for post-implantation mortality in the mouse did not deviate from a linear relationship with dose, Cox and Lyon compared the slopes of the regression (on a linear model for this component) for the three species. The values of the slopes were (in units of 10^{-4} rad^{-1}):

Guinea-pig	3.88 ± 1.18
Golden hamster	6.19 ± 1.18
Mouse	3.57 ± 0.69

80. Thus, although the rate per unit dose for mature oocytes of the golden hamster appears to be higher than that of the other two species, this difference does not reach statistical significance. However, the authors point out that when total dominant lethality in mature oocytes of the three species is compared, the yield in the mouse after 400 rad is significantly below that of the other two species. All these data considered together suggest that the order of species in terms of decreasing sensitivity of the pre-ovulatory oocytes to dominant lethal induction by x rays is: golden hamster, guinea-pig, mouse.

81. For dominant lethal induction in post-meiotic male germ-cell stages (as measured by litter-size reduction), the golden hamster and the mouse appear to have the same sensitivity (in week 1-4 after 200 rad and in week 1 after 600 rad). Likewise, the rabbit and the guinea-pig have similar sensitivities (after 600 rad, week 1-4). The magnitude of litter-size reduction however, is higher in the first two species than in the rabbit and guinea-pig.

5. Summary and conclusions

82. Under conditions where irradiation (or other mutagen treatment) causes germ-cell killing with resultant spermatozoal depletion, the ratio of dead implants to total implants is a better index of dominant lethality than that based on the ratio of dead implants to corpora lutea or the number of dead embryos per pregnant female.

83. There are striking differences between different strains of mice in the amount of x-ray-induced dominant lethality (spermatozoal irradiation), which are as large as those between the mouse and other laboratory mammals studied in this respect.

84. X irradiation of female mice leads to a reduction in the mean number of implants per female (while the number of corpora lutea remain high) and in the ratio of live embryos to total implants; this reduction is more marked in conceptions occurring in the 3rd week than in the 1st week after irradiation; in contrast, in golden hamsters and guinea pigs, the yields of dominant lethals for 2nd-oestrus matings are lower than those for 1st-oestrus matings.

85. X irradiation of female mice (100-400 rad) leads to an increase in post-implantation mortality in conceptions occurring during the 1st and 3rd weeks after

irradiation. The dose-effect relationships are roughly linear, but in the 3rd-week group, the level of mortality is about twice as high as in the 1st-week group. The total dominant lethality (pre- and post-implantation) in the 1st-week group seems to reach a plateau at about 200 rad.

86. In guinea-pigs and golden hamsters, the dose-effect relationships for both post-implantation and total dominant lethality are consistent with linearity. For the induction of post-implantation mortality in pre-ovulatory oocytes, the order of the three species in terms of decreasing sensitivity is: golden hamster, guinea-pig, mouse.

87. For the induction of dominant lethals in post-meiotic male germ-cell stages, the mouse and golden hamster are more sensitive than rabbit and guinea-pig.

88. At a dose of 100 rad, fission neutrons (0.7 MeV) appear to have the same effectiveness as x rays in inducing dominant lethals in female mice.

B. TRANSLOCATIONS

89. Ever since the air-drying technique for making meiotic preparations of mammalian testes was developed by Evans, Breckon and Ford (162), the cytogenetic study of translocations in mammals, especially of those induced by irradiation, has been growing explosively. During the period 1964-1972, a large number of papers were published dealing with dose-effect relationships, dose-rate and dose-fractionation effects, effectiveness of radiations of different quality, the types of translocations and their effects on fertility etc. These were extensively reviewed in the 1972 report of the Committee. The general findings are summarized in the paragraphs that follow (unless otherwise stated, the comments refer to studies with the mouse):

90. Translocations can be induced by ionizing radiation in all stages of spermatogenesis in adults, in embryos *in utero* and in late oocytes. Radiation-induced translocations during spermatogenesis have also been observed in adult male rabbits, guinea-pigs and golden hamsters.

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

92. In spermatogonia, translocation induction by x and fast-neutron irradiation at high dose rates is consistent with a linear kinetics (up to 600-700 rad with x rays and up to 100 rad with neutrons), after which the yield falls off drastically, giving an overall humped curve; the results with gamma irradiation at high dose rates showed that the dose-effect relationship was concave in the range 56-402 R (suggesting the contribution of a quadratic component to the observed yield), although when the data were analysed as a whole (56-816 R) the relationship did not significantly depart from linearity. All these features are very probably the result of secondary distortions of the primary dose-effect relationship, which may well have a more marked square-law component.

93. In rabbit and guinea-pig spermatogonia, the overall dose-response curve after acute x irradiation is also humped (as in mice), but the peak incidence occurs at 200-300 rad.

94. The yield of translocations obtained from spermatogonia is reduced after x and gamma irradiation at low dose rates, the effect being more pronounced with gamma rays; at high doses, protracted neutron irradiation is more effective than acute irradiation whereas the reverse is true at low doses; the effects of fractionation are dependent on total doses and fractionation procedures. Especially important is the observation that the fractionation of a total dose of 300 rad of x rays into several small fractions of 10 or 5 rad each leads to a significant reduction in translocation yields, as compared with the effects of a single dose.

95. Some translocations induced in spermatogonia can successfully pass through the remaining stages of spermatogenesis and can contribute to zygotic populations; all the dominant lethality observed after spermatogonial irradiation can be accounted for by the segregation of unbalanced haploid genomes from spermatocytes with translocation multivalents.

96. A marked discrepancy was found between the frequencies of translocations diagnosed cytologically (in spermatocytes following spermatogonial irradiation) and genetically (in F_1 progeny) in that the observed frequency in the F_1 is only one half that expected on the basis of the frequencies observed in primary spermatocytes; these experiments had been carried out at an exposure level of 1200 R administered in 2 equal fractions separated by a period of 8 weeks.

97. Certain translocations (autosomal) can be fully viable in the heterozygous state and yet cause male sterility through a failure of 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; this is also true for translocations involving the X chromosome.

1. Dose-effect relationships in mouse spermatogonia

(a) *X and gamma irradiation*

98. Preston and Brewen (419) re-examined the problem of dose-effect relationship using x irradiation at a high dose rate (50-1200 R; 11 exposure levels). There was one important methodological difference between their work and that of other authors: while in most of the earlier investigations, a single sampling time (ranging from about 56 to 120 days, depending on the investigator, following irradiation with different exposures) was employed, Preston and Brewen staggered the

sampling time so that it coincided with the first wave of meiotic activity following recovery of the germinal epithelium. In other words, the time interval between irradiation and killing of the animals (to make preparations of the testes) varied with the exposure.

99. Their results are reproduced in table 21. In the range 0-500 R, the data best fit a model that incorporated both a linear and a quadratic component. The value for these two components are given by the equation

$$Y = 1.08\ 10^{-4}D + 5.00\ 10^{-7}D^2$$

Above 600 R, however, the yield of translocations decreased with increasing exposures, leading to a humped curve over the whole range studied, as has been observed by several workers previously. The authors suggest that in earlier work, the use of a single sampling time might have led to the sampling of different populations of stem cells at different exposures and that these might be responsible for the discrepancies between their results and the earlier ones with respect to the shapes of dose-response curves up to exposures of 600-700 R.

100. It is worth pointing out here that Wennström (605), on the basis of somewhat limited data, had earlier reached the conclusion that, in the dose range 25-400 rad, the dose-effect relationship for the induction of translocations in mouse spermatogonia was supra-linear. At all the dose levels, the interval between irradiation and observation was 2 months. The frequencies of cells with multivalents were (per cent): 0.33 (control), 0.33 (25 rad), 0.66 (50 rad), 2.7 (100 rad), 3.7 (200 rad) and 4.0 (400 rad). The numbers of cells analysed were respectively 1521, 300, 300, 300, 273 and 400. These data appear to suggest that, in the particular strain of mice used by Wennström (albino mice of the NMRI strain), the yield peaks at a rather low dose level. The author did not attempt any mathematical curve-fitting.

101. Pomerantzeva, Ramaiya and Ivanov (415) studied the gamma-ray (^{137}Cs) induction of translocations in mouse spermatogonia at three different exposure rates (710, 0.07 and 0.007 R/min). At the highest exposure rate, the frequencies were (per cent): 3.3 ± 0.7, 5.1 ± 0.7, 4.7 ± 0.8, and 4.2 ± 0.8 per cent at exposure levels of 300, 600, 900 and 1200 R, respectively. At the middle rate, the frequencies were lower, being 2.8 ± 0.5 per cent at 300 R, 3.8 ± 1.4 per cent at 600 R and 1.8 ± 0.7 per cent at 900 R. At the lowest rate, the frequencies were consistent with a linear increase with exposure (300, 600, 900 and 1200 R), the equation of best fit being

$$Y = (0.225 \pm 0.086)\ 10^{-2} + (1.74 \pm 0.21)\ 10^{-5}D$$

where Y is the translocation frequency and D, the exposure. In a subsequent paper, Pomerantzeva, Vilkina and Ivanov (417) demonstrated that when the exposure rate was further reduced to about 0.003 R/min, the response over the range of exposures from 100 to 920 R (5 levels) was nearly the same as that obtained with the lowest rate (0.007 R/min) above.

102. Searle *et al.* (516) analysed the available low dose-rate data obtained with chronic gamma irradiation (^{60}Co or ^{137}Cs) down to 0.003 rad/min (82, 83, 89, 90) (with total doses varying from 100 to 1200 rad) and concluded that (a) the yield fell with decreasing dose rate, with little further decrease in rate of induction below 0.02 rad/min, and (b) it seemed unlikely that the minimal rate would be less than $1.0\ 10^{-5}$ rad^{-1}.

(b) *Neutron irradiation and RBE estimates*

103. Muramatsu *et al.* (354) have reported the results of their studies on RBE of 2-MeV neutrons relative to 250-kVp x irradiation for the induction of reciprocal translocations in mouse spermatogonia. A wide range of doses was used (48-672 rad of x rays, 8 levels; 24-267 rad of neutrons, 8 levels). The dose-response curve for the neutrons was linear up to 94 rad beyond which the yield fell off, giving an overall humped curve, as had been observed earlier with 0.7-MeV neutrons (29). For x irradiation, the picture was essentially the same, except that the peak was at 672 rad. The regression coefficient for the linear part of the neutron curve was $11.4\ 10^{-4}$ and that for the x-ray curve, $2.69\ 10^{-4}$. The RBE for 2-MeV neutrons was estimated as 4.2, the ratio of these values, which is close to that of 3.7 estimated by Searle, Evans and West (518) and that of 4.4 recorded in the work of Pomerantzeva *et al* (414) for 1.5 MeV neutrons.

104. Valentin (596) carried out experiments on translocation induction in mouse spermatogonia with 14.5-MeV neutrons at acute dose levels of 75, 150 and 250 rad (dose rate, 1.7 rad/min). It was found that one mouse in the 150-rad series (out of the 11 used) contributed significantly to the variation between the mice (10 out of 16 affected cells were from this mouse, which in addition carried 2 translocations in one of the cells scored). The frequency of affected cells at doses of 75, 150 and 250 rad were, respectively, 0.7 ± 0.2 per cent, 0.8 ± 0.2 or 0.4 ± 0.1 per cent (depending on whether the aberrant mouse is included or excluded) and 1.6 ± 0.2 per cent. Between 1700 and 2500 cells were scored for each dose point in addition to 4700 cells in controls, where no translocations were found. The author points out that the dose response was consistent with linearity in the range studied, although if the aberrant male is excluded, the data give a better fit to a curvilinear relationship. Since no comparable x-ray data were obtained in her experiments, Valentin has refrained from making any RBE estimate except to point out that, in comparison with the results of Searle, Evans and West (518) with 0.7-MeV neutrons, the 14.5-MeV neutrons used in her study were presumably not as efficient and that the RBE value may be much lower than 3.7.

105. Bajrakova *et al.* (22) examined the effects of irradiation with 4.1-MeV neutrons (from a Pu-Be source) delivered at a low dose rate of about 0.08 rad/h on the induction of translocations in male mice. They found that the dose-effect relationship up to 52 rad (3 levels) was linear with an increase of about $3.3\ 10^{-4}$ translocations per rad, beyond which the curve saturated. The frequencies at the two high doses used were 1.91 ± 0.45 per cent, at 98 rad, and 1.65 ± 0.40 per

cent, at 150 rad. Since no comparable x- or gamma-ray experiments were conducted, the RBE value cannot be estimated. However, in comparison with the results of Pomerantzeva *et al.* (415) with chronic gamma irradiation, 4.1-MeV neutrons (also delivered chronically), appear to have an RBE of about 20.

2. Dose rate

106. It may be recalled that the earlier results of Searle *et al.* (517) discussed in the 1972 report (para. 66) demonstrated that, at the 600-rad level, a lowering of the gamma-irradiation dose rate from 83 rad/min to 0.02 rad/min led to a concomitant lowering of the yields of translocations and that the frequency at 0.02 rad/min was about one ninth of that at the higher rate. The results of Pomerantzeva *et al.* (415, 417) discussed earlier in this report (para. 101) corroborate the above finding and extend the data to more exposures and still lower exposure rates. They show that (a) when the exposure rate is reduced from 710 R/min to 0.007 R/min, the translocation yields are lower by at least an order of magnitude (range of exposures, 300-1200 R); (b) over this range of exposures, the data are consistent with a linear exposure-frequency relationship (see paras. 101, 102); and (c) a further lowering of the exposure rate to 0.003 R/min (range, 100-920 R) does not lead to any additional sizeable reduction in the frequencies over that already observed after irradiation at 0.007 R/min.

107. In more recent experiments, Searle *et al.* (516) demonstrated that when a gamma-ray dose (^{60}Co) of 1128 rad was administered to male mice over a 28-week period (0.004 rad/min) the frequency of translocations per spermatocyte was 1.70 ± 0.4 per cent, corresponding to a rate of induction of $1.40\ 10^{-5}$ rad^{-1}. This rate is quite similar to that of Pomerantzeva *et al.* (415), $1.74 \pm 0.21\ 10^{-5}$ R^{-1}. Relative to the yields after acute x irradiation (163) and after acute gamma irradiation (511), the results of Searle *et al.* (516) at 0.004 rad/min show that the efficiency of these is only about 0.05 and 0.01, respectively.

108. Van Buul and Roos (72) irradiated male mice with 400 R of x rays or gamma rays at exposure rates of 60 R/min and 130 R/min respectively and compared the yields of translocations obtained with those observed after a similar exposure of gamma rays delivered at rates of 1.92 R/min and 0.03 R/min. The data show that the yield after 400 R of acute gamma rays is slightly lower than that after x rays (abnormal cells, 6.6 per cent compared with 8.6 per cent; translocations, 7.0 per cent compared with 9.0 per cent; number of cells scored, 1400 and 1200) but not significantly so. With gamma rays at 1.92 R/min, the yield fell to one half of that observed after acute gamma irradiation (3.0 per cent abnormal cells or 3.2 per cent translocations; 1300 cells scored) and at 0.03 R/min, to 1.9 per cent (1400 cells scored). It is of interest to note that the magnitude of reduction observed here (by a factor of 3.5) is lower than that recorded by Searle *et al.* (517) at a dose level of 600 rad (83 rad/min compared with 0.02 rad/min; 12.1 per cent to 1.4 per cent), where it is by a factor of about 9.

109. In the study of Valentin described earlier (596), the effects of 250 rad of 14.5-MeV neutrons administered at rates of 1.67 rad/min and 0.01 rad/min were compared. The frequency of abnormal cells with either dose rate was nearly the same (1.6 ± 0.2 and 2.2 ± 0.3 per cent with high and low dose rates, respectively), thus showing no demonstrable dose-rate effect at this dose and with the range of dose rates employed.

110. In the work of Bajrakova *et al.* with 4.1-MeV neutrons (22) discussed in paragraph 105, no irradiations were carried out at high dose rates, and consequently the question of whether a dose-rate effect is present with that kind of neutron cannot be answered. However, their data show that at high exposures of 98 and 150 rad delivered at 0.0013 rad/mm (80 mrad/h), the frequencies of translocations remained at approximately the same level as after 52 rad. This finding is in marked contrast to that with 0.7-MeV neutrons (518) administered at about the same dose-rate as in the experiments of Bajrakova *et al.* (22), where a markedly high translocation yield (21.7 per cent at 214 rad) was recorded.

3. Dose fractionation

111. As was mentioned earlier, fractionation of the radiation exposure affects the yield of translocations recovered from irradiated spermatogonia and this depends on the magnitude of the doses and the fractionation procedures used. For convenience, the various results obtained will be considered under the headings "short intervals" (less than 24 h), "medium intervals" (24 h to a few days) and "long intervals" (a few weeks). The earlier results reviewed in the 1972 report will be briefly summarized to facilitate comparisons. Unless otherwise stated x irradiation was used.

(a) *Short intervals*

112. Léonard and Deknudt (282) studied the induction of translocations in mouse spermatogonia by using an x-ray exposure of 500 R divided into two equal fractions separated by time intervals from 60 min to 24 h. It was found that after a single 500-R exposure, the incidence of spermatocytes carrying translocations was 8.1 per cent; with an interval of 2 h between the fractions, the frequency fell to 5.7 per cent, rose to 8.8 per cent at 4 h, fell again to 4.4 per cent at 7 h, rose to 8.4 per cent at 16 h, with some suggestion for a further fall to 6.8 per cent at 24 h. In the study of Searle *et al.* (515), in one experiment, when a total dose of 300 rad was delivered in two equal fractions separated by a 30-min interval, the frequency of affected spermatocytes was 8.1 per cent compared with 14.5 per cent observed after the unfractionated exposure. (As the authors themselves point out, the latter frequency is higher than the one expected at this dose (7.5 per cent) on the basis of their earlier studies.) With a 2-h interval, the frequency rose to 10.8 per cent, a just significant increase. In the next experiment, there was again an initial fall from 8.8 per cent (unfractionated) to 6.6 per cent (60 min) and a rise later to 8.1 per cent at 6- and 8-h intervals; none of these changes was statistically significant.

113. In the experiments of Preston and Brewen (420), an unfractionated exposure of 500 R gave a yield of 17.2 per cent; with intervals of 30, 90 and 150 min between the two 250-R fractions, the yields appeared to decline to 16.2 ± 1.8 per cent, 14.0 ± 1.5 per cent, 12.5 ± 1.4 per cent, respectively. In further experiments reported in the same paper, the authors investigated the effects of fractionation of a 1000-R exposure (an exposure which is on the descending part of the dose-effect curve) into two equal fractions separated by intervals of 3, 6 and 18 h. The yield of translocations following a 1000-R single exposure was 5.2 per cent; following fractionation however, the yield increased to 17.1 per cent for a 3-h interval and reached a maximum of about 41 per cent for an interval of 18 h when it was equal to the yield expected on the basis of additivity of yields of two separate 500-R exposures. On the basis of these results and the finding that the yields decreased with 48- and 72-h intervals to 23.8 and 19.5 per cent, respectively, the authors suggest that the pattern of response observed in the above experiments could be explained in terms of the killing of cells in the sensitive stage of the cell cycle by the first exposure, resulting in a partially synchronous population being irradiated by the second exposure. The stage of the cell cycle in which the population exposed to the second fraction is expected to be in, will vary with time between the two fractions, assuming that over the time period used in these experiments, the cells are still partially synchronous. Preston and Brewen predicted that with a time interval of, say, 96 h one would expect the yield to return to that for 1000 R. The results obtained by Cattanach and Moseley (92) indeed show that the expectation is fulfilled (para. 118).

(b) *Medium intervals*

114. The early experiments of Lyon and Morris (311) demonstrated that the yield of translocations after two 500-rad doses to spermatogonia (24-h interval), was not enhanced above that extrapolated from lower single doses, although it was above that for a single 1000-rad dose, which was beyond the maximum of the humped dose response curve. In further work with similar fractionation procedures, Morris and O'Grady (345) adduced further evidence which showed that the yields after fractionated exposures involving total doses from 100 to 600 rad were remarkably close to those for single ones (i.e., no enhancement); at higher doses (up to 1400 rad) the yields continued to increase approximately linearly after fractionation and were consistent with the additivity of yields of the two respective fractions. These observations are in marked contrast to those made by W. L. Russell (471), which showed that after fractionation of an exposure of 1000 R (two fractions, 24 h apart) the yield of specific locus mutations was much greater than expected by extrapolation from lower single doses. The suggested explanation for the enhancement of specific locus mutations was that the first radiation dose synchronized the cell divisions of the surviving spermatogonia in such a way that at the time of the second dose 24 h later, they were particularly sensitive to gene mutations. Morris and O'Grady speculated that the difference in response between specific lcous mutations and translocations could be understood if it is assumed that in the latter case, the cells were synchronized at a stage with relatively unchanged sensitivity to chromosomal damage (see the 1972 report (589) for details of the data).

115. The work of Wennström (605), in which the effects of fractionation of a 200 rad dose into two, three, four or six fractions (24-h interval between fractions) also demonstrated that the yields with these different régimes were not significantly different from the one obtained after a single dose of 200 rad. (The finding that, with four or six fractions there was no change in the yield relative to that of the single dose is at variance with that of Lyon *et al.* (315), which showed that with a 300-rad total dose split into five daily fractions of 60 rad, there was a reduction in frequency from 6.3 to 3.4 per cent.)

116. Van Buul and Léonard (71) examined the extent to which the magnitude of the first fraction of a split dose affected the subsequent response of the cells 24 h later to the second fraction. In these experiments, male mice were irradiated with a total x-ray exposure of 600 R delivered either singly or in two equal fractions or in two unequal fractions of 100 and 500 R or 500 and 100 R. Translocations were scored 70-120 days after the completion of the radiation treatments. The results showed that when the irradiation was delivered either singly or in two equal fractions, the frequencies of abnormal cells were not significantly different (7.6 compared with 8.4 per cent), as had been found by other workers. With the 100 + 500 R régime, the frequency was 10.2 per cent (1600 cells scored), whereas when the order of the exposures was reversed, it was 4.9 per cent (1700 cells scored). These data suggest that the net result of interaction between the postulated cell synchronization by a first fraction of a dose and the alteration of the sensitivity of the surviving spermatogonial cell population is dependent on the magnitude and sequence of the exposure fractions.

117. In a more recent paper, Cattanach, Heath and Tracey (93) adduced further evidence for the thesis that with 24-h intervals, the size and sequence of the exposure fractions determine the radiosensitivity of the stem cells to translocation induction. They found that when 1000 R was administered as 100 R followed by 900 R, the recovered translocation yield (22 per cent) was similar to that which can be obtained by extrapolation from lower exposures and also that of a 500 R + 500 R, 24-h fractionation. However, when the 900 R preceded the 100 R, the response was much lower (7.4 per cent), yet still higher than that produced by a single 1000 R treatment (4.5 per cent). The same order of effectiveness was observed for the length of the sterile period. From these results the authors have concluded that 24 h after the initial exposure (a) the surviving stem cells are more sensitive than formerly, both to killing and genetic damage, and (b) they are no longer heterogeneous in their radiosensitivities so that increasing yields of genetic damage may be obtained with increasing exposures.

118. Cattanach and Moseley (92) irradiated males with a single x-ray exposure of 800 R or in two fractions of 500 and 300 R (in that order), separated by intervals

ranging from 24 h to 12 days. Translocations were scored in spermatocytes descended from the irradiated gonia, 100 days after the completion of the irradiation treatment. The data showed that the 24-h fractionation increased the translocation yield significantly above that of the acute treatment (18.3 per cent compared with 7.9 per cent) but not higher than expected on the basis of additivity of individual fractions; with a 48-h interval, the frequency was 16.0 per cent, still being consistent with the expectation of additivity. On the other hand, with intervals of 3-12 days, the translocation yields were much lower and little different from that obtained after acute exposure. These latter results are consistent with the prediction made by Preston and Brewen (420) on the basis of their experiments in which a 1000-R exposure was given in two fractions separated by periods ranging from 3 h to 3 days (para. 113).

119. In the work of Cattanach *et al.* (93) discussed in paragraph 117, evidence was also obtained showing that the subadditive translocation yields recorded with the 800-R treatments (500 R + 300 R, and in that order with intervals of 3-12 days) was maintained with further intervals of up to 15 days and that additivity was regained by the end of the third week. Sterile period data indicated that with these intervals (of up to 15 days), the germinal epithelium had recovered sufficiently from the first fraction for spermatogenesis to restart before the second fraction was given. These results permitted the authors to conclude that (a) 3-15 days after the first exposure, the surviving cells proliferate rapidly to repopulate the testes and at this time they are either resistant to genetic damage (though still relatively sensitive to killing) or are sensitive to both, but with the two events being correlated, and (b) the onset of spermatogenesis is associated with the re-establishment of a heterogeneity in radiosensitivity among the stem cells.

120. Earlier work from Lyon's laboratory (315) had shown that when repeated small radiation doses are delivered to mouse spermatogonia, the yield of translocations was less that that after the same total dose given in a single exposure. Two main explanations were considered: (a) each individual small dose has less effect than the comparable fraction of a large single dose, and (b) repeated irradiation in some way changes the spermatogonial cell population so as to either decrease the sensitivity to induction or increase the elimination of mutational changes. The evidence obtained for (b) was the following: a dose-response curve was plotted for varying numbers of 10-rad doses (total dose ~620 rad). The form of the curve suggested that later doses were having less effect than earlier ones and hence was consistent with the idea that the spermatogonial cell population was becoming more resistant with repeated irradiations. If this were true, one would expect that if a single exposure to a large dose of irradiation were given *after* a succession of repeated small doses, it would have less effect than if the procedure were reversed.

121. Lyon, Phillips and Glenister (317) tested the above possibility in experiments by comparing the yields of translocations obtained with a dose of 300 rad of gamma rays to spermatogonia administered before, 24 h after, or 8 days after exposure of the animals to 30 daily doses of 10 rad each. The results showed that under conditions when the 300-rad single exposure was given 24 h after the 30 X 10-rad exposure, the yield was significantly lower (7.3 per cent) relative to those from the other two régimes (9.4 per cent: 300 rad + 30 X 10 rad; 9.7 per cent: 30 X 10 + 300 rad, 8 days later). That is consistent with the hypothesis that the repeated irradiation had temporarily increased the resistance of the spermatogonial cell population to translocation induction. The authors consider the above interpretation tentative.

(c) *Long intervals*

122. So far, there have been only four reports on the effects of dose fractionation on translocations induced by x rays in spermatogonia where the interval between the doses was of the order of several weeks. The first of these relate to the work of Ford *et al.* (170), who found that when an exposure of 1200 R was administered in two equal fractions separated by a period of 8 weeks, the yield of translocations was high (being 41.6 and 32.5 per cent in two different experiments) but not higher than what would be expected from additivity of yields, although much higher than what had been found following single acute exposures of similar magnitude. Similarly, Pomerantzeva *et al.* (415) found that when a total exposure of 900 R was given in three equal fractions separated by 4-week intervals, the frequency of translocations was 9.1 per cent, this being roughly three times that after a single 300-R exposure (3.3 per cent) and about twice that after a single 900-R exposure (4.7 per cent).

123. More recently, Pomerantzeva and Ramaiya (412) made a study with fractionated gamma-ray exposures delivered to mouse spermatogonia (900 R in three equal fractions) and varied not only the interval between the exposure fractions (1 to 4 weeks) but also the interval between the completion of the irradiation and the sampling of the spermatocytes (12 to 48 weeks). Considering first the effect of *sampling interval*, increasing it from 12 to 28 weeks led to no significant change in translocation frequency (9.3 ± 1.2 per cent compared with 10.0 ± 1.4 per cent; pooled data: 9.7 ± 1.0 per cent). However, when the interval was extended to 48 weeks, there was a slight decrease in the frequency (7.3 ± 0.8 per cent compared with 9.7 ± 1.0 per cent). The latter finding is qualitatively similar to that recorded earlier by Evans *et al.* (163), namely, that after a single dose of 800 rad the frequency fell from 20.5 per cent with an 11-week interval to 12.8 per cent with a 30-week interval. (See table 11 in the 1972 report, Annex E.)

124. Concerning the effects of *interval between exposure fractions* on the yield of translocations, Pomerantzeva and Ramaiya found that, with a 7-day interval between the fractions (12-week sampling interval), the frequency was 8.6 ± 0.8 per cent and did not change with a 28-day interval (9.3 ± 1.2 per cent). In either case, the yield was lower than expected on the basis of additivity (yield after a single 300-R exposure: 5.2 ± 0.6 per cent). The first of these findings is similar to that of Cattanach *et al.* (93 and paragraph 119) whereas the second one is not: Cattanach *et al.* noted

that with a total exposure of 800 R administered in two unequal fractions of 500 and 300 R, additivity was regained 21 days after the completion of the radiation treatment, whereas this has not been the case in the work of Pomerantzeva and Ramaiya. One possible reason for the discrepancy between the two sets of results may be that, since both the total exposures and the fractionation régimes are different, the pattern of recovery of the germinal epithelium under these conditions is also different (see paragraph 125).

125. In their experiments (500 + 500 R), Preston and Brewen (420) found (see also paragraph 113) that with an 18-h interval, the yield was equal to that expected on the basis of additivity (40.7 per cent compared with 2 X 17 = 34 per cent) but dropped to 23.8 per cent with a 48-h interval and remained at approximately the same level up to an interval of 4 weeks. By 6 weeks, additivity was once again regained (32 per cent compared with 2 X 17 = 34 per cent).

126. In other experiments reported in the same paper, Preston and Brewen irradiated male mice with repeated 400-R x-ray exposures up to a total exposure of 2800 R, the interval between successive exposures being 8 weeks. The expectation was that under such conditions, the interval between one exposure and the next would be long enough for the cells to re-assort back to a normal cycle, with cells present at all stages, and consequently additivity of yields should be obtained. The data presented in table 22 show that the expectation is indeed realized. The time elapsed between irradiation (after different numbers of exposures) and the sampling of spermatocytes was adjusted to sample the first wave of meiotic cells following the recovery and repopulation of the geminal epithelium, as emphasized in an earlier publication (419). This varied from 6 weeks following four 400-R exposures up to 17 weeks following the last 400-R exposure of the 2800-R total exposure. As will be obvious, the interpretation for the observed effect in this kind of experiment, involving long intervals between exposures, is different from that advanced for similar effects observed with fractionation régimes involving 24-h intervals.

4. Interaction with chemicals

127. The 1972 report presented the data of Ehling (148, 152, 153) and of Pomerantzeva and colleagues (209, 411) on the effects of pre-treatment with different chemicals, such as chloramphenicol, mitomycin C, aminoethylisothiourea (AET), 5-methoxytryptamine and cysteamine, on radiation-induced dominant lethals. Similar studies have now been carried out to examine the effects of chemical pre-treatment on radiation-induced yield of translocations in spermatogonia. Léonard and Deknudt (283) who studied the effects of AET pre-treatment, found that the compound exhibited no protective effect and explained the failure of AET to modify the rate of translocation induced by x irradiation on the basis of some germinal selection or, more likely, the stage specificity of AET (Ehling's finding was that a protective action was manifest only in early spermatids). In a subsequent paper, Léonard and Deknudt (284) demonstrated that a mixture of reduced glutathione (GSH), AET, mercaptoethylamine (MEA), cysteine and serotonin creatinine sulphate or 5-hydroxytryptamine (5-HT) did have a protective effect: the frequency of abnormal spermatocytes in the "radiation alone" series was 8.1 per cent and that in the "mixture plus radiation", 5.9 per cent (500-R exposure to spermatogonia). Using Adeturon (ATP salt of AET), Bajrakova (21) found that this chemical did offer some protection against radiation-induced translocations in spermatogonia. The frequencies of abnormal cells after radiation alone or radiation plus chemical are, respectively, 2.6 per cent and 1.6 per cent (150 R), 4.8 per cent and 2.5 per cent (300 R) and 6.1 per cent and 2.6 per cent (450 R). Between 800 and 1000 cells were scored for each data point.

128. In a further elaboration of earlier work (411), Pomerantzeva and Vilkina (413) examined the effects of pre-treatment with cysteamine on gamma-ray induction (100, 300 and 600 R) of translocations in spermatogonia. No protective effects were found. However, in parallel experiments on dominant lethal induction in post-meiotic and meiotic stages, the pre-treatment led to a reduction in induced dominant lethality after 300 R. The effect was negligible after 100 R and completely absent after 600 R.

5. Comparison between cytologically observed and genetically recovered frequencies of reciprocal translocations

129. Despite the phenomenal progress that has been made in the cytological screening for radiation-induced reciprocal translocations induced in spermatogonia, there is a great paucity of experiments designed specifically to examine the relationship between the frequencies obtained in spermatocytes and those observed in genetic tests of F_1 male progeny. In the only paper thus far published, Ford *et al.* (170) determined this relationship at a high exposure (600 R + 600 R, separated by 8 weeks; adult males were irradiated and the frequencies of translocations in spermatocytes derived from irradiated spermatogonia were determined, and in parallel experiments the frequency of heritable translocations in the F_1 progeny of similarly irradiated males was also ascertained). It was found that the frequency of transmissible reciprocal translocations was only about one half of that expected on the basis of the cytological data. The interpretation was that this discrepancy could arise if some selection process operated on translocation-carrying diploid (rather than haploid) genomes between the meiotic metaphase and fertilization.

130. For better use of the latter kind of data in hazard evaluations, information on whether or not the relationship found at high exposures will hold at lower levels and the extent to which this is likely to be modified under other conditions of radiation exposure (low dose rate, fractionation etc.) is of paramount importance. To rectify this deficiency in our knowledge, Generoso *et al.* (186) and Brewen *et al.* (56) have embarked on an extensive study aimed at determining, for a series of acute exposure levels (150, 300, 600 and 1200 R of x rays), the frequencies of translocations in spermatocytes and those of translocation heterozygotes in the F_1 male progeny, in parallel cytological and genetic tests.

131. The data available until now are given in table 23. It can be seen that (a) the frequency of translocation heterozygotes (genetic tests) increases with exposure up to 600 R (although the difference between the yields after 150 R and 300 R is not significant), followed by a marked falling off at 1200 R; this humped characteristic of a exposure-frequency curve is in general agreement with the cytologically scored reciprocal translocations; (b) at exposure levels of 300 R and above, the ratio of translocation recovery in the genetic tests to cytologically determined frequency is roughly one eighth, as was found earlier by Ford *et al.* (170); and (c) at the lower exposure level of 150 R, this ratio is one quarter (although not significantly different from one eighth in view of the still relatively small number of F_1 males tested). Thus at the exposure level of 150 R, there is a suggestive indication that, on the basis of the conditions assumed by Ford *et al.* (170), the rate of recovery of balanced translocations among progeny might be that which is expected from the cytological frequency.

132. In a recent study in which male mice were given fractionated gamma-ray exposures (3 X 300 R, separated by 28-day intervals between fractions), Pomerantzeva, Ramaiya and Vikina (416) found that the frequency of cytologically diagnosed translocation heterozygotes among F_1 males (derived from irradiated gonia) was 2.8 ± 0.9 per cent (9/319). Although no direct determination of the translocation frequency was made in the irradiated males themselves, such data collected by the same group of workers in another study (para. 123) under similar radiation conditions may be used to compute the ratio of the frequency of translocation heterozygotes in the F_1 to the frequency of translocations in irradiated males. This ratio, 0.28 (2.8/10.0), is not significantly different from either 0.25 or 0.125, in view of the small number of F_1 males screened.

6. Summary and conclusions

133. Since the publication of the 1972 report of the Committee, a sizeable amount of information has accumulated from studies on the induction of reciprocal translocations. Most of this has come from continuing studies with mice.

134. In contrast with the results from earlier work in which the frequency of reciprocal translocations induced in spermatogonia (and scored in spermatocytes) by x irradiation at high dose rates was found to show a linear dose-effect relationship, the results of Preston and Brewen (and those of Wennström to some extent) show that the data best fit a model which incorporates both a linear and a quadratic component. In the work of Preston and Brewen, the interval between irradiation and killing of the mice was varied (depending on the dose) in such a manner that the time of sampling of the spermatocytes coincided with the first wave of meiotic activity following recovery of the germinal epithelium; in earlier work, most often, a single sampling time (irrespective of the dose and depending on the laboratory) had been used.

135. With chronic gamma irradiation delivered at a rate of 0.007 R/min, the exposure-frequency relationship is linear over the entire exposure range investigated (up to 1200 R). Further lowering of the exposure rate to 0.003-0.004 R/min does not lead to any significant reduction in the rate of induction. An analysis of all the available data obtained with chronic gamma irradiation at low dose rates down to 0.003 rad/min (with doses varying from 100 to 1200 rad) shows that the minimal rate of induction is unlikely to be lower than $1.0\ 10^{-5}$ rad^{-1}. At these very low dose rates, the efficiency of chronic gamma irradiation is at least an order of magnitude lower than that of acute gamma irradiation or acute x irradiation.

136. Irradiation of mouse spermatogonia at high dose rates with 2-MeV neutrons is about four times as effective for translocation induction as similar irradiation with 250-kVp x rays. Neutrons at 14 MeV appear to be much less efficient in this respect. The dose-effect relationship for chronic neutron irradiation (4.1 MeV) is consistent with linearity up to 52 rad, after which a saturation effect is observed. Relative to chronic gamma irradiation, similar irradiation with 4.1-MeV neutrons is about 20 times more efficient. No dose-rate effect can be demonstrated with 14-MeV neutrons in the range 0.01-1.67 rad/min.

137. Fractionation of the irradiation exposure affects the yield of translocations recovered from irradiated spermatogonia in a way that depends on the magnitude of the radiation dose and the fractionation régimes employed.

138. With total doses below 600 rad (i.e., those on the ascending part of the humped dose-effect curve), irradiation in two equal fractions separated by intervals of up to about 2 h leads to a decrease in the yield of translocations (relative to single doses). This might be due to two reasons: (a) the change in the characteristics of the spermatogonial population caused by the first fraction of the dose (for instance, the killing of cells in certain phases of the spermatogonial cell cycle and the consequent synchronization of the remaining cells), the degree of which may vary with the dose and (b) the decreasing possibility of interaction between chromosome lesions produced by the first fraction and the subsequent one. The former possibility seems, however, most unlikely.

139. When the interval between the fractions is equal to or more than the maximum time that seems to be required for rejoining of the chromosome lesions produced by the first fraction (2.5-4 h), the total yield is approximately equal to the sum of the yields of individual fractions, as expected. The limited data beyond the 4-h interval between fractions in short-term fractionation studies suggest that there is a further drop in the frequency (7-h interval) followed by a tendency for an increase (16-h) and then a decrease (24-h). Such a cyclical response may be interpretable on the assumption that with the different fractionation intervals, the partially synchronized cells receiving the second fraction may be in sensitive or resistant phases of the cell cycle.

140. With a high total dose such as 1000 rad (which is on the descending part of the humped dose-response curve), administered in two equal fractions and with time intervals ranging from 30 min to 72 h, again, there is a cyclical pattern of response; but this is a different one. With a 30-min interval the yield is close to that of

the total single dose of 1000 rad. With increasing intervals, the frequency increases up to a maximum with an 18-h interval when it is equal to the yield expected on the basis of additivity. The yield subsequently decreases and after 72 h, falls back to the level expected from a single 500-rad exposure. With extended intervals of up to 4 weeks, the increase is only gradual, and by 6 weeks, additivity is once again regained.

141. With unequal fractions (500 R + 300 R and in that order), additivity of yields is first observed with a 24-h interval (intervals shorter than this were not used) followed by a drop and a slow rise in the yield (with intervals ranging from 3 to 15 days), and by the end of the third week, additivity is once again regained. These results are in general agreement with those described in the preceding paragraph.

142. The interpretation of these results (paras. 140-141) might be that (a) the spermatogonial stem cells have different sensitivities in the different stages of the cell cycle; (b) the 500-rad fraction kills the most sensitive cells, leaving a partially synchronized population which passes to a cell cycle stage in which the cells are sensitive (18 h); (c) the subsequent decline in yield indicates a progression into a more resistant stage; and (d) by about 6 weeks after the first fraction of the dose, the cells have become re-assorted to a normal ("asynchronous") population.

143. In experiments involving a total irradiation exposure of 2800 R administered in seven equal fractions of 400 R and separated by 8-week intervals, additivity of yields is expected and in fact has been found.

144. With unequal fractions separated by 24-h intervals (100 R + 500 R, 500 R + 100 R, 500 R + 300 R, 100 R + 900 R, and 900 R + 100 R), the recovered translocations yields are dependent on the size and sequence of the exposure fractions. With a small fraction preceding the large one, the yield is high and is equal to that extrapolated from lower exposures. With the reversed sequence of exposures, the yield is low, but higher than that after a single acute exposure. These observations are interpreted on the assumption that 24 h after the first fraction, the surviving stem cells are in a more sensitive stage than before, both to killing and to translocation induction and that they are less heterogeneous in their radiosensitivities at the time they receive the second fraction (the degree of synchrony being dependent on the magnitude of the first fraction).

145. When total dose as large as 300 rad is split into several small fractions (30 of 10 rad or 60 of 5 rad) and administered over daily intervals, the frequency of translocations recovered is much lower, being only about one quarter of that after the single dose. This finding has been explained on the assumption that the spermatogonial population becomes progressively radioresistant under conditions of repeated irradiation. The possibility was tested in experiments in which a 300-rad gamma-ray dose followed an exposure régime involving 30 daily doses of 10 rad each and vice versa. The yield under the first set of conditions was lower than under those of the second.

146. Pre-irradiation injection with chemicals such as Adeturon or a mixture of reduced glutathione, AET, MEA, cysteine and serotonin creatinine sulphate has a protective effect with regard to the induction of translocations in spermatogonia. However, cysteamine has no effect.

147. A comparison of the frequencies of cytologically observed reciprocal translocations and genetically recovered ones (following spermatogonial irradiation) has shown that at exposure levels of 300 R and above, the ratio of translocations recovered in the genetic tests to cytologically determined ones is about 1 to 8; at a lower exposure of 150 R, this ratio is 1 to 4, though this result is not significantly different from 1 to 8. In another study involving a total exposure of 900 R delivered in three fractions separated by 4-week intervals and cytological examination of the parental males and their F_1 sons (thus no semi-sterility tests), the ratio of the frequency of translocation heterozygotes in the F_1 males to the frequency of translocations in the parental males was 1 to 4, but again, the result was not significantly different from 1 to 8.

7. Other male germ-cell stages

148. Searle *et al.* (520) have reported on the kinetics of dose response for the induction of reciprocal translocations in mouse spermatozoa. In these experiments, adult male mice were given gonadal doses of 0-1200 rad acute x-irradiation (nine levels) and mated the same day. The 531 sons conceived within a week after irradiation were tested for fertility and their testes examined cytologically for chromosome aberrations in spermatocytes. A total of 55 of the 57 diagnosed as semi-sterile and 35 out of 40 of those diagnosed as sterile were judged to be heterozygous for one or more reciprocal translocations. There was a close agreement between fertility diagnosis and the presence or absence of reciprocal translocations. Numbers of 0, 1, 2, . . . translocations per mouse showed a good fit to a Poisson distribution, in contrast to previous finding with spermatogonial irradiation. Again, in contrast to findings after spermatogonial irradiation, the translocation frequency after spermatozoal irradiation steadily rose with dose and showed no decline at higher levels.

149. The equation of the straight line that fit the data best was

$$Y = (4.78 \pm 0.43)\ 10^{-4} D$$

where Y is the yield and D the dose. Although the fit to linearity was good ($P = 0.57$), it was noted that deviations of observed values from expectations were all negative at low doses (50-400 rad) but positive at the higher doses of 1000 and 1200 rad; this suggested that the true relationship was curvilinear. The equation of best fit to a quadratic model was

$$Y = (2.25 \pm 1.03)\ 10^{-4} D + (3.09 \pm 1.26)\ 10^{-7} D^2 \quad (P = 0.84)$$

and to a power law model was

$$Y = (3.03 \pm 3.98)\ 10^{-5} D^{(1.41 \pm 0.20)} \quad (P = 0.87)$$

150. Data on the types and relative frequencies of aberrations induced in spermatocytes by different x-ray exposures (50-400 R) have been reported by Wennström (605). They show that when testes preparations were made 1 day after irradiation of the mice (to sample spermatocytes treated at the diplotene stage), the frequency of cells with multivalents increased non-linearly ($Y = 0.2805\ 10^{-2} + 0.746\ 10^{-6}\ D^2$); this was also true of chromatid breaks and fragments. In one experiment in which the time interval between irradiation and killing of the mice was varied from 2.5 h to 7 days, maximum yields of all aberrations were obtained with the 1-day interval (3.3 per cent multivalents, 3.7 per cent chromatid breaks and 11 per cent fragments) followed by a decline thereafter. The decrease for multivalents at the 3- and 7-day intervals (sampling of spermatocytes irradiated at early and late pachytene stages, respectively) was not significant.

151. In a similar study, Tsuchida and Uchida (579) found that in preparations made 1 day after irradiation with 300 R of gamma rays, the frequency of multivalents was 6 per cent; those of chromatid breaks and fragments were respectively 6.4 and 10.5 per cent. Five days after irradiation (sampling of mid-pachytene stages) the frequency of multivalents was nearly the same (7 per cent) whereas those of chromatid breaks and fragments increased (15 and 10 per cent). The overall frequency of abnormal cells was 20.7 per cent with the 1-day interval and 32 per cent with the 5-day interval.

152. Although most of the studies on translocation induction in male mice had been concerned with the irradiation of adult males, some studies have been carried out using males of other ages. Fazylov and Pomerantzeva (164) reported that the sensitivity of the germ cells of new-born male mice was only one third of those in the adult (as judged by cytogenetic analysis of spermatocytes) and that the exposure frequency relationship was linear in the range 20-400 R. Ivanov and Léonard (245) irradiated male mice soon after birth or when 90 or 450 days old with x-ray exposures of 100, 200 or 300 R (100 R/min). One hundred days after the exposure, the animals were killed and meitoic preparations of the testes were made. The results that Ivanov and Léonard obtained however, were at variance with those of Fazylov and Pomerantzeva: there were no differences in sensitivity between gonocytes and spermatogonia at any of the exposure levels. Data similar to those of Ivanov and Léonard have also been collected by Brewen and Preston (53) (50-300 R; five levels); again there were no significant differences in sensitivity between new-born and adult male mice.

153. Another finding of Fazylov and Pomerantzeva (164) relates to their work on irradiation of 16-day-old foetal males (*in utero* irradiation; translocations scored later in spermatocytes of adults). At the 20-R level, the translocation frequencies were higher in foetal than in adult males; at exposure levels of 50 R or more, the reverse was true. The authors concluded that the reason for the lower mutation frequency in foetal males was that there was a positive correlation between mutational response and radiosensitivity to cell killing and that at exposures of 50 R and above, only gonocytes with a lower mutational response survived. They did not, however, take full account of an important feature of their data, namely, the likely occurrence of clusters. Clusters of translocations in a few males could have explained the higher frequency of translocations they found following the 20-R exposure, thereby invalidating their statistical analysis and their conclusion (526).

154. In similar work but with irradiation of 13.5-day-old male foetuses (this stage was chosen because at day 13.5 of gestation, the sex cords are just developing) at x-ray exposure levels of 100, 200 and 300 R, Ivanov *et al.* (246) failed to obtain any evidence for translocation induction. Tsuchida and Uchida (578) also reported essentially similar results after 150 R of gamma irradiation to 12-day-old male foetuses (*in utero* irradiation). The aberrations scored included multivalents, chromatid breaks and fragments. The data obtained by Brewen and Preston (53), however, are at variance with the above in showing that translocations can be induced in the dividing gonocytes present in the 13.5-day-old foetus. The frequencies were: 0.24 per cent (50 R), 1.4 per cent (100 R), 0.90 per cent (150 R), 2.8 per cent (200 R) and 3.4 per cent (300 R). These results demonstrate that the gonocytes in the 13.5-day-old foetus are about one half as sensitive as those of adult spermatogonia, at least at exposure levels of 150-300 R.

8. Female mice

155. While a number of studies have been carried out in recent years on the induction by radiation of translocations in male germ cells of the mouse, our knowledge of similar effects in female germ cells has remained meagre. The reasons for this contrast have been more connected with technical difficulties than with any expectation that the level of translocation induction would be negligible. In the 1972 report, mention was made of the early work of L. B. Russell and Wickham (463) and of the preliminary results of Searle and Beechey in Harwell. In the former experiments (carried out before the technical advances which made cytological examination possible), after maternal x-ray exposure to 400 R, 1 out of 320 F_1 male progeny was judged to be a semi-sterile offspring and thus presumably heterozygous for a reciprocal translocation. However, a few others were sterile and so could have carried translocations.

156. Searle and Beechey (513) have now completed their experiments, and in addition, two papers by Gilliavod and Léonard (192, 193), one by Tsuchida and Uchida (579) and another by Brewen, Payne and Preston (58) have appeared in the literature; besides, some preliminary results of Krishna and Generoso (267a) have also become available. In the first experiments of Gilliavod and Léonard (192), female mice were irradiated with 50 or 200 R of x rays (whole-body, high dose rate) and caged individually with unirradiated males for one year. The F_1 male progeny were killed when mature and meiotic preparations of the testes were made. In parallel experiments, female mice irradiated at similar x-ray exposure levels were killed 24 h after irradiation and meiotic chromosome preparations of the oocytes were made. Although such preparations of the oocytes themselves showed the presence of translocation configurations in some first meiotic metaphases (2 out

of 50 in the 50 R series and 0 out of 50 in the 200 R series), no translocations could be diagnosed in the spermatocyte preparations of the F_1 males examined (101 sons in the 50 R series and 78 sons in the 200 R series).

157. In their subsequent paper, Gilliavod and Léonard reported on cytological observations on control and irradiated oocytes at exposure levels of 25, 50, 100 and 200 R. The frequencies of translocation configurations observed (R-IV and/or CH-IV) were (per cent): 0 (0/101, 0 R); 3.2 (2/63, 25 R); 3.7 (4/108, 50 R); 1.0 (1/100, 100 R); and 0 (0/85, 200 R). At the 100-R level, there were 6 oocytes bearing chromosome fragments. The authors attribute the finding that the frequency of oocytes carrying translocation configurations declines already at 100 R (in contrast to the observations of Searle and Beechey (513) to be described later) to the relatively small numbers of dividing oocytes that could be examined, although one cannot rule out sensitivity differences in the strains used by the two groups of investigators (C57BL in the experiments of Gilliavod and Léonard and F_1 females from C3H/HeH X 101/H in the work of Searle and Beechey).

158. In the experiments of Tsuchida and Uchida (579), a comparison was made between the radiosensitivity of the dictyate oocytes and of spermatocytes in diplotene and mid-pachytene. Gamma-irradiated (300 R) female mice (2-3 months old) were killed 1 and 5 days after irradiation and the oocytes collected cultured *in vitro;* they were then processed for the scoring of aberrations at metaphase I. (No hormonal treatments were administered to stimulate oocyte maturation or superovulation.) The results showed that there were no significant differences in the frequencies of abnormal cells at the two time intervals (18.9 per cent compared with 21.6 per cent; number of cells analysed, 366 and 352). At the first interval, the frequencies of chromatid interchanges, fragments and chromatid breaks were respectively 4.4, 7.4, and 10.7 per cent. At the second interval, the comparable frequencies were 6.3, 9.1, and 8.8 per cent. Thus, in terms of both the overall frequencies of abnormal cells and of the kinds of aberrations, the dictyate oocytes were found to be somewhat less sensitive than mid-pachytene spermatocytes (see paragraph 151).

159. Brewen, Payne and Preston (58) irradiated female mice 2-3 months old with x rays and used hormonal treatments to induce superovulation. Attention was focused on (a) correlating the yield of aberrations with the time interval between irradiation and ovulation, (b) studying the relationship between exposure and the yield of aberrations at the time interval in which highest frequencies of aberrations were observed, and (c) estimating the frequency of translocation heterozygotes in the F_1 progeny of irradiated females from cytogenetic observations on irradiated oocytes. Data on the first problem are given in table 24. It can be seen that the frequency of interchanges is low at the 1-, 3- and 5-day intervals, increases 6-fold at the 7-day interval and finally reaches a peak at the 14-day interval followed by a decline thereafter. The observation that very few interchanges are recovered with 1-, 3- and 5-day intervals is in accordance with the results of Gilliavod and Léonard (192, 193; 1-day interval). The variation in yield of aberrations as a function of time between irradiation and ovulation agrees well with the variation in sensitivity to dominant lethal induction over the same time period (458, 513).

160. Results that bear on the exposure-frequency relationship obtained in the above study are given in table 25. Only the 14-day interval was used. It can be seen that the frequency of chromatid interchanges increases faster than linearly with the exposure, indicating a significant two-track component. An examination of whether or not the distribution of cells with various numbers of interchanges fit a Poisson distribution revealed that the fit was good at all the exposures except at 300 R where there was an excess of cells with two interchanges and a deficit of those with one interchange. The authors attribute this deviation to the low numbers of cells scored.

161. At the 300-R level, 50 metaphases were stained for C-bands and analysed. Of the 11 interchanges found, 5 had two centromeres on one chromatid plus an acentric fragment and were classified as asymmetrical interchanges. The other 6 were classified as symmetrical interchanges since each chromatid had a single centromere. Thus, these very limited preliminary data indicate that the two interchange events are recovered at the 14-day interval.

162. The 400-R data were used to estimate the expected proportion of oocytes carrying a balanced translocation (that will be transmitted) as well as the amount of dominant lethality that will be generated. The assumptions used were the following: (a) symmetrical and asymmetrical interchanges occur at about the same frequencies; (b) there is no preferential segregation of exchange or non-exchange chromatids into polar nuclei; (c) the inclusion of any deleted chromosome, dicentric chromosome, or duplication deficiency in the mature ova will result in dominant lethality. Of the 100 metaphase-I oocytes analysed, 36 had no visible aberrations; 18 had a single isochromatid break or, rarely, a chromatid deletion; 19 had a single interchange; 6 had a single interchange plus a single deletion; and 8 had two interchanges. The remaining 13 cells contained three or more aberrations (exchanges and/or deletions) and were not considered in estimating the frequency of transmissible translocations; however they were included for calculating dominant lethality.

163. The estimated frequency of transmissible balanced translocations was 1.8 per cent; if the same calculations were made for the 300-R data, the predicted recovery rate was 0.6 per cent, in very good agreement with the observed rate recorded in the genetic tests of Searle and Beechey (513): 4 out of 680 progeny of irradiated females (0.6 per cent) given 300 rad 1-42 days prior to conception (para. 166). The predicted dominant lethality at the 400-R level was about 43 per cent, consistent with the figure of 35 per cent post-implantation mortality (although somewhat lower than that of 59 per cent total dominant lethality) recorded in the experiments of Searle and Beechey (table 17) after 400 R given to females 21 days before conception.

164. The experiments of Searle and Beechey (513) were designed to study the induction of reciprocal translocations in oocytes. In one set of experiments, mature

females were irradiated with 300 rad of x rays and mated after irradiation. Up to two litters were produced before sterility ensued. The sons were tested for fertility by mating to outbred females, and the method of Carter *et al.* (84) was used for fertility diagnosis. The daughters were crossed to males and those giving small litters (less than 9 in the first, less than 12 in the first two) were allowed to continue breeding, further tests for fertility being performed in their offspring. The production of 12 or less new-born in three litters was considered as presumptive evidence for semi-sterility. The diagnosis of translocation heterozygosity (or XO condition) in these tests was cross-checked by making appropriate chromosome preparations. As in the experiments of Gilliavod and Léonard (193), meiotic preparations of the oocytes of irradiated females (100 and 400 rad) were also made and screened for exchange configurations, fragments etc.

165. The second set of experiments involved exposure to 0.7-MeV fission neutrons (100 and 200 rad; 49-55 rad/min). Mature females were irradiated and both sons and daughters were tested for fertility as before. In addition, sons and daughters of females exposed *in utero* to fission-neutron irradiation in an earlier study of Searle and Phillips (514) (108.5 rad with 20.5 R gamma contamination: exposure period of about one week to pregnant females between days 0.5 and 11, post-coitum; oogonial irradiation) were also tested for fertility by the same methods as described for the x-ray experiments.

166. The results showed that 0 out of 386 sons of females given 300 rad of x rays showed evidence of semi-sterility or translocation heterozygosity, but 9 out of 294 daughters were diagnosed as semi-sterile because of the small size of their litters. Further tests led to good evidence for translocation in 3. In the others, the semi-sterility was not proved heritable. At least one of these probably carried a translocation but the induction of XO females or other causes seemed more likely to be responsible for the low litter size in the rest. The frequency of translocations that can be estimated from the total data (sons and daughters combined) is 4 out of 680, or 0.6 per cent, at 300 rad. Correcting for the spontaneous frequency (3/1443 when contribution by both males and female gametes is considered or 1.5/1443 for females; see Lüning and Searle (302)) and assuming linearity, this would correspond to a rate of $0.16\ 10^{-4}$ rad^{-1} per gamete. This rate is roughly one half of that estimated in the male for spermatogonial irradiation.

167. Examination of oocytes at metaphase I during the first and third week after irradiation with 100 and 400 rad of x rays revealed both multivalents (some of the ring quadrivalent type) and fragments. The aberration frequency in oocytes rose with dose and, at the 400-rad level, with time after irradiation, reaching a maximum of 10 per cent multivalents and 22 per cent fragments in the third week. As was mentioned earlier under "dominant lethals in female mice" the increase in levels of chromosomal damage with dose and time after irradiation was reflected in dominant lethals after the same radiation-conception intervals and doses.

168. In the neutron experiments (513), no evidence for the induction of translocations either in maturing oocytes or in oogonia was obtained, although there were indications for X-chromosome loss. However, sample sizes were small.

169. In the work of Krishna and Generoso (267a), a total of 288 female mice 11-17 weeks old received 300 R of x irradiation and were divided into four groups. Females from the first group were caged with males immediately after irradiation, while females from the other groups were caged with males on the 5th, 11th and 15th day after irradiation. They were kept together until the females ceased to produce young. With the radiation exposure used, 24 females from the first group and 12 females from the second (caged on the 5th day post-irradiation) produced two litters, while females in the other two groups produced only one litter.

170. Both male and female progeny are being tested for translocation heterozygosity. The currently available results from tests of 800 male progeny show that 4 (0.5 per cent) were partially sterile and 2 (0.25 per cent), completely sterile. The four partially sterile males were cytologically confirmed as translocation heterozygotes, and tests on the sterile males are not yet complete. Even if comparisons are restricted to partially sterile males only, these results indicate a significant induction of heritable translocations in dictyate oocytes that were recovered in male progeny (4 in 800 compared with 1 in 4392 in controls). These results may be compared with those of Searle and Beechey (513 and para. 166), who failed to find any translocation heterozygotes in 386 sons of female mice given 300 R. However, there is no statistically significant difference between the two sets of data ($P = 0.2$ by Fisher's exact test).

9. Other species

171. In the 1972 report, the data of Lyon and Smith (313) on translocation induction in pre- and post-meiotic germ-cell stages of golden hamster, guinea-pig and rabbit were presented and compared with those obtained in the mouse. The results showed that (a) translocations are induced in the spermatogonia in all the experimental species although the dose-effect relationship differs from that in mice; (b) in both rabbits and guinea-pigs, the overall dose-response curves are humped, as in mice, but the peak incidence occurs around doses of 200-300 rad (compared with 600-800 rad in mice); (c) the pattern of translocation induction in the post-meiotic male germ cells of the golden hamster (after 200 rad x irradiation) resembles that of the mouse showing (in the limited data then available) that spermatids are more sensitive than spermatozoa.

172. More data have now become available not only for the three species mentioned in the preceding paragraph, but also for the Chinese hamster and the marmoset. In addition, the available cytogenetic data derived from studies on translocation induction in human spermatogonia have also been published.

(a) *Post-meiotic cells*

173. Cox and Lyon (122) irradiated adult male golden hamsters with 200 or 600 rad of x-irradiation and mated them to females for four successive weeks; similarly,

adult guinea pigs and rabbits were exposed to, respectively 600 rad and 100 to 600 rad of x rays and likewise mated to females. The male offspring collected from these matings were cytogenetically screened for the presence of translocations; in the case of the golden hamsters irradiated with 600 rad, the F_1 female progeny were also kept and tested for the presence of translocations by examination of their male progeny.

174. After a dose of 200 rad to golden hamsters, the yield of translocations increased from week 1 to week 3 and fell in week 4 (1.5, 2.0, 6.4 and 3.9 per cent; the number of animals tested were, respectively 66, 50, 47 and 51). After 600 rad, there appeared to be a similar pattern but with higher yields, but the number of offspring obtained from matings in weeks 3 and 4 was very low. The pattern was roughly the same in tests of female F_1 progeny. In the guinea-pig too, there was a similar pattern of translocation induction after 600 rad, but again the number of animals tested was too small to make accurate estimates. In the rabbit, although translocations were found, too few offspring were tested, and precise estimates of the pattern of sensitivity change are not possible. Averaging the data over all weeks and doses, Cox and Lyon established a tentative rank order of sensitivity of the post-meiotic cells of the different species, taking into account the earlier mouse data. This is as follows: mouse > rabbit > guinea-pig > hamster.

175. Comparing these translocation data with those on dominant lethality collected in the same experiment (by the litter-size reduction criterion), the authors found that (a) the yields of both translocations and dominant lethals induced in spermatozoa were higher in the mouse than in guinea-pig; (b) in the golden hamster, the litter-size reduction was as high as in the mouse, but the translocation yield appeared lower. These comparisons suggest, as was mentioned in the 1972 report, that at present there are no sure grounds for extrapolating from one stage or type of genetic damage to another and from one species to another (see paragraphs 23 and 105, Annex E, 1972 report (589)).

(b) *Spermatogonia*

176. Our knowledge on the sensitivity of spermatogonia of mammals other than the mouse to the radiation induction of reciprocal translocations has considerably expanded since the publication of the 1972 report, in which the available data on guinea-pigs, rabbits and golden hamsters were reviewed. Additional information for those three species and new data for the rat, Chinese hamster, rhesus monkey, marmoset and man have now become available.

177. In work which is essentially an extension of that reported in an earlier paper (313), Lyon and Cox (309) investigated the response of the spermatogonia of the guinea-pig, rabbit and the golden hamster to the induction of reciprocal translocations. Adult male golden hamsters, guinea-pigs and rabbits aged 3, 6 and 9 months respectively, were given single acute x-ray exposures in the dose range 100-600 rad (88 rad/min; part-body exposures). After the irradiation, the animals were left for varying periods of time (3-7 months for the golden hamster, and 3-9 months for the guinea-pig and rabbit) to allow for recovery of spermatogenesis, after which cytological preparations were made of the testes of these animals.

178. The data that bear on dose-effect relationships are summarized in table 26 along with those of Brewen and Preston (54) for the guinea-pig and Chinese hamster. (In the latter case, young male guinea-pigs were whole-body irradiated and Chinese hamsters were given part-body irradiation; no details of when the testes preparations were made are given.) It can be noted that, in the experiments of Lyon and Cox (309) the frequency of translocations in guinea-pigs and rabbits increased with dose up to 300 rad and then declined sharply giving an overall humped curve, as in the mouse. (A similar increase is observed in the data of Brewen and Preston for the guinea-pig and the Chinese hamster, but the data at higher doses are not yet available.) The kinetics of increase in the dose range up to 300 rad is consistent with linearity. In the golden hamster on the other hand, although the mean translocation frequencies at all dose levels are higher than in the controls, they are not significantly different from each other, presumably due to a severe distortion of the primary dose-response curve. The net result is a flat-topped curve without any distinct peak. It may be noticed that in this species, the yields of translocations at 500 and 600 rad are higher than those in rabbit and guinea-pig.

179. Using the model $y = a + bD$, the authors estimated the values of the slopes (the b values)[12] up to 300 rad in the rabbit and guinea-pig and up to 200 rad in the golden hamster and found that these were significantly higher in rabbits than in the other two species (definite translocations: rabbit, $(1.48 \pm 0.13)\ 10^{-4}\ \text{rad}^{-1}$ compared with $(0.94 \pm 0.07)\ 10^{-4}\ \text{rad}^{-1}$ in guinea-pigs and $(0.93 \pm 0.09)\ 10^{-4}\ \text{rad}^{-1}$ in the golden hamsters; definite plus possible translocations: rabbit, $(1.70 \pm 0.14)\ 10^{-4}\ \text{rad}^{-1}$ compared with $(1.10 \pm 0.10)\ 10^{-4}\ \text{rad}^{-1}$ in guinea-pigs and golden hamsters).

180. Lyon and Cox (309) compared their data with those obtained in the mouse by other authors (163, 280, 353), who also found that the dose-effect relationship up to doses below the peak of the humped curve was consistent with linearity; that showed that irrespective of whether the data were expressed in terms of translocations per cell or proportion of cells with translocations, the slopes of the mouse lines were higher, except for the comparison of rabbit data with the mouse data of Léonard and Deknudt (280). In the latter situation, the slopes (rabbit, mouse) were not significantly different. On the basis of all these, it can be concluded that in the dose range up to 300 rad, the rank order of sensitivity is golden hamster $\cong$ guinea-pig $<$ rabbit $\leqslant$ mouse.[13]

[12] The slopes estimated are for translocations per cell; "possible translocations" refers to cells containing a configuration which resembles a multivalent, in size and shape but in which the individual chromosomes are not distinct; "definite translocations" are those in which the identification is unequivocal.

[13] The authors' conclusion: golden hamster < guinea-pig < rabbit < mouse.

181. The results of Brewen and Preston (54) for the guinea-pig (table 26) show that at comparable exposures, their frequencies are higher than those of Lyon and Cox (309); the frequencies in Chinese hamsters are roughly similar to those recorded for the mouse by Preston and Brewen (419).

182. Gilliavod and Léonard (191) compared the induction of translocations in mouse and rat spermatogonia after an x-ray exposure of 300 R and found similar yields in both species. Lyon *et al* (314) and van Buul (70) have recently published some data on the induction of translocations in the spermatogonia of the rhesus monkey *(Macaca mulatta)*. The experiments of Lyon *et al.* were carried out on two colonies, one maintained in Birmingham, England (United Kingdom) and the other in Rijswijk (the Netherlands), whereas those of van Buul were on the latter colony only. The animals were irradiated (testicular exposures) unilaterally or bilaterally with x-ray doses of 100, 200 and 300 rad (about 63 rad/min for the English monkeys and 30 rad/min for the Dutch monkeys). The recovery of the testis from radiation injury was monitored by volume measurements and biopsies at different intervals after irradiation. The data are presented in table 27.

183. It can be seen that (a) there are between-colony, between-monkey and between-testis variations in the response to the induction of translocations; (b) when the pooled data of Lyon *et al.* as well as those of van Buul are considered, the dose-effect relationship suggests a peak at about 200 rad, but the differences in frequencies between the different doses are not statistically significant; and (c) the frequencies of translocations are clearly lower than those observed in the mouse (see table 21 for the latter). The authors noted that monkeys of both colonies showed seasonal variation in spermatogenesis and in addition, some monkeys of the Birmingham colony were not in breeding condition. The effect of this seasonal variation on the yield of translocations is not known but in any case could explain some of the variation in response observed between the different monkeys.

184. Brewen *et al.* (57) have now published their complete results on translocation induction in the spermatogonia of the marmoset and man. Mature marmosets were exposed to testicular irradiation with acute doses of 25, 50, 100, 200 and 300 rad of x rays delivered at high dose rates. At various intervals after irradiation (depending on the dose), bilateral castration was performed and preparations were made by the method of Evans, Breckon and Ford (162) with some modifications. Biopsy material was obtained from nine human volunteers who had received testicular irradiation with x rays at doses of 78, 200 or 600 rad (225). As in the marmoset, the interval between irradiation and sampling varied, depending on the dose. Cytological preparations were made using the same methods as those employed for the marmoset.

185. The results are given in table 28, where mouse data are also presented for comparisons. It can be seen that in the marmoset, peak yields are obtained at about 100 rad and the human data do not exclude such a possibility. Under the assumption that the sensitivity of the spermatogonia of both species is approximately the same and that the yield increases with dose up to 100 rad (and using both sets of data at and below 100 rad), the authors estimated that the average rate is $7.7\ 10^{-4}$ rad^{-1} per cell. A weighted regression analysis performed on the human and marmoset data (the 78-rad point for man and the 25-, 50- and 100-rad points for the marmoset, all considered together), assuming that the regression passes through the origin at zero dose, gives a good fit ($P = 0.2$) to the equation $Y = (6.94 \pm 0.92)\ 10^{-4}\ D$; however, the data also fit ($P = 0.1$) the model $Y = (7.64 \pm 3.94)\ 10^{-4}\ D - (0.0089 \pm 0.05)\ 10^{-4}\ D^2$, the negative quadratic component not being significantly different from zero ($P = 0.9$) (495). For this reason, the estimate of risks is based on the linear model.

186. It should be pointed out that the primate data, taken as a whole, show considerable heterogeneity (tables 27 and 28). Translocation frequencies obtained in the Rhesus monkey are much lower than those at comparable doses in man and marmoset, while there are discrepancies at the 100-rad level between the two sets of results on the Rhesus monkey. Yet in all these experiments, peak yields of translocations are found at lower dose levels (100-200 rad) than in the mouse. It is impossible to tell at present whether the marked differences in yields between the man and the Rhesus monkey (which are more closely related than man and the marmoset) are due to fundamental differences in radiosensitivity (akin to those already established within rodents) or just artefactual, in view of the limited amount of material and possible differences in techniques or irradiation and scoring (314).

187. Under these circumstances, it has been decided to use data from man and the marmoset (which are in close agreement) for the purpose of risk estimation. It should be stressed, however, that the amount of direct information on man is still very limited (table 28), so that any estimate derived from it must be regarded as only approximate. It should also be noted that this information would suggest that human germ cells are about three times as sensitive as those of the mouse with respect to translocation induction in spermatogonia, while the latest information (41) suggests approximately equal sensitivity with respect to the induction of dicentrics in lymphocytes in these two species.

(c) *Fractionation effects*

188. Experiments similar to those carried out in the mouse to study the effects of dose fractionation on the response of spermatogonia to the induction of reciprocal translocations have now been conducted in two other mammalian species, the golden hamster and the guinea-pig (308). The fractionation régimes chosen were similar to those already investigated in detail in the mouse. Male guinea-pigs were irradiated with a total dose of 400 or 600 rad of x rays given in 2 equal fractions separated by either 24 h or 8 weeks; in another series, a total dose of 600 rad was administered in 12 fractions of 50 rad at weekly intervals. In parallel experiments, golden hamsters received 400 rad divided into 2 equal fractions separated by either 24 h or eight weeks. In addition, total doses of 400 or 600 rad were delivered in

8 (or 12) 50-rad fractions delivered a week apart. The time interval between irradiation and cytological examination depended on the species and the fractionation procedures employed, but were similar to those used for single doses (309).

189. The results obtained are given in table 29 along with data collected at pertinent single exposures to facilitate comparisons. Considering first the data on 24-h fractionation, it can be seen that in both species the yields are higher than after single doses of comparable size. Statistically, the yields after fractionated exposures did not deviate significantly from those predicted by extrapolating the curves for single exposures (up to 200 rad in hamster and up to 300 rad in guinea-pigs) to higher doses. The estimated slopes for both the guinea-pig and golden hamster are the same, $(0.93 \pm 0.07)\ 10^{-4}\ \text{rad}^{-1}$. These results show that the situation after fractionation is the same as after unfractionated exposures. (The authors, however, considered that the guinea-pig was more sensitive than the golden hamster after single exposures up to 300 rad, and therefore the situation after fractionation was construed as being different.)

190. Turning now to the data obtained with fractionation at 8-week intervals, it can be seen that the yields are again higher than after unfractionated total doses of comparable size; however, at both the dose levels, 400 and 600 rad, the yields are lower than those obtained after 24-h fractionation, i.e., the yields are lower than those expected from additivity of the effects of the dose fractions. These differences (24 h compared with 8 weeks) are significant only for the guinea-pig at the 600-rad level. It may also be noticed that in the guinea-pig, the yield after 2 X 300 rad (8-week interval) is nearly the same after 2 X 200 rad (8-week interval), which in turn is similar to that in the golden hamster under the same conditions.

191. The finding in the mouse (420) that successive 400-R x-ray fractions to mouse spermatogonia (up to a total of 2800 R, separated by 8-week intervals) resulted in yields consistent with the additivity of effects of individual fractions favour the view that the 8-week interval is long enough for the spermatogonial cells to return to normalcy. In contrast, in the guinea-pig and the golden hamster, the translocation data as well as those on the lengths of the sterile periods observed in the 8-week fractionation series support the notion that such normalcy is not restored in these species within this interval of time.

192. In both the guinea-pig and the golden hamster, the results of multiple small fractions show that the translocation yields are much lower than those expected by extrapolation of the dose-response line for single exposures (up to 300 rad) to higher doses. In comparing these results with those obtained in the mouse with similar fractionation régimes, it should be borne in mind that although the total doses are the same (600 rad), in the case of the mouse, this dose point is still on the ascending part of the dose-response curve, whereas in the other two species, it is beyond the dose of peak yield. In the mouse, the yield after fractionation (12 X 50 rad) is roughly half that obtained after a single unfractionated dose of 600 rad (316); in the golden hamster, the yield after 12 X 50 rad is roughly twice that after the single dose of 600 rad; and in the guinea pig, after similar fractionation (600 rad) the yield is about six to eight times higher than after the unfractionated dose. Thus it appears that the response after fractionated small doses is dependent on whether or not the total dose administered is on the ascending or the descending part of the curve. Further data for the mouse using total doses greater than that which gives the maximum yield for single exposures administered in small fractions as above are required to confirm this suggestion.

10. Types of translocation and their effects on fertility and viability

(a) *The mouse*

193. From earlier work reviewed in the 1972 report it is known that (a) certain autosomal translocations recovered from treated post-meiotic male germ-cell stages may be fully viable in the heterozygous state and yet lead to male sterility through failure of spermatogenesis; (b) such failure may not be specific to a particular stage or cell type but occurs with variable incidence throughout the meiotic process and possibly at earlier steps in the germ-cell sequence and (c) all the known X-autosome translocations also cause sterility with failure of spermatogenesis and small testes.

194. Lyon and Meredith (310) found that in translocation lines where males were sterile, virtually all of the quadrivalents were chains rather than rings, in contrast to the "semi-sterile" lines in which almost two thirds were rings. The same tendency can be found in the data of Léonard and Deknudt (281) on translocation induction by post-meiotic irradiation. Searle (505), who analysed the data of Léonard and Deknudt (281) for single translocations, found that in those males with few or no spermatozoa, the frequencies of chain configurations (quadrivalent or trivalent) were almost twice that in males which had no obvious shortage of spermatozoa. Furthermore, there was a positive correlation between the severity of the sterility effect and the frequency of chain quadrivalents, the latter being 99 per cent when the sperm count was nil (507). In the F_1 males that were derived from post-meiotic germ-cell irradiation and which had enough sperm, the proportion of multivalent associations of the chain type (44.9 per cent) was decidedly higher than that found after spermatogonial irradiation (23.6 per cent), as calculated from previous results of Léonard and Deknudt (280). Thus there is evidence for a selective process which effects the frequency of chain quadrivalents and also for a qualitative difference between the types of translocation observed after post-meiotic and pre-meiotic irradiation.

195. Cacheiro *et al.* (73) made histological and cytological analyses of the testes of 42 sterile sons of males treated with ethyl methanesulphonate (EMS), butylated hydroxytoluene (BHT) plus EMS, or 200 R of x rays (treated germ cells: spermatozoa and spermatids). A very high percentage (36 out of 42) of these males carried chromosome aberrations and a majority of them carried translocations. While translocations are impli-

cated to explain partial sterility (which results in the death of the offspring of the carrier) and complete sterility (where the effects are on the carrier himself) the data of Cacheiro *et al.* (73) show that this difference is not due to single versus multiple translocations. The evidence indicates that the difference between translocations that cause sterility and those that cause partial sterility may be correlated with the position of the break points as revealed by cytological evidence: translocations that cause sterility in males appear to be those in which at least one of the breaks occurs close to one end of a chromosome (position effect). A similar suggestion, namely that breakage in or near centromeric heterochromatin is associated with male sterility, has been made by Searle (507).

196. These suggestions have now been confirmed, as shown by the recent results of Cacheiro *et al.* (74). In this work, 30 sterile F_1 sons of x-irradiated males were studied, 12 of which were derived from irradiated spermatids (158 F_1 males tested) and 18 from treated spermatogonia (4286 F_1 males tested). Cytological analyses were carried out in mitotic metaphases from dividing spermatogonia (orcein staining), kidney cultures (quinacrine and Giemsa banding techniques) and in meiotic cells at diakinesis wherever spermatogenesis proceeded to that point or beyond. Of the 12 sterile males derived from spermatid irradiation, 10 were found to carry reciprocal translocations as determined by banding (with 7 of these also revealed by conventional staining through the presence of a small marker chromosome): 2 of these had Y-autosome translocations and two others had two autosomal translocations each. Except where the Y was involved, at least one of the breaks in each male was close to the centromere (c) or telomere (t); in fact, all but two of these males had c/t-type translocations. Wherever diakinesis could be studied, it was found that only a small number of multivalent configurations were of the ring-IV type, in keeping with the breakpoint locations near chromosomal ends. Such c/t-type translocations often lead to long and short marker chromosomes and seem especially liable to give rise to viable tertiary trisomies which are usually sterile in the male (though not in the female) and may have specific abnormalities (310, 507).

197. In sterile sons derived from irradiated spermatogonia, however, only 4 out of 18 had translocations and, although at least one break was near a chromosomal end, none was of the c/t type. It thus appears that sterility here may be predominantly due to causes other than reciprocal translocations with near-end breaks; possibly small deficiencies or point mutations could play a role.

198. It is worth pointing out in this context that Jacobs *et al.* (248), who analysed the breakpoints of structural rearrangements in man (lymphocyte cultures, quinacrine and/or Giemsa techniques), noted that within chromosome arms there appeared to be an excess of breaks in the terminal regions, an excess of c/t translocations where ascertainment was through a balanced carrier, and a possible excess of terminal/median translocations where ascertainment was through an unbalanced carrier. The authors, however, were careful to point out that there might be observational biases. Similar observations showing an excess of breaks in the terminal region of chromosomes have been made by other human cytogeneticists (278, 580).

199. Although most of the translocations causing sterility in mice have been recovered from treatment of males, especially of post-meiotic stages, there is now evidence that such translocations could also be obtained from irradiation of oocyte stages of female mice (513).

(b) *Other species*

200. Among experimental mammals, most of the information thus far available on properties of reciprocal translocations has been collected from studies with mice. The work of Cox and Lyon (123) extends such information to guinea-pigs, golden hamsters and rabbits. These investigators made a cytological study of translocations induced by post-meiotic male germ-cell irradiation in the F_1 (and in some cases in the F_2) sons of irradiated males. It was found, as in the case of the mouse (see review by Léonard, 279) that the frequency of spermatocytes displaying multivalent configurations varied with the translocation, but the average percentage appeared to depend on the species, the latter being 57 per cent in the case of the hamster and 83 per cent in the case of the guinea-pig; too few rabbits were examined to make meaningful estimates of the kind or the average frequency of multivalents. Chain quadrivalents were more abundant than ring quadrivalents at meiosis for the guinea-pig and the hamster, in contrast to the mouse. An attempt was made to estimate the size of chromosomes involved in the various translocations from the size of the multivalent configurations in each of the animals. It appeared that in the hamsters, the longest, one of the shortest, and the X, as well as the long, medium and short chromosomes, had all been involved in translocations, although there was some suggestion that more exchanges tended to take place between the longer than the shorter chromosomes. This is expected if the longer chromosomes present a larger target for the radiation and if the effect of x rays is random. In the guinea-pig, the situation was roughly similar.

201. One possible explanation for the greater abundance of chain quadrivalents in hamster and guinea-pig translocation carriers would be that a greater proportion of breaks were near the ends of the chromosomes than in the mouse; if true, the cause could be in the greater number of chromosome arms (and hence chromosome ends) in these species than in the mouse or in the greater range of sizes of the chromosomes.

202. In all three species, as in the mouse, translocations were found which caused male sterility, due to partial or complete failure of spermatogenesis, although most translocations caused semi-sterility. In the hamster, chain quadrivalents were more abundant than ring quadrivalents of two of the sterile males, and in another sterile male only chain multivalents were obtained. However, translocations which lead only to chain multivalents are not necessarily associated with sterility here, since two other males for which all the quadrivalents were chains proved to be fertile. It is likely that sterility in the hamster is associated with the size of

the chromosomes involved, since the translocations in the four sterile males all involved the longer chromosomes, but more data are needed to substantiate this viewpoint.

203. In the guinea-pig, the situation was nearly the same: only chain quadrivalents were observed in the three sterile males; however three of the seven fertile males also carried only chain quadrivalents. Thus the presence of only chains in this species, as in the hamster, is not always associated with sterility. In contrast to the hamster, sterility here does not appear to be correlated with the size of the chromosomes involved in the translocation. In the rabbit, two of the four translocation carriers were sterile; one of the fertile males carried two translocations and the other, one. Both of them, as well as the two sterile males, carried translocations which involved the longer chromosomes. Hence, there is no evidence here of any association between size of chromosomes involved and sterility.

204. It is instructive to compare these results with those available in man. Only a very few human reciprocal translocations with known breakpoints have been studied at meiosis; however, in those which have been described, a preponderance of ring-IV configurations was found in three male translocation heterozygotes showing a normal spermiogram (98, 99, 100, 166) while chain-VI configurations were found in the majority of spermatocytes from a sterile translocation heterozygote (99). Unequal bivalents, indicative of telomeric breakpoints, occurred in one patient who was a t(Cp– Eq+) heterozygote, and he too was sterile through spermatogenic breakdown (99).

(c) *Embryonic mortality in the progeny of translocation heterozygotes*

205. If the chromosomes involved in a translocation between non-homologous chromosomes segregate randomly at anaphase-I of meiosis, four classes of gametes are produced in the proportion 1:1:2 of balanced normal, balanced-translocated and unbalanced. Thus, about 50 per cent of the zygotes derived from a mating of a balanced translocation heterozygote and normal animal will die. As the number of translocations per cell increase, the proportion of unbalanced gametes will also increase (see for instance table 1 in Ford *et al.*, 170). Mice heterozygous for a single translocation are therefore expected to be semi-sterile whereas those heterozygous for two or more translocations would be expected to show a much higher order of infertility, it not outright sterility, due to death of unbalanced embryos.

206. In their translocation experiments involving irradiated mouse spermatozoa, Searle *et al.* (520) compared the embryonic lethality in the progeny of males with or without detectable types of chromosomal aberrations (mainly translocations). As the data given in table 30 shows: (a) the mean number of live embryos per female in the cytologically abnormal category is less than one-half that in the normal category; (b) the mean number of implants is also markedly reduced by a clearly significant amount. That suggests that some pre-implantation loss is occurring, which may be in part due to reduced fertilization of eggs and in part due to early death of some very unbalanced zygotes. The embryonic survival (in terms of live embryos per female) in those with one reciprocal translocation is 42.4 per cent of the survival in those without detectable abnormality (3.08/7.3). Since a value of 50 per cent is expected with normal disjunction, the observed figure suggests an average level of adjacent-2 disjunction of around 15 per cent (519). However, this must be regarded as an upper limit, for the comparative embryonic survival in terms of the ratio live embryos/total implants is 50.1 ± 1.9 per cent (ratio 369/806 for translocation carriers divided by the ratio 6161/6743 for normal males).

207. Cox and Lyon (123) have presented similar data for the golden hamster and guinea-pig. They found that, in general, both the frequency and the time of embryonic death in the progeny were the same as in the mouse. In the hamster (table 31), although with some translocations, the number of implants per female was lower than in the controls; on the average, the difference was relatively small (10.3 compared with 11.1). However, there was a large increase in the number of small moles among the translocation series, suggesting that for all these males, the death of unbalanced zygotes was occurring predominantly in the early post-implantation period. Thus, the golden-hamster resembles the mouse in this respect (170, 245). Inspection of table 31 will also reveal that among females mated to some males (Nos. 3, 4 and 13) there is a slight reduction in the number of implants which could be due to some excess pre-implantation loss. In 6 of the 7 animals with one translocation, the frequency of post-implantation loss was close to 50 per cent which is as expected if chromosomal disjunction is normal and unbalanced zygotes die; in the single male with two translocations, the post-implantation death was 75 per cent, which again is as expected. The remaining male with one translocation (male No. 8) had a low proportion of embryonic deaths, 34.9 per cent, and the mean number of live embryos in females mated to him was 6.9, or 75 per cent of the control value.

208. In the guinea-pig, the data are limited, most of the results pertaining to 3 of the 7 males tested. In general, the number of implants per female was similar to that in controls, but there was a marked increase in small moles suggesting that here too, the death of unbalanced translocation products occurs mainly in the early post-implantation period. For male No. 6, post-implantation death was 50 per cent, but for Nos. 4 and 9, it was significantly less than 50 per cent. These latter results and similar ones obtained in the golden hamster can be explained on the assumption that one of the unbalanced translocation zygotic types is viable, although other interpretations cannot be excluded. In the mouse, at any rate, there is evidence for the survival of some unbalanced zygotes (89, 310, 334, 335).

11. Summary

209. After spermatozoal x irradiation in mice, the frequency of translocations increases with dose and does not show a decline at high dose levels; this finding is in

contrast to that after spermatogonial irradiation, where the frequency increases with dose up to about 700 rad followed by a decline thereafter.

210. There does not appear to be any significant differences in sensitivity to the induction of translocations between new-born and adult male mice. Contradictory data have been reported with regard to the induction of translocations in the germ cells present in male foetuses 12-13.5 days old, some showing that translocations can be induced and others showing that they are not. In those experiments in which positive evidence has been obtained, the data show that the germ cells in the foetuses are about one half as sensitive as adult spermatogonia.

211. Studies on the induction of translocations in female mice have gained momentum during the last few years. The development of techniques for culturing oocytes has facilitated this line of inquiry. Oocytes derived from irradiated females 14 days after irradiation show the highest response in terms of aberration recovery: the frequency of chromatid interchanges induced in oocytes increases faster than linearly with x-ray dose. The frequency of genetically recoverable translocations (after irradiation of females) predicted from the kinds and number of aberrations observed cytologically are in accordance with one another, at least at the 300-R level.

212. In terms of both the overall frequencies of abnormal cells and of the kinds of aberrations, mid-pachytene spermatocytes (sampled 5 days after irradiation) are more sensitive than dictyate oocytes if the same sampling interval is used; however, if the sensitivity of the latter at the 14-day interval is used, the reverse is true.

213. For the x-ray induction of translocations in post-meiotic male germ cells, the mouse is more sensitive than the rabbit, the latter more sensitive than the guinea-pig, and the guinea-pig more sensitive than the golden hamster. After spermatogonial irradiation, the rank order of sensitivity is mouse $>$ rabbit $>$ guinea pig $\cong$ golden hamster.

214. Human spermatogonia and those of the marmoset appear to be roughly three times as sensitive as those of the mouse for the x-ray induction of translocations. In contrast, the spermatogonia of the Rhesus monkey appear to be less than one half as sensitive as those of the mouse. The primate data as a whole shows considerable heterogeneity. In using the data from marmoset and man for hazard evaluations, the Committee wishes to stress the uncertainties involved; it further notes that the estimate so derived can only be regarded as approximate.

215. In the guinea-pig and the golden hamster, as in the mouse, the effects of dose-fractionation on the yield of translocations (after spermatogonial irradiation) are dependent on the time interval between the fractions and the size of the fractions.

216. In translocation lines where the males are sterile (derived from irradiation of post-meiotic male germ cells in the mouse), there is a predominance of chain quadrivalents in contrast to the semi-sterile lines, which are characterized by a predominance of ring quadrivalents; in addition, there is a positive correlation between the severity of the sterility effect and the frequency of chain configurations, the latter being 99 per cent when the sperm count is nil.

217. With banding techniques, the breakpoints involved in the translocations that were recovered have been localized. In male-sterile translocations obtained from post-meiotic germ cell irradiation, at least one of the breaks was close to the centromere (c) or telomere (t) and the majority of the translocations were of the c/t type. In sterile sons derived from irradiated spermatogonia, however, this was not the case.

218. Apart from the qualitative difference between translocations recovered from irradiated post-meiotic versus spermatogonial cells, there is also a quantitative difference in that, in semi-sterile sons, there are more ring than chain quadrivalents after spermatogonial irradiation, whereas the reverse is true after post-meiotic germ-cell irradiation.

219. In the golden hamster and the guinea-pig, in translocation lines in which male semi-sterility is found (derived from post-meiotic germ-cell irradiation), chain quadrivalents are more abundant than ring quadrivalents, in contrast to the situation observed in the mouse (see paragraph 216 above).

220. In the golden hamster, guinea-pig and rabbit, although most translocations recovered from post-meiotic germ cell irradiation cause semi-sterility in males, there are some which cause male sterility; in the hamster, sterility is not necessarily associated with translocations leading to chain multivalents, and the situation is the same with the guinea-pig. In the hamster, sterility seems to be associated with the size of the chromosomes involved in the translocation (involvement of longer chromosomes) but this is not true in the guinea-pig or rabbit.

C. INVERSIONS

221. Roderick (436) and Roderick and Hawes (438) have published the results of a continuing study concerned with the identification, recovery and genetic properties of chromosomal inversions in mice. (Earlier work by the authors was reviewed in the 1972 report (589) in paragraphs 126-132 of Annex E.) So far, 26 paracentric inversions have been induced (by x rays or other mutagenic agents), out of which 18 seem to be simple autosomal ones, with almost no deleterious effect on viability. In animals homozygous or heterozygous for any two inversions on different chromosomes, there was no significant lowering of reproductive performance. However, there were two instances in which the animals heterozygous for two inversions on different chromosomes were sterile (437).

222. Using inversion In(1)1Rk on chromosome 1 (earlier designation, In(13)1Rk), which has now been demonstrated to be about 43 cM long (3.5 per cent of the genome), Roderick (436, 437) has uncovered two

recessive mutations in a sample of 400 chromosomes tested. One of them causes lethality at various stages of embryonic development and hours after birth. The other mutation may be allelic with the *leaden* (ln) locus.

223. In the course of an experiment designed to measure sex-chromosome loss after x-irradiation of spermatogonia, L. B. Russell *et al.* (454) found, within the presumed X^MO (i.e., paternal loss) class, a female that had a submetacentric chromosome in a complement of 39. Further genetic and cytological work showed that the submetacentric segregated independently from the 39-chromosome (XO) condition, indicating that the abnormal chromosome was wholly autosomal. Meiotic preparations from animals heterozygous for the submetacentric failed to yield multivalent configurations and had only 20 bivalents. Since the short arm of the metacentric is of considerable length, multivalent configurations would be expected, if the abnormal chromosome were the result of a reciprocal translocation. A pericentric inversion was, therefore, suggested by this finding. The suggestion has been tentatively confirmed by the results of cytological banding studies which indicate that the affected chromosome is No. 8. A breeding stock has now been established, and homozygotes have proved to be viable and fertile. Current attempts are directed at introducing chromosome-8 markers in order to determine the effects of the presumed inversion on recombination. The submetacentric may prove to be a useful cytological marker chromosome.

224. Evans and Phillips (161) recently reported on the finding of an X-chromosomal inversion among the descendants of a male that had been exposed to a fractionated dose of x irradiation (12 X 50 rad separated by weekly intervals; spermatogonia). It was found associated with Bare-patches *(Bpa)*, a mutation which proved to be sex-linked and male-lethal (509). The original *Bpa*/+ females produced amongst their progeny considerable numbers of XO daughters, all of which were of the rare OX^P type. This enhanced capacity to produce XO progeny was subsequently shown to be separable from *Bpa* and was given the symbol *Fxo* (410). The presence of *Fxo* also suppressed crossing over between *Bpa* and *Ta* or *Blo*, and it was suggested that a structural change of the X-chromosome might be involved; this suggestion has now been confirmed cytologically in *Bpa Fox*/++ animals and shown to be absent from *Bpa*+/++ animals. Consequently, the symbol *Fxo* has now been withdrawn and replaced by In(X)1H in accordance with the standardized nomenclature for the mouse (117).

225. Preliminary observations suggest that this X-chromosomal inversion is a long one, covering about 85 per cent of the physical length of the X-chromosome; the genetic data obtained are consistent with a length greater than 48 cM (the map distance between the markers *spf* and *Blo*). Strong crossover suppression occurs between *Bpa* and *Ta*, between *Bpa* and *Blo* and between *Bpa* and *spf* as well. The latter result indicates that there is crossover suppression on both sides of *Bpa*. In addition, recombination between *Bpa* and either of the markers *Ta* or *spf* leaves In(X)1H on the unmarked chromosome. This evidence implies that *Bpa* lies within the inversion and is separable from it by a double crossover (one exchange on either side of *Bpa*). The frequency of such separation has been estimated to be about 8 per cent. The authors believe that while the occurrence of a double crossover would reduce the efficiency of the use of the inversion in the detection of sex-linked lethals, the inversion may still prove useful in experiments aimed at estimating rates of induction with more accuracy than has hitherto been possible.

D. TANDEM DUPLICATION

226. In the course of studies on induced mutations involving the mouse haemoglobin loci, L. B. Russell *et al.* (460) found the daughter of an x-irradiated female (SEC/Rl, Hba^b/Hba^b; Hbb^s c^{ch}/Hbb^s c^{ch}) and an unirradiated male (101/Rl, Hba^a/Hba^a; Hbb^d C/Hbb^dC) whose haemoglobin was not of the usual type by the criteria of electrophoretic pattern (fast-moving band relatively fainter), solubility (low), and crystal pattern. The presumed mutant was also of small stature. The abnormal haemoglobin pattern was not transmitted in backcrossing to SEC/Rl (although the small size was), but was transmitted in backcrossing to 101/Rl, together with the small size. Crosses of the presumed mutant to an albino stock (Hbb^dc/Hbb^dc) yielded offspring which were c^{ch}/c^{ch} in coat colour (instead of the expected c^{ch}/c), possessed the abnormal haemoglobin phenotype, and were of small size. Subsequent cytological analysis by the use of quinacrine banding clearly showed a chromosome 7 which was approximately 20 per cent longer than normal and in which there was a repetition of the bright E band and adjacent sub-bands (D and F). The combined genetic and cytological findings indicate a tandem duplication within chromosome 7 which involves a segment including at least the *Hbb* and *c* loci. The abnormal haemoglobin phenotype is consistent with the presence of two doses of c^{ch} and one of *c*. This tandem duplication, the first recorded in experimental mammals, provides a valuable new tool in mouse genetics, for example, in the study of gene-dosage effects, and has the advantage that involves the *c* locus region, which is well characterized as a result of complementation analysis and also is involved in a number of X-autosome translocations.

E. LOSS OR ADDITION OF CHROMOSOMES

1. Sex-chromosome losses

(a) *Spontaneous rates*

227. L. B. Russell (450) has recently reviewed the results that bear on the spontaneous incidence and rates of induction of numerical sex-chromosome anomalies in mammals. In this paper, among other things, data on spontaneous frequencies which have accrued from the controls of various mutagenesis experiments, from the treated groups of mutagenesis experiments where the anomalous type must have originated in the untreated parent and from the routine maintenance of stocks that carry X-linked markers have been compiled. They show that for maternal X-chromosome losses (scored as OX^P

exceptions), the overall average based on 49 176 females in these various sets (excluding those in which the pre-existing XO condition was not ruled out) is 0.053 per cent. The corresponding figure for paternal X-chromosome losses (scored as X^MO exceptions), as judged from a comparison of the frequencies of X^MO and OX^P exceptions (in crosses where these could be simultaneously scored), is 5-10 times higher.

(b) *Induction in male germ cells*

228. The complete results of the sex-chromosome loss experiment carried out by L. B. Russell and Montgomery (reported in a preliminary form in the 1972 report) have now been published (450, 457). In this study the incidence of sex-chromosome anomalies after x irradiation of mouse spermatogonia and spermatozoa was studied. A 600-R x-ray exposure was used, delivered either singly or in two fractions, 100 R and 500 R, separated by a 24-h interval. The breeding scheme involved crossing irradiated or control (101 X C3H)F_1 males to females homozygous for the dominant sex-linked gene, Greasy *(Gs)*; this permitted the phenotypic detection of paternal or maternal sex-chromosome losses (by the occurrence of X^{Gs}/O or O/X^+ female progeny, respectively), paternal non-disjunction ($X^{Gs}/X^+/Y$ progeny) and certain translocations. All exceptional progeny were examined cytologically and through breeding tests. Mothers of presumed XO progeny were likewise tested. It turned out that in 9 out of 14 cases of O/X^+, there was a pre-existing XO condition indicating the importance of performing such tests.

229. The results obtained confirmed the conclusions reached in the 1972 report, namely, that (a) there were no significant differences between the effects of single or fractionated exposures; (b) after spermatozoal radiation (pooled data of the single and fractionated exposure series), 2 exceptional females were recovered among 421 offspring (0.48 per cent); there was none in the controls. The induction rate (on the assumption of linearity) is therefore 0.8 10^{-5} R^{-1} per gamete; (c) after spermatogonial irradiation, the frequency of sex-chromosome loss was 0.20 per cent (16/8155), which is not significantly different from that in controls (0.24 per cent (12/4994)).

(c) *Induction in female germ cells*

230. In the 1972 report, the data of W. L. Russell *et al.* (489) on the induction of X-chromosome losses in female mice irradiated with 400-R gamma rays at exposure rates of 80 R/min and 0.6 R/min were presented. They showed that the frequency was significantly lower at the lower exposure rate. These results have now been confirmed and extended to a lower dose rate of 0.006 R/min and besides, additional data, hitherto unpublished, have become available (450). These are summarized in table 32.

231. It can be seen that (a) the exposure-frequency relationship for the induction of X-losses (in maturing dictyate oocytes sampled up to 6 weeks after acute irradiation) is non-linear, (b) there is a continuous drop in the frequency with the lowering of the exposure rate, and (c) the interval effect which had earlier been documented for the induction of specific-locus mutations is even more pronounced for the induction of X-losses: in mice irradiated with 400 R at 0.006 R/min, the exceptional progeny are almost exclusively recovered from conceptions that occurred during the first 6 weeks followed by a steep decline to control levels thereafter. These data thus confirm the earlier observations with reference to specific locus mutations and extend them to yet another measure of genetic damage. From the standpoint of hazard evaluations, these findings are of great importance: (a) the rate of induction at low exposure rates, such as 0.006 R/min, is extremely low (even after a high total exposure); (b) there is a marked interval effect; (c) after relatively low exposures, such as 50 R, delivered at high exposure rates, the rate of induction is negligible.

2. Non-disjunction

232. In recent years, there has been a growing interest in the study of non-disjunction. The reasons are (a) the increasing realization that non-disjunction is an important cause of spontaneous abortion in man (autosomal trisomies and X-monosomy together constituting over 20 per cent of karyotyped abortuses and over 70 per cent of all those that are chromosomally abnormal (45, 127 and tables 12 and 13)); (b) the findings that about 0.4 per cent of live-born children carry sex-chromosomal and autosomal numerical anomalies (table 11); and (c) the fact that, with the exception of maternal age, the roles of factors affecting the frequency of non-disjunction (including the effects of radiation) are not unequivocally established in our species.

(a) *Mouse*

(i) *Male germ cells*

233. Spontaneous non-disjunction of sex-chromosomes at meiosis in the male mouse is an exceedingly rare event (450, 457, 462). L. B. Russell and Saylors (462) and L. B. Russell (452) used a breeding scheme which would allow the genetic detection of XXY mice as males heterozygous for the sex-linked dominant gene Tabby (*Ta*). Only one such exceptional mouse was recovered from irradiation of spermatocytes (200 R of x rays) in 6214 classified offspring, compared to none in controls.

234. In a cytological investigation with male mice carried out by Ohno *et al.* (384), every one of the 1460 second meiotic metaphase divisions examined contained either a single X or a single Y and not one instance of abnormal segregation which might lead to the formation of XY or O sperm was noted. In a recent cytological study, Szemere and Chandley (558) examined the effects of radiation on non-disjunction in male mice. Mice of the random-bred Q strain were irradiated with 100 or 200 rad of x rays; half of the irradiated animals were killed 5 days after irradiation (to sample metaphase-II cells treated at the pachytene stage of the meiotic prophase), the remainder being killed at 12 days (to sample cells treated at pre-leptotene). In the 200-rad

series, one group of males was killed 43 days after irradiation to sample cells treated as early spermatogonia. Estimation of the irradiation-to-killing intervals was made using timings established earlier for the Q strain of mice (261). Additional experiments at 100 rad involved mating of irradiated males to females, first after 4 weeks for a 1-week period (to sample cells treated as early spermatocytes) and subsequently remating them for another week (to sample late spermatogonia). The pregnant females were killed and dissected when the foetuses had reached 9 days of gestation. It was thought that the foetal age chosen would permit the detection of at least some of the trisomic offspring (168). Appropriate controls were maintained.

235. The estimates of non-disjunction frequencies were made on scores obtained at metaphase-II in control and irradiated groups as the ratio of the number of cells containing 19 and 21 dyads to the number of cells containing 19, 20 and 21 dyads. For each dose and treated stage, 200 cells were analysed. The results showed that in controls there were no hypoploid or hyperploid cells, in agreement with the data of Ohno *et al.* mentioned earlier. In the x-irradiated groups, aneuploid cells were found, particularly in cells treated as pre-leptotene spermatocytes, 12 days prior to scoring. The non-disjunction frequency for this treated stage was 4.5 per cent at 100 rad and 6.0 per cent at 200 rad; the frequencies for other stages were relatively lower. The foetal karyotyping gave no evidence for pure trisomics although some mosaics were present. In addition, and of special interest, was the finding of two triploid ($3n = 60$) foetuses in the treated group; both were from matings in week 5 and could have therefore arisen following the irradiation of early spermatocytes.

(ii) *Female germ cells*

236. Yamamoto *et al.* (626) examined the maternal age-dependence of chromosome anomalies in unirradiated mice. The procedure was briefly as follows: female mice (strain CF 1) were mated at ages of 3-5 months (control), 11-13 months, and 14-16 months to young adult males. On day 10.5, the pregnant mice were sacrificed and the foetuses recovered and analysed cytogenetically. The proportion of aneuploid foetuses was 2/149 in the control, 5/117 in the 11-13 month group and 5/39 in the 14-16 month group. In the young group, both the aneuploids were mosaic monosomies and in the two, the breakdown was 3 trisomies, 1 monosomy and 6 mosaics. The incidence of aneuploidy in the two "old groups" considered together was significantly higher than in controls. However, a comparison of the control with the 11-13 month group alone did not reveal any significant difference in frequency whereas that of the control with the 14-16 month group showed that in the latter it was higher.

237. In a subsequent study, Yamamoto *et al.* (627) irradiated female mice of different ages with 5 R of x rays and conducted a cytological study similar to the one described in the preceding paragraph. Chromosome analyses revealed that the number of aneuploid foetuses was 2 (1.3 per cent), 4 (3.6 per cent), 10 (6.4 per cent) and 7 (16.3 per cent) in the groups, non-irradiated young, x-irradiated young, non-irradiated aged and x-irradiated aged, respectively. The number of foetuses examined in the different groups was 149, 111, 156 and 43, respectively. While the difference in the incidence of chromosome abnormalities between non-irradiated and x-irradiated young adults was not significant, there appeared to be a definite increase in the incidence of aneuploid foetuses in the x-irradiated aged as compared with the x-irradiated young. The authors concluded that the incidence of aneuploid foetuses in aged mothers is further increased by 5 R of x irradiation. Of the 7 aneuploids in the aged group (irradiated) there were 4 trisomies, 1 double trisomy and 2 mosaics (39/40 and 40/41).

238. Gosden and Walters (197) have reanalysed the above data statistically for evidence of interaction between age and x irradiation using three procedures: (a) comparing the appropriate function of the percentages with its standard error; (b) partitioning the three degrees of freedom into an age, irradiation and interaction effect; (c) examining the proportionate increase apparently due to irradiation. None of these tests showed any significant difference of the kind claimed by Yamamoto *et al.* (627). The other objection which Gosden and Walters raise to the conclusion of Yamamoto *et al.* pertains to the fact that the authors have used as controls the earlier published results detailed at the beginning of this section; such a procedure would be open to question in view of possible differences in environmental conditions.

239. Uchida and Lee (582) made use of the recent advances in the technique of culturing mouse oocytes *in vitro* to obtain preparations of meiotic chromosomes (in metaphase II) of oocytes derived from irradiated females. C3H X ICR/Swiss F_1 hybrid females aged 3 and 6 months were exposed to 10, 20 and 30 R of whole-body gamma irradiation; the irradiated females and their paired controls were sacrificed and the ovaries removed within one week of radiation exposure. The oocytes were teased out of the ovaries and those containing a germinal vesicle were incubated in foetal calf serum for 18-23 h to obtain cells in metaphase II and subsequently processed for cytological examination.

240. In a total of 15 713 viable oocytes collected, 1151 and 1054 metaphase-II oocytes (in the irradiated and control groups, respectively) were analysable (428 oocytes at 10 R, 368 at 20 R, and 355 at 30 R). Since there were no significant differences between the two age groups used, the results were combined. Six of the irradiated oocytes had an extra chromosome (i.e., 21 chromosomes), Two of these were in the 10 R series, three in the 20 R series and one in the 30 R series. No hyperploid cells were found among the controls. Assuming that for each oocyte with 21 chromosomes, a complementary cell with 19 chromosomes must have been produced (and formed the polar body) and barring preferential segregation of one type of abnormal product into the polar body, there should be an equal number of hyperploid polar bodies with their complementary hypohaploid oocytes. The total number of non-disjunctional products should therefore be closer to twice the observed frequency of hyper-haploid oocytes, i.e., 12 (1 per cent) among the irradiated oocytes. The

numbers of non-disjunctional events in the sample are still too scanty to permit comparisons between the different exposure levels. From the work of Uchida and Lee, it would therefore appear that non-disjunction can be induced in young females by *in vivo* radiation exposure.

241. Uchida and Freeman (581a) have now performed another experiment on the same lines as that of Uchida and Lee described above, but with the use of older female mice, aged 12 months instead of 3-6 months. The frequency of hyperhaploid metaphase-II oocytes was significantly higher than in controls and was also higher than in young females. The incidence of non-disjunction among aged controls was 0.6 per cent, increasing to 2.7 per cent on irradiation, as compared with a rate of 1 per cent in irradiated young females. The authors consider that these results support the suggestion that the risk of producing trisomic offspring among humans is increased with exposure of the abdomen to diagnostic x rays. They point out that 9 out of 11 epidemiological studies have shown an increase in non-disjunction with radiation exposure, although in some series these increases were not statistically significant.

242. In a genetic study on the effects of maternal age on spontaneous and x-ray-induced (200 R) sex-chromosome non-disjunction and loss in the mouse, L. B. Russell and Montgomery (457a) compared the response of mice about 3 months old with that of mice about 9 months old. The experiments, which are still continuing, have so far generated no unequivocal instances of maternal non-disjunction, although there has been one case of paternal (spontaneous) non-disjunction. In a new series, also still in progress, in which the females are even older (11.5-12 months at the time of irradiation and/or mating), no cases of maternal non-disjunction have so far been recovered (457a, 467).

243. Lüning, Eiche and Lüning (303) conducted some pilot experiments with the CBA strain of mice to check whether or not exposure of females to low doses of x rays induce non-disjunction in oocytes; should this be the case, then the nullosomic and disomic gametes that result from non-disjunction should lead to the production of aneuploid foetuses. Such foetuses, except possibly some trisomies, are expected to die *in utero* and the frequency should be ascertainable under proper conditions. The experimental scheme included (a) irradiated and control females of various ages, with and without a history of having produced a few litters prior to the commencement of the experiments; (b) acute exposures of 2, 4, 8, 12, 16 and 32 rad to females as well as fractionated doses (4 X 4 rad; 2 X 8 rad at 1-week intervals), (c) exposure of foetuses at various times before parturition; and (d) intervals ranging from 0 to 182 days between radiation exposure and mating. The females were killed 11-20 days after mating for uterine examination.

244. The results obtained in this study show that (a) in the irradiated series, there is no excess intra-uterine death at a more or less late stage in development and (b) the age of the females at the time of mating has a considerable effect on the intra-uterine death rate (varying from about 8 per cent in young females to 25-30 per cent in older females) in both the irradiated and control series. It should be pointed out that conclusion (a) cannot be considered a definitive one; in spite of the variety of pilot tests performed, the absolute numbers in any one series are relatively small and this makes it difficult to detect small increases in intra-uterine death.

(b) *Hybrids between the house mouse and tobacco mouse*

245. The diploid chromosome complement of the tobacco mouse, *Mus poschiavinus*, consists of only 26 chromosomes, 14 of which are metacentric (206) in contrast to the all-acrocentric complement of 40 in the house mouse. The fundamental numbers are the same ($2n = 40$), however, and both meiotic (572) and mitotic studies (628) with the two species and their F_1 hybrids have demonstrated that the chromosomes are, in fact, homologous. It is believed that the tobacco mouse metacentrics were derived from acrocentric chromosomes of the house mouse by Robertsonian fusion/translocation. During the last few years, stocks of mice have been constructed each carrying a different metacentric chromosome derived from *M. poschiavinus*. These are being extensively utilized to examine systematically the involvement of specific chromosomes in meiotic non-disjunction and as a good model system to gain an insight into the conditions leading to aneuploidy in mammals, including man.

246. The F_1 hybrids between the house mouse and the tobacco mouse are fully viable, but have greatly reduced fertility. Studies on first meiotic division cells have shown that the seven metacentrics regularly form trivalents with the 14 homologous *M. musculus* acrocentrics. The finding that in the F_1 hybrids more than one half of the metaphase II figures are genetically unbalanced suggests that anaphase-I disjunction is disorderly leading to gametic aneuploidy, which in turn leads to the production of aneuploid inviable zygotes (572). Tettenborn and Gropp (572) suggested that the non-disjunction observed in the hybrids could result either from genetic heterozygosity *per se* or from structural heterozygosity leading to trivalent formation.

247. Cattanach and Moseley (91) found that in males, each of the 7 tobacco mouse metacentric chromosomes when carried heterozygously with the house mouse acrocentrics led to non-disjunction, but the frequency was not the same for each chromosome. In addition, considerable heterogeneity existed between individuals heterozygous for any one metacentric and the non-disjunction frequencies did not appear to be correlated either with chromosome size or centromere position. The authors also obtained some evidence of non-disjunction in metacentric homozygotes, small testis size and reduced sperm production among homozygotes and crossover suppression between the metacentrics and the homologous acrocentrics in heterozygotes. On the basis of these data, they concluded that a large part of the non-disjunction observed when the tobacco mouse metacentrics are carried heterozygously with the house mouse acrocentrics may result not from structural heterozygosity *per se*, but rather from other genic or

minor chromosomal differences which reflects the fact that the chromosomes are derived from two different species.

248. The results obtained by Ford and Evans (169) and Gropp, Giers and Kolbus (204) in the backcross progeny of single metacentric heterozygotes are essentially the same: depending on the metacentric involved, heterozygous males caused different rates of non-disjunction and the estimates based on metaphase-II counts and on karyotying of pre- and post-implantation embryos were in agreement with one another. The work of Gropp, Giers and Kolbus (204) also demonstrated that aneuploidy (trisomy) of the zygotes was considerably more frequent in the progeny of heterozygous females than in those of heterozygous males and has led to the suggestion that this disparity might be the result of a higher non-disjunction rate in female gametogenesis. The authors have entertained the possibility that mechanisms of selection against unbalanced male germ cells might operate on a small scale. Ford and Evans, however, suggest that in their work there was no evidence of selective elimination of spermatids or spermatozoa with unbalanced genomes up to the third day of gestation.

249. So far, all the trisomic conditions reported in this system cause pre-natal death, which in some of them occurs at an early stage (day 10-13) while in others, at a later one (day 14-16), and the developmental profiles differ considerably according to which individual autosome is involved (169, 203, 204, 205). Consistent with this, Ford and Evans found a considerable reduction in the number of trisomic embryos between the earlier gestational period (8-11 days) and the later period (12-15 days). Taken together, these observations with primary trisomics contrast with the findings with partial (tertiary) trisomics (in the house mouse) which may be viable and not infrequently fertile (40, 154, 310). One further finding relates to the observation that very few monosomic zygotes survived to be identified subsequent to implantation in the *M. musculus* X *M. poschiavinus* heterozygotes for any of the metacentrics thus far tested (169).

(c) *A method to measure non-disjunction in the laboratory mouse using Robertsonian translocations*

250. Lyon *et al.* (319) have recently devised a method for measuring non-disjunction in mice using Robertsonian translocations of the tobacco mouse. Since a high frequency of non-disjunction occurs spontaneously in mice heterozygous for Robertsonian translocations (see the preceding section), it was thought that it should be possible to measure this by a method similar to that used for measuring adjacent-2 disjunction in mice with reciprocal translocations. In the latter method, animals heterozygous for the translocation and homozygous for different alleles of a marker gene are mated together. Offspring homozygous (rather than typically heterozygous) for the marker arise through adjacent-2 disjunction (or non-disjunction) and their frequency can be recorded (519).

251. In the scheme of Lyon *et al.*, homozygotes for the Robertsonian translocation are crossed to homozygotes for the chosen genetic market, and the offspring intercrossed. The intercross progeny are tested for the presence of the translocation by biopsy of the testis, spleen or ear skin. By this means a stock homozygous for both the translocation and the marker is constructed. Animals from this stock are crossed to homozygotes for the marker, but not carrying the translocation. All the offspring from these matings will then be heterozygous for the translocation and homozygous for the genetic marker and are used in the experiments. They are mated *inter se* and the young are observed at birth, 7-14 days later, and at weaning for the presence of recessive markers. All such marker-carrying young are tested for fertility and their karyotypes are determined by biopsies of ear, skin or corneal preparations.

252. Using this method, Lyon *et al.*, estimated that the non-disjunction frequencies ranged from less than 5 per cent to about 15 per cent in the different crosses. In addition, the finding that all the marked young were heterozygous for the Robertsonian translocation suggests that the gametes involved are derived from non-disjunction at the first meiotic division. A search for genetic or environmental factors affecting the frequency of exceptional progeny revealed that with one Robertsonian translocation, the frequency increased steadily with increasing maternal age whereas with another, the maternal age of the marked young was not significantly below that of the total progeny.

(d) Microtus oeconomus

253. Another mammalian species which may be potentially useful in the study of non-disjunction, specifically of the sex-chromosomes, is the northern vole, *Microtus oeconomus*. The use of C-banding techniques have shown that in this species with a diploid chromosome number of 30, while all the 14 pairs of autosomes exhibit tiny dots of centromeric heterochromatin, the X-chromosomes have large blocks of these and the Y-chromosome is C-band positive along its entire length (636).

254. In an exploratory study, Tates, Pearson and Geraedts (570) found that in testes preparations processed for C-banding, cells having a single X or Y, two Ys, two Xs and XYs can be identified in early and mid-term spermatids (some of the spermatids were larger in size and appeared to be polyploid). In a subsequent study, Tates (569) irradiated males with 50, 100 and 200 R of x rays and made preparations 1, 2, 4, 8 and 12 days after irradiation. Such a procedure would enable the sampling of cells irradiated as spermatocytes and possibly as spermatogonia (in the later interval series). There is definite evidence for the induction of non-disjunction even at the lowest level of exposure used, but the calculation of the exact frequencies is complicated by the occurrence of great variability between animals within exposure levels, and within and between the different sampling intervals. The author is at present collecting more data.

3. Summary

255. Additional data on the x-ray induction of sex-chromosome losses in mouse spermatogonia that have accumulated since the publication of the 1972

report support the conclusion reached earlier, namely that there is no evidence for an increase in the frequency at which exceptional XO progeny are recovered (relative to controls) after spermatogonial irradiation (600-R level). After spermatozoal irradiation however, the frequency is significantly higher than in the controls.

256. X-chromosome losses can be readily induced by irradiation of mouse females at high dose rates; there is a marked dose-rate effect in that a lowering of the exposure rate from 80 R/min to 0.6 R/min down to 0.006 R/min (total exposure of 400 R) results in a progressive reduction in the frequency. This continuous reduction in frequency with lowered dose rates parallels the results obtained for the induction of specific-locus mutations; in addition, the frequency of X-chromosome losses in conceptions occurring later than a few weeks after irradiation is not significantly higher than in the controls. Thus there is evidence for the operation of an interval effect for this end-point of genetic damage as well.

257. Studies on non-disjunction have been receiving more attention during the past few years. In male mice, the results show that non-disjunction can be induced by irradiation of pre-leptotene spermatocytes. In female mice, although there are indications for the radiation induction of non-disjunction in oocytes, the data do not permit reliable risk estimates to be made.

258. Use has been made of the presence of the seven pairs of metacentric chromosomes in the tobacco mouse to construct mouse stocks carrying known metacentric chromosomes to study the process of non-disjunction in more detail. Although up to now no radiation experiments have been carried out with these stocks, the studies have provided interesting insights into how the frequencies of non-disjunction vary depending on which metacentric is involved, the time of death of the different trisomics and their developmental profiles.

259. A new method has been developed to measure non-disjunction in mice using Robertsonian translocations of the tobacco mouse. This is likely to prove valuable in studying radiation-induced non-disjunction in mice.

260. Attempts are being made to study non-disjunction of the X and Y chromosomes in the males of the northern vole, *Microtus oeconomus*, in which techniques are now available to score for non-disjunctional spermatids derived from spermatocytes and spermatogonia.

F. POINT MUTATIONS

1. Specific-locus mutations in male mice

261. The complete results of the work of Selby (discussed in the 1972 report in a preliminary form, Annex E, paragraph 174) on the x-ray induction of specific locus mutations at the seven loci in the germ cells of male mice of different ages (new-born, and age groups from 2 to 35 days[14] after birth at irradiation) have now been published (526, 527) (table 33). These results, with only a slight increase in the number of progeny tested (over those presented in the 1972 report) confirm the conclusions reached earlier: (a) for new-born mice, the rate is $1.37\ 10^{-7}\ R^{-1}$ per locus, which, statistically, is significantly lower than that of $2.91\ 10^{-7}\ R^{-1}$ per locus for spermatogonia in adults (300-R level for both). The former rate does not significantly differ from the one reported by Carter *et al.* (194) ($1.83\ 10^{-7}\ R^{-1}$ per locus, 200-R level) for 17.5-day-old foetal males; (b) none of the mutation rates in the other age groups was significantly higher than that of the adult, although this conclusion does not rule out the possibility that individual age groups may have mutation rates somewhat different from that of the adult.

262. The combined mutation rate for the nine age groups of young males (day 2-35), $2.63\ 10^{-7}\ R^{-1}$ locus, is significantly higher than that of the new-born males, $1.37\ 10^{-7}\ R^{-1}$ per locus, and almost the same as that of similarly irradiated adults. An examination of the data for the different age groups suggests that the change in mutational response may have occurred by day 8; day 8 has the second-highest mutation frequency of all nine groups. All three age groups before it have lower mutation frequencies than the adult. The mutation frequencies on days 2 and 4 are the lowest found among the nine groups, both being very similar to the rate found for new-born males. The point estimates for the data are arbitrarily grouped according to their indication in table 34, in which estimates for new-born and adult are also shown for comparison.

263. The incidence of clusters of specific-locus mutations following irradiation of new-born males was significantly higher than the cluster incidence reported by W. L. Russell for similar irradiation of adults. This presumably indicates the survival of relatively fewer reproductive cells following irradiation of the day-0 testes. In the other age groups, clusters were found only on day 21 (two clusters of two mutations each). In new-born males, although there were suggestive indications for differences in the distribution of mutations among the loci studied (relative to that in the adults) the differences were not significant: for the other age groups, the distribution appeared similar to that in adults.

264. Cattanach and Moseley (92) investigated the effects of irradiating mouse spermatogonia with a total exposure of 1000 R of x rays delivered in two equal

[14] Between birth and 35 days of age, the testis undergoes many developmental changes, and by 35 days, the cell population resembles that of the adult. Widmaier (609) found that primary spermatocytes were commonly observed 7-8 days after birth in the mouse strain he studied. Some spermatids were present by 20 days and at 25 days, almost all tubules contained secondary spermatocytes and spermatids. At 30 days, the germinal epithelium was fully developed. Widmaier concluded that the first cycle of the seminiferous epithelium begins on day of birth in the mouse and that the cycle proceeds essentially as described by Oakberg (368). Oakberg (369) has shown that the time required for type-A spermatogonia to develop into mature spermatozoa averages 34.5 days in the adult male. However, spermatogenesis has been shown to proceed more rapidly in juvenile rats than in the adult (236).

fractions separated by 4 and 7 days; the yields of specific-locus mutations (at the loci) were compared with those obtained in the experiments of W. L. Russell (472) with single 1000-R exposures, two 500-R exposures separated by 2 and 24 h and two exposures of 600 R and 400 R; 15 weeks apart. These are shown in table 35 from which it can be seen that (a) neither the 4- nor the 7-day interval results in yields which are similar to that of the 24-h fractionation, (b) the frequencies are higher than after the single acute exposure of 1000 R, and (c) the data for the 4- and 7-day fractionation periods are consistent with the expectation of additivity of yields such as that seen in the long-term fractionation experiments of W. L. Russell (472), 600 R + 400 R, > 15 weeks apart.

265. One of the early attempts to screen for biochemical variants induced by irradiation of the mouse was that of Feinstein *et al.* (165) who devised a rapid semi-quantiative screening method for blood catalase; in the progeny of irradiated male mice (derived from W. L. Russell's experiments), the authors observed two with low blood catalase in a total sample of 12 000 mice.

266. A programme designed to study the radiation-induction of haemoglobin variants in the mouse, initiated a few years ago at Oak Ridge is continuing and has produced some interesting results (461, 492). The work consists of screening blood samples of F_1 progeny from experiments in which either 101 or SEC strain mice are x irradiated and mated to non-irradiated mice of the other strain. The parental strains differ from each other for alleles at the *Hba* chromosome 11 and *Hbb* loci chromosome 7. The haemoglobin characters of F_1 offspring analysed included electrophoretic pattern, solubility and crystal pattern. Blood samples were also checked for possible alterations in serum albumin and red-cell lysis.

267. Of a total of 8621 F_1's so far analysed for these characters, 6918, 875 and 828 were derived from irradiated spermatogonia, post-spermatogonial stages and oocytes, respectively. Five haemoglobin variants have been found. Two of these appear phenotypically to be alpha-chain deletions or inactivations, the third a sterile male (with spermatogenic block in pachytene), a result of an independent translocation T(3;16), the fourth a tandem duplication (para. 226) involving *Hbb*, and the fifth presumably carries two No. 7 chromosomes from the 101 mother and none from the irradiated SEC father. No serum albumin mutants were detected and four anaemic F_1's probably did not transmit their abnormality. The authors note that the per-locus mutation rate based on the 3 *Hba* mutants (which may be either small deficiencies or intragenic changes) are not out of line with earlier specific-locus results, although confidence limits are still very wide.

268. Work similar to that outlined above, but using nine electrophoretically detectable markers (*Es*-1, *Es*-3, *Gpd*-1, *Gpi*-1, *Id*-1, *Mod*-1, *Pgm*-1, *Dip*-1 and *Hbb*) is currently underway at Research Triangle Park (321a, 591, 592). Irradiated DBA/2J mice (500 R + 500 R gamma rays, 24 h apart, spermatogonial irradiation) were crossed to C57BL/6J and the F_1 progeny screened by electrophoretic methods for variants. Thus far, 4 mutants (two of which are haemoglobin variants) have been found in 2600 progeny. Work is continuing.

269. In two recent papers, Kohn and Melvold (262) and Kohn, Melvold and Dunn (263) have summarized the results of their x-irradiation mutagenesis studies in mice using the histocompatibility *(H)* system. Before describing the results, it will be useful briefly to review the system and the methodology. This system is comprised by a group of co-dominant histocompatibility genes that are located throughout the genome and on whose action the acceptance or rejection of dermal grafts depends. For operational reasons, the *H* loci are divided into two classes which can be distinguished from one another in the F_1 hybrid of the B6 and C lines that are employed (B6 = C57BL/6Kh; C = BALB/cKh). The class-I loci, 30 in number[15] have different alleles in the parental lines and are therefore heterozygous in the F_1 hybrid. The class-II have similar alleles in the parental lines and are therefore homozygous in the hybrid. The number of class-II loci is unknown.

270. The *H*-test involves five major steps (264, 266): (1) mating of selected parents, one of which can be treated with a mutagen or x irradiated (C♂ X B6♀); (2) skin-graft testing of the F_1 progeny; each animal exchanges one graft with each of two other animals in a "reciprocal circle"; (3) identification of suspected mutants; (4) backcrossing of each of these to a parental line; (5) graft-testing of the resultant backcross progeny to establish the transmission of the suspected mutation.

271. The mutations are classified on the basis of their graft-rejection patterns as "gains" (appearance of a new antigenic specificity, i.e., grafts donated by the putative mutant rejected), "losses" (loss of a specificity, i.e., grafts placed on the putative mutant rejected) and "gains and losses" (one specificity replaced by another, i.e., reciprocal rejection occurs between the putative mutant and other animals). Class-I mutations are distinguished from class-II by their loss or gain-and-loss phenotypes for loci on autosomes and X-chromosomes: class-II mutations could produce only gains unless the Y-chromosome was involved.

272. Male mice (C-line) were x irradiated (60-65 rad/min; 350, 500, 650 and 800 rad acute x irradiation; unequally fractionated doses of 500, 650 and 800 rad) and mated to the B6 line. The F_1 progeny (derived from irradiated spermatogonia) were graft-tested, the mutations identified and subsequently verified as mentioned in the preceding paragraph. The results showed (262) that among a total of 13 614 F_1 progeny, including 11 279 experimental (all radiation groups) and 2335 controls, a single class-I mutation was recovered, which was of the "gain-and-loss" type (in a series in which the male parents were irradiated with a fractionated dose of 300 + 500 rad, 24 h apart). The mutant rejected skin from the C parental line but accepted skin from the B6 maternal line. No class-I mutations were detected in the controls. Four other class-I mutations that were detected (1 loss and 3 gain-and-loss) had occurred in the B6 alleles, i.e., in the unirradiated maternal gametes.

[15] Minimum number of loci at which the B6 and C strains differ (19).

273. In tests for class-II mutations (263), a total of 36 mutants was found, of which 8 were in the controls and 28 in the different irradiated groups. Neither the frequency of mutations in any of the irradiated groups nor the total frequency of mutations recovered was significantly different from the control frequency.

274. The failure to obtain evidence for radiation-induction of histocompatibility mutations is attributed by the authors, not to the failure of the system to detect mutations, but rather to the very low mutability of these loci (relative to the seven or the six loci used in the Oak Ridge and Harwell studies) after radiation exposures. They surmise that this can happen if x-ray-induced histocompatibility mutational lesions are more efficiently repaired or if spermatogonia carrying x-ray-induced histocompatibility mutations fail to result in viable progeny. Obviously, further research is needed. It would be especially of interest to determine if similar results would be obtained after spermatozoal irradiation.

275. In a subsequent paper, using target theory considerations, Kohn (265), estimated the sizes of genes involved in mutation experiments with specific loci in the mouse and in other organisms. The author's main conclusions were: (a) mutation rates tend to be much lower than predicted by target theory; (b) selection and/or repair are major factors that determine the rates; and (c) the mouse seven-loci test, which provides a principal data-base for the standards of human radiation protection, may not provide adequate overall representation of the mutability of the mammalian genome. However, since the author himself points out "estimates of gene size by target theory most likely will be incorrect, depending on the magnitude of balancing of a number of factors", it does not appear profitable to enter into a detailed discussion of this paper. Furthermore, in this report, the Committee has used the specific-locus data from the mouse only in the context of assessing the effect of various physical and biological variables on mutation rates, in the sense they were originally intended to be used (480).

2. Dose-rate effects

276. Lyon, Phillips and Papworth (318) published a paper in 1972 in which they re-examined the published data on dose-rate effects with low-LET irradiation for the induction of specific-locus mutations in mouse spermatogonia besides some new data from Harwell hitherto unpublished. Based on earlier results, the conclusion was that in adult spermatogonia, the maximal effect of reducing the exposure rate is already obtained at 0.8 R/min (namely a reduction of the yield to about 30 per cent of that at high exposure rates), such that a further reduction in exposure rate has no measurable effect. The paper of Lyon *et al.* (318) raises questions about this conclusion.

277. The arguments are as follows: (a) in Russell's work, the mutation rate per unit of exposure at an exposure rate of 0.001 R/min is actually higher than at 0.009 R/min, suggesting an inverse relationship between mutation rate and exposure rate, but the difference is not statistically significant; (b) from work at Harwell, new data (recorded in the paper) at low dose rates have become available, and the same trend towards higher mutation rates at very low dose-rates is observed; (c) when, using all the available data, the induced mutation rates are plotted against $\log_e I$ (where I is the exposure rate), some kind of curvilinear relationship is indicated. Statistically, the data fit well a model in which the mutation rate varies continuously with the exposure rate and in which there is a dose-rate I_{min} at which the mutation rate is minimal. The maximum likelihood estimate of I_{min} is about 0.03 R/min (95% confidence limits, 0.002 and 0.09); (d) although the quadratic model used gives a good fit to the data, the model of zero slope below exposure rates of 0.8 R/min also fits the data; (e) an examination of whether the true relationship is not a smooth curve but rather two independent straight lines, one with a negative slope over the range of 0.001 and 0.02 R/min, and the one with a positive slope thereafter, reveals that the data also fit this model.

278. In the opinion of Lyon *et al.*, the biological basis for two separate mechanisms that might conceivably operate (one on either side of I_{min}) may be related to the number of cell cycles, duration of the irradiation and cell population changes. A relevant consideration may be that the position of I_{min} is such that at exposure rates below it, the period of irradiation necessary in order to give the total dose extends over many cell cycles, whereas above I_{min}, the total exposures are delivered within one cell cycle. Published results of Oakberg and Clark (375) suggest that at a rate of 0.001 R/min, the survival of type-A spermatogonia is much higher than at 0.009 R/min and above. At 300 R, the survival of type-A spermatogonia expressed as experimental/control ratio is 0.372 at 0.009 R/min and 0.846 at 0.001 R/min. The pattern of survival at 0.009 R/min resembles that at high exposure rates (600-R level).

279. The essence of the argument then is that cell killing may be a plausible explanation for the dip in the curve with relatively little depression of the mutation rate due to cell killing at 0.001 R/min and lower rates. At environmental levels of radiation, one might therefore expect a response more similar to the one at 0.001 R/min than at 0.009 R/min (see also Oftedal (383) for a discussion of the problem).

280. The implication of the analysis of Lyon *et al.* for hazard evaluation is this: on the basis of Russell's interpretation that the mutation rate remained constant at exposure rates below 0.8 R/min, it was reasonable to suppose that it would still remain constant at environment radiation levels (0.1 R/year or $1.9\ 10^{-7}$ R/min). This means that for low-dose conditions applicable to man, the assumption that the induction rate is one third of that seen at higher dose rates is justified. On the other hand, if the efficiency of the irradiation increases at very low dose rates, the rate used in risk evaluations should need upward revision.

281. The paper of Lyon *et al.* has been re-examined by W. L. Russell (467, 481). This reanalysis bears out the following conclusions: (a) at rates below 0.8 R/min, the Oak Ridge data for induced mutation rates do show an upward trend; however, the confidence intervals are sufficiently large such that they fit the model of zero slope; (b) the Harwell data at low dose rates show a less

pronounced upward trend if Harwell controls are used to correct for spontaneous rates; furthermore, the confidence limits for the Harwell data are also wide, indeed very wide, for the 0.001-R/min point. Thus there is no firm evidence for a negative slope at lower dose rates.

282. Additional data that have since become available seem to lend support to the earlier conclusion of W. L. Russell that at exposure rates below 0.8 R/min to mouse spermatogonia there is no further significant reduction in mutation rate and that at low exposure rates (0.8 R/min or lower) the induction rate is about one third of that obtained at high dose rates. In experiments specifically focusing attention on the problem of determining mutation rates at very low exposure rates, W. L. Russell and Kelly (486, 487) irradiated male mice with a total gamma-ray exposure of 300 R (^{137}Cs) at rates of 0.0007 R/min and 0.0056 R/min (delivered over a 10-month and a 38-day period, respectively). Two factors that were not considered in earlier experiments have been controlled in the current series: (1) in the earlier work, animals of all groups entered the experiment at about the same age. Thus, those that required the longest exposure time were irradiated over a more advanced age than the rest; (2) their matings did not start until they were considerably older.

283. In the new experiments, the animals exposed at 0.0056 R/min for 38 days were divided into four groups. The exposure period of these groups was distributed at approximately 3-month intervals of age to cover the age range involved in the 0.0007-R/min group that was exposed for over 10 months. All groups, including controls were not mated until the 0.0007-R/min group had received its exposure.

284. The data thus far available show that at a rate of 0.0007 R/min there are 11 presumed mutations in 42 020 offspring and at 0.0056 R/min 19 presumed mutations in 74 842 offspring. The frequencies are highly significant above the controls and differ from each other by less than one mutation from that expected on the null hypothesis of no difference between the two groups. The tests are continuing.

285. In studies initiated to complement those of W. L. Russell and Kelly (discussed in the preceding paragraphs) on spermatogonial stem cell survival, Oakberg and Palatinus (378) demonstrated that there was no reduction in the stem cell population after 300 R of ^{137}Cs gamma rays given to mouse spermatogonia at a rate of 0.0007 R/min, whereas it was 37 per cent of controls after the same exposure delivered at 0.0056 R/min. Thus, in these experiments, a change in cell killing has not been accompanied by a change in mutation frequency, contrary to the arguments presented in paragraphs 278-279.

3. Specific-locus mutations in female mice

(a) *Dose-fractionation effects*

286. In earlier studies (reviewed in the 1972 report), W. L. Russell (476, 480) had found that a single exposure of 50 R of x rays to female mice gave a lower mutation rate per unit exposure (conceptions within the first seven weeks after irradiation; maturing oocytes samples) than those of 200 or 400 R and that the yield after 8 X 50 R (75-min interval between the fractions) was 8 times that of a single exposure (i.e., much lower than the effect of a single 400-R exposure; see the 1972 report, Annex E, table 16).

287. Lyon and Phillips (312) have now investigated the mutational response of maturing oocytes to irradiation with a total dose of 200 rad given in 20 fractions of 10 rad each, over a period of five days (10 rad at a time, four times a day; interval between fractions in a day, approximately 2 h) or over four weeks (10 rad per day for five days in a week (Monday through Friday) 24-h interval between fractions). The effects observed were compared with those in females irradiated with single doses of 200 rad, of which there were two groups. One group was mated soon after irradiation and the other after a week's delay. All x irradiations were delivered to mice 9-10 weeks old at about 52 rad/min. The offspring conceived within seven weeks (and later) after the irradiation were scored for mutants at the seven loci of the PT stock. There were no controls.

288. The results showed that there were no significant differences in mutational yields between the two single-exposure experiments (although in one, mating of the irradiated mice was delayed for a week) and between the two fractionation régimes (although in one, the irradiation was delivered in five days and in the other, over four weeks). Therefore, the data of the two acute groups were pooled and, likewise, those of the two fractionation groups were pooled; these data are given in table 36. Inspection of table 36 will reveal that (a) among the offspring conceived within seven weeks after irradiation, the mutational yield after single exposures is 9/34 813 (a rate of 1.85 10^{-7} rad^{-1} per locus) and after fractionation, 1/39 887 (a rate of 0.18 10^{-7} rad^{-1} per locus); and (b) if the comparisons are restricted to the progeny conceived within the first three weeks after irradiation, the corresponding figures are 7/21 578 (a rate of 2.32 10^{-7} rad^{-1} per locus) and 1/20 398 (a rate of 0.35 10^{-7} rad^{-1} per locus). Thus, there is a reduction in mutation frequency by factors of about 10 or 7, depending on which progeny groups are included in these comparisons.

289. The rates given above have not been corrected for controls.[16] If this is down, the induction rates will be even lower. For instance, the induction rate obtained at the 200-rad level (even by correcting with the minimal spontaneous rate) is lower than that of W. L. Russell at the same exposure: for the first seven-week conceptions, W. L. Russell obtained a rate of 3.95 10^{-7} R^{-1} per locus, which is twice that which can be estimated from the data of Lyon and Phillips (1.75 10^{-7} rad^{-1} per locus). The latter authors suggest two possible reasons

[16] The Oak Ridge control data currently stand at three independent mutations (one of which was a cluster of six) among 166 826 progeny. This, combined with the earlier data of Batchelor *et al.* (28) (no mutations among 37 813), bring the total number of progeny scored to 204 639. Based on the method used in the 1972 report (Annex E, paras. 145-146) a minimal and an upper rate of spontaneous mutations can be estimated. These are, respectively, 2.1 10^{-6} per locus per gamete and 5.6 10^{-6} per locus per gamete.

for this discrepancy: (a) the stocks of mice used (originally descended from those of Russell) have come to differ in the twenty-year period since they were founded, and (b) there was a difference in the dose rates used: about 90 R/min in W. L. Russell's experiments and about 53 rad/min in those of Lyon and Phillips. W. L. Russell (467) has pointed out that when his oocyte data are split up into those pertaining to "older" and "younger" females the discrepancy between his data and those of Lyon and Phillips is very much reduced.

(b) *Dose-rate effects*

290. One of the difficulties in measuring the effect of protracted irradiation on mutation frequency in maturing oocytes of mice is that the length of radiation exposure time necessary to accumulate a sizeable dose may approach the duration of the oocyte stage being studied. This was recognized in the earliest work on mutation induction in females by W. L. Russell. When he later discovered (475a) that the mutation frequency apparently drops to zero for conceptions occurring more than six or seven weeks after irradiation, he pointed out, at that same time, that the lowness of a total mutation frequency in offspring collected during a period of several weeks after the end of a chronic irradiation exposure could have resulted partly from an effect of low dose rate on maturing oocytes and partly from a portion of the dose having been given to oocytes in immature stages, more than six weeks before conception. However, it was shown that a very low mutation frequency from chronic irradiation, compared with acute, would be demonstrated, even for maturing oocytes, by restricting the comparison to conceptions occurring within a short time after accumulation of the dose.

291. In order to utilize data collected from conceptions occurring over a longer period, and still restrict the measurement to maturing oocytes, it is necessary to compute the proportion of the dose received while the oocytes are in maturing stages. This was done, in a crude approximation, in computations used to arrive at the figure of 1/20 which was accepted in the 1972 UNSCEAR and BEIR reports as the ratio of effects of chronic to acute irradiation in maturing oocytes.

292. W. L. Russell (483a) has now presented previously unpublished data which have enabled him to make more precise estimates of the effects of protracted irradiation on maturing oocytes. The data shown in table 37, were collected to determine as sharply as possible the time interval after irradiation at which mutation frequency drops, and to find out if this is a sudden or gradual change. No mutations were obtained in conceptions occurring more than six weeks after irradiation, and the frequencies in the fifth and sixth weeks showed no decline compared with earlier weeks. Thus the drop in mutation frequency with time after irradiation is very sudden. Russell also points out that, on the basis of these and other data, offspring conceived in the first week after irradiation appear to have a lower mutation frequency than those conceived during week 2-6 after irradiation.

293. On the basis of the above results, an "effective dose" can be computed for offspring conceived at various intervals after a period of protracted irradiation. By "effective dose" is meant the portion of the total dose that was received six weeks or less before ovulation. Russell applied this analysis, computing a weighted mean effective dose, to his own experiments and to the data of Lyon and Phillips (312) and Carter (84a), as shown in table 38. A mutation rate for protracted irradiation of mature and maturing oocytes was then determined by fitting a weighted least-squares regression line to the frequencies for all six irradiation experiments and the control. Actually, four regression slopes were calculated, one set of two based on all the data and the other set excluding an experiment that used only old females. Each set had two fits, one for each of the two different estimates of the control mutation frequency.

294. The mutation rates estimated in this way for low-level irradiation of mature and maturing oocytes, when compared to the mutation rate for his 400-R acute irradiation data, yield ratios of 1/18, 1/24, 1/29 and 1/46. The ratios might have been even lower if the proportion of oocytes that received most of their dose in the first week before ovulation (when mutational sensitivity is somewhat less) had been as high in the chronically irradiated animals as it was in the acutely irradiated ones. In any case, with the more precise estimation of the effective doses than was available for the preparation of the 1972 UNSCEAR and BEIR reports, and with the addition of the new data of Lyon and Phillips, it turns out that the Committees' statements of a ratio of 1/20, for the relative effectiveness of chronic to acute irradiation, did not underestimate the risks from low-level irradiation of mature and maturing oocytes.

295. In his paper, Russell also compared the specific-locus mutation rates obtained for mature and maturing oocytes with the mutation rate for chronic irradiation of spermatogonia. The mutation rate, for the same seven loci, in mouse spermatogonia irradiated at dose rates of 0.0009 R/min and below, was calculated in the review paper by Searle (506) to be $6.59\ 10^{-8}\ R^{-1}$ per locus. The four rates for low-level irradiation of mature and maturing oocytes estimated by Russell are only 0.17, 0.27, 0.33 and 0.44 times as effective, and only in the highest of these is the induced rate in oocytes significantly above the control rate. Thus the ratio of effectiveness to the spermatogonial mutation rate could be zero. Similarly, the ratio of effectiveness to acute irradiation given in the previous paragraph could also be zero.

296. With regard to the validity of extrapolating mutation rates in mouse immature arrested oocytes to human immature arrested oocytes, which has been questioned because of marked differences in the sensitivity of these oocytes to cell killing, Russell indicates that his data have an indirect bearing on this problem. The mutation frequency is lower for the most mature oocytes, those which produced the offspring for week 1 in table 37, than for the less mature oocytes that produced the offspring listed for weeks 2-6. The fully mature oocytes are less sensitive to killing than the less mature ones. Thus here there is a positive correlation between killing and mutational sensitivity. That is in contrast to the negative correlation found for immature arrested oocytes, which give no evidence of mutation

induction but which are highly sensitive to killing. It appears from the lack of consistent correlation, that mutation induction and killing are independent events. In support of this view, Russell cites the new data of Cox and Lyon (121), on x-ray induction of dominant lethal mutations in mature and immature oocytes of guinea-pigs and golden hamsters. In both species, they found lower mutation yields from immature than from mature oocytes, despite the fact that the immature oocytes of the guinea-pig are less sensitive to killing than the mature ones, while the reverse is true for the golden hamster, which, therefore, resembles the mouse. Thus, as these authors state, there is "no general pattern ... of correlation, either positive or negative, in the sensitivity of oocytes to killing and to dominant lethal induction".

297. In Russell's view, there would now seem to be less reason than before for rejecting an application of data on the mouse immature arrested oocyte to the human immature arrested oocyte. In the immature oocytes, the specific-locus mutation frequency in the mouse and the dominant lethal frequency in guinea-pig and golden hamster are all low compared to the frequencies in mature oocytes. The fact that this is so, despite the tremendous differences in sensitivity to cell killing, and in chromosomal morphology, in the immature oocytes of these species, weakens the contention that the sensitivity to cell killing of the mouse immature oocyte is an argument against using it to predict the mutational response of the human immature oocyte.

298. Russell concludes that if, on the side of caution, one continues to consider the possibility that the human immature arrested oocyte might be as mutationally sensitive as the most sensitive of all oocyte stages in the mouse, namely the maturing and mature oocytes, then one can use his estimates of mutational frequency from low-level irradiation in these stages. The rates range from 0.17 to 0.44 times that in spermatogonia, but it should be kept in mind that in three of the four estimates the frequencies are not significantly above control values. Thus, even in the event that the human immature arrested oocyte does not respond like the mouse arrested oocyte, but more like the most sensitive oocyte stages in the mouse, it seems likely that the genetic hazard of radiation in the female will still be less than in the male.

4. Mutation processes at low and high radiation doses and dose rates: criticism of a current model

299. Since the discovery of the dose-rate effect with low-LET irradiation for the induction of specific-locus mutations in mice in 1958 (491), the question of whether the phenomenon mainly involves the repair of only two-track mutational events or primarily the repair of one-track events has been repeatedly discussed (473, 475, 477, 479). In their 1958 paper, W. L. Russell *et al.* pointed out that one-track events can show a dose-rate effect (and a supra-linear dose-effect curve at high dose rates) if the repair system is damaged or saturated at high doses and at high dose rates. For various reasons that have been enumerated in detail (473, 475, 477, 479; also discussed in the 1972 report, Annex E, paragraphs 182-195) the hypothesis of repair of one-track mutational events has been favoured.

300. In 1967, Wolff (617) listed several arguments (based essentially on dose-rate effects and RBE considerations) which in his opinion lend support to the view that mutations scored in specific-locus experiments are predominantly chromosome rearrangements that result from two-track events. More recently, this view has been modified in papers by Wolff (618) and Abrahamson (3) and Abrahamson and Wolff (4a) in which the mutationed lesions are assumed to be a mixture of one- and two-track events. Attention will be focused on the last paper since this contains the most definitive account of these authors' views.

301. Abrahamson and Wolff fitted the specific-locus data obtained in the mouse to an equation of the form $Y = C + \alpha D + \beta D^2$, where Y is the expected yield of mutations, C = control rate, D = the dose of sparsely ionizing radiations, and α and β the linear and quadratic coefficients, respectively. For the oocyte data, the α and β terms were estimated by a least-squares regression analysis of the data obtained at 50 R, 200 R and 400 R (acute x-ray exposures). The estimated values, if a cluster of six mutants was counted as one mutation, were $C = 1.67\ 10^{-6}$, $\alpha = 1.26\ 10^{-7}$ R^{-1} and $\beta = 1.14\ 10^{-9}$ R^{-2}. From these, the expected number of mutations in other experiments was computed. For spermatogonial data, Wolff and Abrahamson used all the low dose-rate data available (below 1 R/min to derive α, and this was then used with the 300-R acute x-irradiation data to derive β. The values so obtained were $C = 8.3\ 10^{-6}$; $\alpha = 6.88\ 10^{-8}$ R^{-1} and $\beta = 6.57\ 10^{-10}$ R^{-2}. These values were used to calculate expected frequencies of mutations in the other experiments.

302. Considering first the oocyte data, the predicted and observed mutation frequencies were similar except in three low dose-rate experiments (in which exposures lasted for 12, 20 and 31 days, respectively) and in an acute irradiation experiment from Harwell (312) in which the yield of mutations was lower than expected from Oak Ridge data. Abrahamson and Wolff suggested that the low observed frequencies in the chronic exposure experiments "are the consequence of some of the oocytes having been irradiated during the less mutable stages rather than resulting from repair of pre-mutational damage in a static cell population". The authors made some "crude corrections" to allow for this postulated admixture with less sensitive stages, which brought the expected numbers into reasonable agreement with the data.

303. Turning now to the spermatogonial data for acute irradiation, many discrepancies were found between observed and expected values. However, these were mainly in single or fractionated irradiation experiments with individual exposures of more than 500 R and with a general pattern of less than expected yields. The authors attributed these discrepancies to differential cell killing and selection, in line with previous views (e.g., ref. 470).

304. The main implications of this reanalysis for the evaluation of radiation hazards are the following: with sparsely ionizing radiations at low doses and low dose rates, it is the linear component that predominates, and the magnitude of the risk of induction of mutational

damage will be determined by this. For males, where the hazard evaluation is in fact based on the *observed* linear dose-effect relationship (506, 617) at low dose rates over a wide range of doses, the above reanalysis adds nothing new. For females however, the risk (on some methods of calculation) will be expected to be higher than what had been hitherto assumed by UNSCEAR (589) and BEIR (34).

305. The above model and, especially, the modifications of the mouse data which the authors perform to fit their model have been criticised by W. L. Russell (482, 483, 483a). With regard to the model itself, Russell points out that the data also fit his hypothesis, in which the mutational lesions themselves are one-track events, but multiple tracks are involved in damaging or saturating the repair process. The data will fit still other models. Therefore, Russell argues that the fit of the data to the model proposed by Abrahamson and Wolff does not prove their hypothesis to be superior to other hypotheses which also give acceptable fits. He contends that other criteria which he has discussed elsewhere (473, 475, 477, 479) provide more cogent evidence on the nature of the mutational lesions.

306. Russell's strongest objection is against the modification of the mouse oocyte data carried out by Abrahamson and Wolff. The two fits preferred by the authors fail to give good agreement with the low dose-rate experiments considered by them, essentially the data of Carter and Russell's 258-R and 400-R data shown in table 38. Abrahamson and Wolff recognize this and, in an extensive discussion, try to explain away the discrepancy by claiming that the effective doses in the chronic irradiation experiments are very low, much lower than the values calculated by Russell (paras. 293-294 and table 38). Russell claims that errors arose when they tried to compute the effective dose on the basis of number of oestrus cycles and an oocyte maturation scheme which, according to Russell, seems to have no basis in reality. Thus they compute that the effective dose in the Carter experiment would be only one half or less of the dose given. The irradiation time in this experiment was 12 days, and all matings occurred within two weeks of the end of irradiation. In view of the data in the table and the well known fact that maturation and loss of oocytes occurs at the same rate in breeding and non-breeding females (370a), Russell finds it hard to see how any of the oocytes in Carter's experiment could have received less than the total dose during the maturing stages that are sensitive to acute irradiation. It is also noted by Russell that the two mutations in the 283-R experiment in table 38 were from conceptions occurring in the 5th week after the end of irradiation, a period long after the time when Abrahamson and Wolff would have had the effective dose down to zero.

307. Although Abrahamson and Wolff include the new Lyon and Phillips data for a single 200-R dose of acute irradiation, they omit any consideration of the fractionated exposures. Russell states that if Abrahamson and Wolff had computed their expected value (assuming cluster = 0) for this set of data, it would have been 9.5 mutations expected where only 1 was observed. With a correct adjustment for effective dose they would still have predicted 7.8 mutations.

308. Abrahamson and Wolff, on the one hand, admit that their corrections for effective dose are crude, but on the other hand, conclude "it is reasonable to assume that the wide discrepancies [between their theoretical expectations and the actual data] are caused by some of the radiation occurring during less mutable stages". Since the data shown in table 38 have already been adjusted for this factor using Russell criteria, it is of interest to see how much discrepancy is still left between his corrected observed values and the Abrahamson-Wolff prediction.

309. Of the two Abrahamson-Wolff fits preferred by them, the slope of one (cluster = 0) exceeds the slopes of the four regression lines derived by Russell (paras. 293-294) by multiples of 4.8, 6.5, 8.0 and 12.7, and the slope of the other Abrahamson-Wolff line (cluster = 1) exceeds the slopes of the Russell lines by 4.3, 5.7, 7.1 and 11.2. Russell calculates that both Abrahamson-Wolff slopes are significantly above all the slopes of the actual data with a high degree of confidence. The lowest t value in these comparisons is 7.84, where the critical value of t for 5 per cent probability is 2.57.

310. Thus the Abrahamson-Wolff derivation from acute irradiation of the mutation frequency theoretically expected, on their hypothesis, for low-level irradiation exceeds the observed value by a factor of 4-13. Russell concludes that their approach is not a reliable one for estimating hazards.

5. Nature of specific-locus mutations

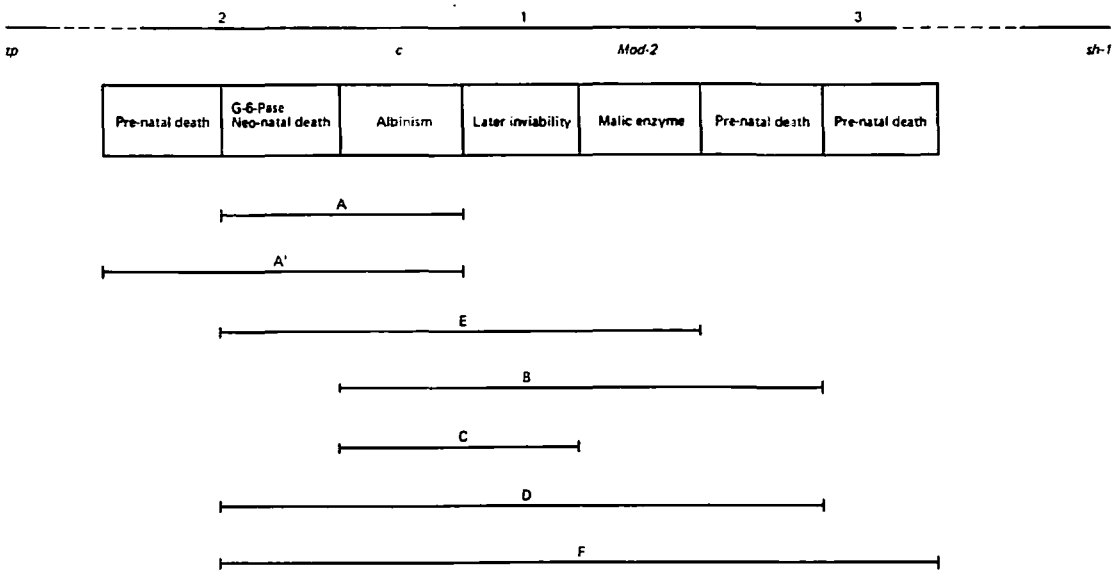
311. The 1972 report considered in some detail the work of L. B. Russell (453), who analysed, by means of complementation tests, the mouse chromosome 9 region between and surrounding *d*, *se* and *sv* (recombination frequency *d-se*, 0.16 per cent; *se-sv*, 2 per cent). This analysis revealed the existence of 16 complementation groups spanning eight or nine functional units. It was found that (a) there was a strong effect of the irradiated germ-cell stage, as well as 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 units as against that of two or more functional units; (b) the frequency of mutations interpreted as aberrations (deficiencies) ranged 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 oocytes; and (c) the recombinational length of most of the aberrations was very small, 75-80 per cent of them spanning less than two crossover units. Even in the groups that had a high total frequency of aberrations (post-gonial stages and oocytes), no more than 23 per cent of all mutations exceeded this length and the frequency was for x- and gamma-irradiated spermatogonia (excluding the 24-h fractionation group).

312. The type of analysis discussed in the preceding paragraph has now been extended by L. R. Russell to another locus, the *c* (albino locus), located in linkage group I, chromosome 7 (213); the mutations used in the present work, as in the earlier one on *d-se* were derived from the specific locus experiments of W. L. Russell and

co-workers and were recovered in the heterozygous state with the c^{ch} allelle carried by the tester stock. Since all but one of the 103 completely, and 12 partially, tested mutants are distinguishable in colour from c^{ch}, testing consists of crossing the original mutant to c^{ch}/c^{ch} and then intercrossing the heterozygotes, for tests on viability, and in the case of lethals, time of death.

313. The results available thus far show that (a) among the 9 control mutants, 8 were viable and 1 subvital in the homozygous condition; (b) among 50 mutants derived from x- or gamma-irradiation experiments (spermatogonia), 35 were viable, 3 subvital and 12 lethal in the homozygous condition (there was no significant effect of dose rate, fractionation or of x irradiation compared with gamma irradiation in the distribution of mutants in the three classes); (c) neutron irradiation of spermatogonia appears to produce a shift in the sense that now 7 out of 19 tested mutants are lethal, 1 subvital and 11 fully viable; and (d) the data for the post-spermatogonial and oocyte stages are limited and the numbers in the subgroups too small to compare the spectra between gamma or neutron irradiation, but, out of a total of 25 mutants tested, 12 are viable in the homozygous condition and the rest are lethal.

314. L. B. Russell *et al.* (454a, 464, 465) carried out complementation and deficiency-mapping tests on a total of 30 independent lethal mutations involving the *c* locus. It was found that these lethal mutants group themselves into at least seven complementation groups. The authors suggest that at least seven functional units must be postulated to account for all the interaction effects on a linear basis. The complementation groups have been designated as A, A', E, B, C, D, and F. Their properties and their relationship to the *c* locus and adjacent markers used in the complementation study can be diagrammed as follows:



(G-6-Pase: glucose-6-phosphatase deficiency; *Mod-2:* mitochondrial malic enzyme (*m*alate *o*xidoreductase *d*ecarboxylate); *tp:* taupe; *sh-1:* shaker-1).

315. Five of the *c*-lethal mutants (A-group) do not involve *Mod-2* (only 1 cM distant from *c*). However, the finding that complementation for neo-natal death can occur without complementation for albinism and separate from complementation for various kinds of pre-natal death suggests that even these mutants may be small deficiencies involving several cistrons. Since the neighbouring marker *tp* is not involved, their maximum length must be less than 3 cM. Among proven deficiencies, the two longest were at least 4 but less than 8 crossover units in length (454a). The remainder of the *c*-lethal mutations were at least 1 but probably much less than 6 cM long. It is likely that the 66 viable *c*-locus mutations found are not deficiencies.

316. Four of the 30 *c*-locus lethal mutants have been studied cytologically using both quinacrine-mustard and Giemsa-banding (454a). In one of these, a B complementation-group mutant, no loss of cytological material was detectable. The two F-group mutants (deficient for *sh-1* as well as *c)* were found to lack most of one of the major bands of chromosome 7 (the E band), one being slightly longer than the other. One of the D-group mutants lacks an amount of chromosome 7 that is almost equivalent in length, but is located more proximally, overlapping with the F-group mutants with regard to cytological sub-band E1. This serves to locate the *c*-locus cytologically within E1 and its flanking markers, *sh-1* and *tp* in E2 (or E3) and proximal to D1, respectively. This finding, and evidence from cytological studies of other chromosome-7 aberrations, indicate that relative cytological lengths in that region of the genome may not correspond closely with relative genetic lengths (454a).

317. Again, on the basis of complementation tests, Erickson *et al.* (160) and Gluecksohn-Waelsch *et al.* (195) concluded that all six lethal albino alleles that they examined which included three of Russell's, could be overlapping deficiencies of various sizes at and around the albino locus. The fact that no complementation could be observed in combinations of c^{25H} with any of the other five permitted these authors to infer that c^{25H} may be the biggest deletion of all.

318. Using Q- and G-banding methods, Miller *et al.* (338) demonstrated cytologically that the above inference is in fact true: in heterozygous animals, the two No. 7 chromosomes could be distinguished from one

another, and the one carrying c^{25H} was found to be 7.6 ± 1.5 per cent shorter than its homologue, providing a direct estimate of the size of the deletion. Since the total genetic length of the mouse genome is about 1250 cM (589) and since chromosome 7 makes up about 5.4 per cent of the haploid genome, a deletion of 7.6 per cent of this chromosome might be expected to be about 5 cM long (1250 X 5.4% X 7.6%); this value is in the range of length based on the available genetic recombination data. However, there are indications that cytological length may not be a good indicator of genetic length for this portion on the genome (see paragraph 316).

6. Autosomal recessive lethals

319. Since the publication of the 1972 report, some new data on recessive lethals in mice (spontaneous and radiation-induced) have become available (298, 299, 300, 416). In addition, several specific problems and statistical difficulties involved in the design and evaluation of experiments on autosomal recessive lethals have become apparent, such as the presence and detection of pre-existing lethals in inbred strains and in the control and experimental groups derived from them, the basis and magnitude of fluctuations from generation to generation in the frequency of heterozygotes for recessive lethals, and what steps need to be taken to make a proper assessment of spontaneous and induction frequencies of these lethals (269, 299, 493).

320. In a recent paper on testing for autosomal recessive lethals, Lüning (198) elaborated further on the methodology that he described earlier (296), with a reconsideration of several aspects that are involved. Among these may be mentioned (a) the identification of individual lethal heterozygotes, (b) the identification of families carrying pre-existing recessive lethals by analysing 10 or more F_1 brothers, and (c) the variance of the rate of heterozygosity for recessive lethals in inbred strains in successive generations and its relevance for estimates of the mutation rate per genome.

321. The general scheme for individual and familial identification of recessive lethals can be diagrammed as follows:

P_1 generation ♂ X ♀

↓

F_1 generation ♂ X ♀♀♀ (at least 10 F_1 sons per parental cross to be tested)

↓

F_2 generation ♀♀♀ X F_1 father (backcross)

Uterine contents of pregnant females are examined to assess the amount of pre-natal mortality; identification of heterozygosity for lethals dependent on the rate of intra-uterine mortality, which is expected to be higher in crosses where a lethal is involved, than in those where it is not.

If an F_1 male is heterozygous for a recessive lethal, then 50 per cent of his progeny will carry the same lethal, therefore, in a backcross involving father-daughter mating, the expected mean mortality is 12.5 per cent over and above the normal mortality of 8 per cent, or a total of 100–(0.875)92 = 19.5 per cent. In practice, this involves the identification of each individual case as belonging to a distribution with a mean mortality of 8 or 19.5 per cent, the precision of which is dependent on the number of implants examined per backcross.

322. The model presented by Lüning (298) enables the identification of lethal heterozygotes and lethal-free animals in the backcross test of F_1 males to their respective daughters. According to the model, when a minimum of 50 implants (50-60; mean, 53) are analysed for each backcross of the F_1 male to his daughters, those which gave 9 or more dead are judged to be heterozygous for a recessive lethal; those crosses which resulted in 4 or less dead are considered lethal-free. An increase of the number of implants analysed increases the accuracy of the above procedure to identify lethal heterozygotes and lethal-free animals.

323. It is known that in inbred strains of mice there are large variations in the spontaneous frequencies of autosomal recessive lethals between one generation and the next. The earlier analysis by Lüning and Searle (302) of three sets of control data (controls for radiation experiments) revealed considerable differences in frequencies between them, and the authors estimated that the mean rate was $2.9\,10^{-3}$ with an upper 95% confidence limit of $6.5\,10^{-3}$ per gamete. In a subsequent paper, Lüning (299) examined the control data accumulated over the years 1964-1971 in his laboratory (and which pertain to four different series, including the three that were considered by Lüning and Searle) and concluded that the mean rate was $5\,10^{-3}$ per gamete with an upper limit of $1\,10^{-2}$. In relatively small experiments involving three strains of mice (CBA, C3H and 101) and their hybrids, Lyon (306) likewise found variability in the frequencies of autosomal recessive lethals and stated that "allowing for sampling error, the results are not incompatible with the hypothesis that in all three strains, recessive lethal genes arise with a frequency of 0.1 per gamete" (an approximate upper limit).

324. Haldane (211) first drew attention to the fact that, although spontaneous recessive lethals arise at a finite rate, the average frequency of heterozygotes for lethals can vary within a strain depending on how the parents are selected to prepetuate the strain and dealt with the problem from a theoretical standpoint. In the case where a pair of full-sib parents heterozygous for the same recessive lethal leave three quarters as many offspring as the others not carrying the lethal, he showed that the average accumulation of lethal heterozygotes is equal to $9.3\,\mu$ where μ is the mutation rate per genome. In the extreme case where the number of offspring from heterozygous parents is not reduced at all, the average accumulation would be equal to $12.7\,\mu$. At the other extreme, where the heterozygous parents leave no offspring, Lyon (306) showed that the average accumulation would equal $6.0\,\mu$. Thus, depending on the breeding scheme, the average frequency of lethal heterozygotes in an inbred strain would be between 6.0 and $12.7\,\mu$. These calculations, however, are based on

the assumption that the strain in question is constituted by an "infinite" number of breeding pairs, a theoretical situation. In practice, due to economic and spatial reasons, a given strain is propagated with only a limited number of breeding pairs. This restriction will cause drastic fluctuations in the frequency of heterozygotes for lethals in the strain when sampling is done at different periods of time, as has actually been observed in the experiments discussed by Lüning and Searle (302) and by Lüning (299).

325. Ryman (493) conducted a computer-simulation study to investigate the magnitude of temporal variation in the frequency of heterozygotes for autosomal recessive lethals in inbred strains, a problem to which little attention has been devoted so far. The simulation studies were performed with special reference to the mouse with the following assumptions: (a) the number of breeding pairs for strain continuation is 10, 20 or 50; (b) each pair produces 1 or 5 litters in every generation; (c) the minimum number of live-born young from a pair whose offspring can be selected to continue the inbred strain is 6 or 0 (1-litter situation) and 30 or 0 (5-litter situation) (The offspring not used to form breeding pairs in the strain were "surplus" and used for "experimentation", and the frequency of lethal heterozygotes was determined in the "surplus" group.); (d) the sex ratio is 1 to 1; (e) the mutation rate of autosomal recessive lethals per generation and the pre-weaning mortality are 0.5 and 16 per cent, respectively, these values being the long-term averages for the inbred CBA strain in the Stockholm Laboratory of Radiation Genetics; (f) lethal homozygotes die before birth.

326. The computer was programmed to trace the temporal pattern (over 200 or 500 generations) in the frequencies of heterozygotes for lethals under the different conditions mentioned above and to estimate the means and variances. The results showed that, with the 200-generation trials with "surplus" animals, there were large and sudden changes in the frequencies of lethal heterozygotes (0-40 per cent) under conditions when the number of breeding pairs per generation was 10. This variation became much less (0-12 per cent) when the latter was 50. The means over all generations and their respective variances were 0.046 (4.6 per cent) and 0.00573 with 10 breeding pairs and 0.038 (3.8 per cent) and 0.0006 with 50 breeding pairs. The estimated variance of the differences between consecutive generations was, as would be expected, much higher under the first set of conditions than under the second (0.00878 compared with 0.00092).

327. The results of simulations for 500 generations (with different maximum number of breeding pairs for the continuation of the inbred strain) revealed that with a low number of breeding pairs (such as 10), the variance of the mean frequency of lethal heterozygotes, as well as that between successive generations, is quite large, irrespective of the number of litters per pair and of whether selection against small litters is practised or not. Furthermore, the number of generations in which there were no lethal heterozygotes ("lethal-free" generations) was also appreciable. An increase in the number of breeding pairs and/or an increase in the number of litters per pair leads to a decrease in the variation and the number of lethal-free generations also becomes reduced.

328. The practical implications of these findings are the following:

(a) Since the maintenance of an inbred line is not an end in itself, great care must be taken in choosing an optimal breeding régime in relation to the type of experiments that are proposed to be conducted;

(b) Since heterogeneity within an inbred line may be considerable, the use of an inbred strain does not automatically guarantee a homogeneous genetic background;

(c) Estimates of spontaneous or induced rates of mutations to recessive lethals will be reliable only when the experiment is repeated at different time intervals or when pre-existing mutations are identified and taken into consideration;

(d) If the strain is not very large, the replications are expected to yield statistically heterogeneous results and it is justifiable to pool the results obtained with a given strain at different time intervals to get a realistic estimate of mutation rates, as was done earlier by Lüning and Searle (302) and by Lüning (299).

329. New data on the x-ray induction (500 R acute) of autosomal recessive lethals in mouse spermatogonia come from the experiments of Lüning and Eiche (300) and those of Pomerantzeva *et al.* (416). Lüning and Eiche used the technique outlined in paragraph 310 and found that the rate of induction (after correcting for the spontaneous rate of $0.5\ 10^{-3}$ per gamete) was $0.9\ 10^{-4}$ rad^{-1} per gamete. This is in complete agreement with the earlier estimate made by Lüning and Searle (302) based on three different sets of data (see the 1972 report for details). In the work of Pomerantzeva *et al.* (416) a total exposure of 900 R of gamma irradiation was administered to male mice in three equal fractions separated by 4-week intervals, and the frequency of autosomal recessive lethals was determined. In one of the two experiments, the induced frequency was 22.65 per cent (or $2.5\ 10^{-4}\ R^{-1}$) and in the other, 5.4 per cent (or $0.6\ 10^{-4}\ R^{-1}$). Pooling the controls and likewise pooling the radiation data, the induced frequency can be estimated as 9.93 per cent which corresponds to a rate of $1.1\ 10^{-4}\ R^{-1}$. As will be obvious, this rate is quite close to that recorded by Lüning and Searle (302) and by Lüning and Eiche (300).

7. Dominant mutations

330. Since the publication of the 1972 report, some new data have become available from studies on dominant mutations in mice. These pertain to the work of Selby and Selby (528), who investigated the gamma-ray induction and transmissibility of dominant mutations affecting the skeleton. Before considering the results of Selby and Selby, it is profitable to examine the background of this kind of work and place it in the perspective of earlier work done in this area.

331. The rationale was, and continues to be, based on the following considerations:

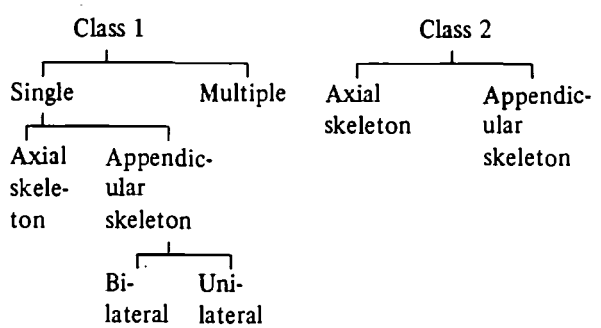
(a) For the evaluation of genetic radiation hazards to a population, it is desirable to obtain more information on the frequency of induced dominant mutations;

(b) Information on dominant visible mutations which has been generated as a by-product of mutation rate studies with specific loci continues to be limited;

(c) The skeleton is a complex system formed over an extended period of development and differentiates in specific ways in various parts of the body to form permanent structures. It is therefore subject to modification by gene action at very many loci;

(d) Consequently, studies on the radiation induction of dominant genetic changes affecting the skeleton are useful because they uncover a portion, perhaps a substantial one, of the overall genetic damage induced by irradiation.

332. In their first studies, Ehling and Randolph (147) showed that, in skeletal preparations of F_1 progeny of irradiated male mice (x rays and neutrons; pre- and post-meiotic germ-cell stages), it was possible to distinguish between the effects of newly induced skeletal variation and that which is normally present in the strains employed. Thus, they demonstrated the feasibility of using skeletal abnormalities as a criterion of radiation-induced, presumed dominant genetic damage and set the stage for further work (149, 150). The abnormalities were classified into Class 1 (those that occurred only once in the whole experiment) and Class 2 (those that occurred more than once). The further subdivision of these classes can be diagrammed as follows:



333. Since the sample sizes in these experiments were such that not more than one mutation would be expected from any particular gene locus, it was thought that the classification into Class 1 and Class 2 would provide for the optimum separation of the existing natural variation from that caused by newly occurring genetic changes. The results showed that there was a statistically significant increase in the incidence of Class 1 abnormalities after irradiation; among these, multiple abnormalities (those with at least one Class 1 abnormality plus others, regardless of whether Class 1 or Class 2) and single bilateral abnormalities of the appendicular skeleton were considered to provide the most sensitive indicators of mutational damage. There was no significant increase in Class 2 abnormalities after irradiation.

334. In the earlier experiments of Ehling (149, 150), since the F_1 mice were killed for making skeletal preparations at four weeks of age, breeding tests of the presumed mutants could not be carried out. Subsequently, however, Ehling (151) designed experiments to permit such breeding tests, the F_1 offspring being sacrificed only after they had produced a litter; 3 out of 5 presumed mutations were found to be transmitted to the second and later generations. In similar work carried out by Tutikawa (quoted in the Committee's 1972 report) in which 11 presumed dominant skeletal mutations were observed, 2 which showed externally visible effects were given breeding tests and both were found to be transmitted. Since the transmission of only a few skeletal mutations had been studied at the time of writing the 1972 report and the transmission pattern of a majority of them did not permit an unequivocal conclusion of dominance, the Committee stated, "It seems probable that many of the presumed dominant mutations may be heterozygous manifestations of recessive mutations".

335. In their work Selby and Selby (528, 528a) focused attention on the problem which had hitherto not been studied in depth, namely, that of the transmissibility of the presumed dominant mutations affecting the skeleton. Male mice received a fractionated gamma-irradiation exposure (100 R + 500 R, separated by 24 h; 60 R/min; the choice of this radiation régime was dictated by Ehling's finding (150) that this gave maximal response for spermatogonial irradiation). The F_1 sons sired subsequent to the sterile period were processed for examination of their skeletons after they were allowed to produce progeny. Thirty-seven of the 2646 F_1 males (1.4 per cent) were judged to have dominant mutations that caused one or more rare skeletal abnormalities; 31 of these were shown to be mutants by breeding tests and the remaining 6, having no progeny, were counted as mutants based only on criteria supported by the data. The frequency of 1.4 per cent recorded in this study is similar to the 1.8 per cent (5/277) obtained by Ehling (150) under similar radiation conditions.

336. In breeding tests, Selby and Selby found that the dominant mutations affecting the skeleton showed variable expressivity and incomplete penetrance for many or all of the effects that they caused. A number of them severely affected viability. With the experimental procedure used, both incomplete penetrance and decreased viability would have caused an underestimate of the mutation rate. Thus, some of the F_1's would not be counted as mutants because they do not show the effect of the mutation they carry and others would not be counted because the mutation they carried caused their death before they could be examined.

337. In comparing the results of Selby and Selby (528) with those of Ehling (149, 150), the following comments are in order. Ehling expected that a critical determination of which of the skeletal abnormalities are actually heritable would reveal that his criteria for detecting presumed mutations would, on the one hand, miss some mutations altogether and, on the other hand, wrongly classify some abnormalities as mutants when in fact they were not. The degree to which these errors might occur was not known at the time Ehling

conducted his studies, but it was hoped that they would not be large. The finding of only one presumed mutation in the control group of Ehling (1/1739) indicated that the second kind of error could not have contributed substantially to the large number of presumed mutations observed in the experimental groups. The work of Selby and Selby (528) indicates that, as expected, the use of Ehling's criteria would lead to some false positives and some false negatives when estimating the mutation rate. It is difficult to estimate, from the Selbys' data, the extent of the two kinds of error, but it appears that they might have approximately balanced each other, since there is close agreement between the total rates obtained by Ehling and the Selbys.

338. The mutation frequency in the experimental series of Selby and Selby is 1.4 per cent. No controls were run, since the main thrust of their work was to find out if the general types of skeletal abnormalities classified as presumed mutations by Ehling's criteria would in fact be transmitted to descendants. However, the authors point out that, although a reliable estimate of the spontaneous mutation frequency needs to be made, Ehling's results indicate that this frequency is very low and would probably not appreciably affect the assumption that 1.4 per cent is close to the induced rate. The authors further conclude that, for the reasons given in paragraph 324, taking 1.4 per cent as the induced rate is more likely to be an underestimate than an overestimate of the true value.

339. While it is true that the number of dominant mutations of spontaneous origin present in any generation must be considerably higher than the spontaneous mutation frequency per generation, owing to accumulation in the ancestral mouse populations, it is important to realize that the experiments of Ehling and of the Selbys were designed to exclude most, if not all, such accumulated mutations in the mutation frequencies recorded in the F_1. For example, in Ehling's experiments any abnormalities caused by mutations accumulated in the stocks should have occurred more than once in his large samples. Thus, they would have been classified as Class 2 abnormalities, and the animals containing them would have been considered non-mutants. In the Selbys' experiments, skeletal examination of the parents, as well as the sibs, of the mutant individuals was performed and supported the conclusion that most, and perhaps all, of the reported mutations were of new origin.

340. In humans, partial trisomies and monosomies are often associated with skeletal abnormalities (see, for instance, references 208 and 349). Although it cannot be ruled out that some of the skeletal abnormalities in the mouse may have resulted from partial trisomies and monosomies, it is unlikely that such chromosomal aberrations made an important contribution to the number of mutations reported, because most of these aberrations are male sterile and derive from spermatozoal rather than spermatogonial irradiation (507). Thirty-one of the 37 mutants reported in the Selbys' experiment proved to be fertile.

341. Many of the anomalies found in the above study are similar to rare dominant and irregularly inherited dominant conditions in man. As will be recalled (chapter I), regular dominant and irregularly inherited dominant diseases (together with multifactorial ones and congenital anomalies) constitute a very substantial proportion of human disorders. Studies on the effect of radiation in inducing mutations affecting the skeleton of mice may thus lead to an independent and direct estimate of the rate of induction by radiation of dominant mutations in man.

342. Bartsch-Sandhoff (27) studied the incidence of skeletal abnormalities in mouse embryos 19 days old derived from germ cells of irradiated males (600 R of ^{137}Cs gamma irradiation). This work was undertaken to verify the presumption that some of the skeletal mutations may be incompatible with post-natal survival and would not have been represented in the four-week-old mice examined in the earlier experiments of Ehling and Randolph (147) and Ehling (149, 150). It must be stressed that not all Class 1 abnormalities in four-week-old mice are presumed dominant mutations as determined by Ehling, and that only the total frequency of Class 1 abnormalities is comparable with the data of Bartsch-Sandhoff.

343. The small sample sizes in the Bartsch-Sandhoff investigation permit only limited quantitative conclusions. The incidence of Class 1 anomalies for the irradiated postspermatogonial stages (9/371) is significantly higher than that for all unirradiated controls (5/1190 in 19-day foetuses; 14/1739 in four-week-old animals), and not significantly different from the incidence (18/569) scored in four-week-old mice for the same irradiated cell stages. The frequency after spermatogonial irradiation (4/343) does not differ significantly either from the controls or from the corresponding frequency in four-week-old mice (13/597). However, there was an important qualitative conclusion. In the 19-day foetuses there were several new abnormalities hitherto undetected in four-week-old animals, some of which were so drastic that they could conceivably have caused death before four weeks of age.

344. Comparing the results of the Bartsch-Sandhoff study with those obtained by the other investigators, it can be concluded, on the one hand, that Ehling and the Selbys would not have seen those skeletal abnormalities which, although apparent in foetal stages, were too severe to survive to the ages studied by them. On the other hand, most of the anomalies scored by Ehling and the Selbys would not have been detected in the 19-day foetuses, owing to the limited degree of ossification present at that stage. Thus the total load of dominant skeletal abnormalities induced by radiation is likely to be greater than the number which can be detected at any one particular stage.

8. Summary and conclusions

345. In the germ cells of new-born male mice, mutations at seven specific loci are induced by x rays at the rate of $1.37\ 10^{-7}\ R^{-1}$ per locus, which, statistically, is significantly lower than the $2.91\ 10^{-7}\ R^{-1}$ per locus recorded for spermatogonia in adults (300-R level in both). In young males 2-35 days old, the rate is $2.63\ 10^{-7}\ R^{-1}$ per locus.

346. When a total x-ray exposure of 1000 R is administered to male mice in two equal fractions (spermatogonial exposures) separated by intervals of 4 and 7 days, the frequencies of specific locus mutations observed are similar to those seen in long-term fractionation experiments (interval between fractions about 15 weeks) and are consistent with the additivity of yields from two separate fractions. These frequencies, however, are higher than those after a single exposure of 1000 R and lower than that after two fractions of 500 R separated by a 24-h interval.

347. The limited data that have been collected on the radiation induction of biochemical variants using nine electrophoretically detectable markers in the mouse show that such mutations can be induced in spermatogonia. Likewise, studies on the induction of haemoglobin variants (at two loci) by x-irradiation of mice (spermatogonia, post-spermatogonial stages and oocytes) have yielded positive results: a total of five haemoglobin variants have thus far been found in a sample of over 8000 F_1 animals screened.

348. In mouse spermatogonia, the rate of x-ray induction of mutations in a large group of histocompatibility loci is very much lower than that at the seven specific loci.

349. The reanalysis of the data on dose-rate effects for the induction (by low-LET irradiation) of specific locus mutations in mouse spermatogonia by Lyon and colleagues raised the possibility that at dose rates below 0.03 rad/min the mutation rate might be higher than that at 0.8 rad/min. Recent work that is being carried out by W. L. Russell at Oak Ridge to test this (at rates of 0.0007 R/min and 0.0056 R/min, 300-R gamma-ray exposure) shows, however, that in over 40 000 and 74 000 F_1 progeny so far scored at these two exposure rates, the mutation frequencies are the same.

350. Histological studies carried out by Oakberg and Palatinus demonstrate that the survival of type A_s spermatogonia (stem cells) is practically unaffected when a gamma-ray exposure of 300 R is delivered to the testis at a rate of 0.0007 R/min, whereas it is reduced to 37 per cent of that in controls when the irradiation is delivered at 0.0056 R/min. This result, viewed in conjunction with the mutation data reported in the preceding paragraph, suggests that the cell-killing interpretation of Lyon *et al.* proposed to explain the possible differential yield of mutations at low and lower dose rates may not be tenable.

351. In female mice, fractionation of an x-ray dose of 200 rad into several small fractions leads to a very pronounced reduction (an order of magnitude or more) in the yield of specific-locus mutations relative to unfractionated irradiation. These data, along with the earlier data on chronic irradiation of the same oocyte stages, indicate that the mutation rate in mature and maturing oocytes is lower than that of spermatogonia. The results therefore support the conclusion that, even if the mutational sensitivity of the human immature arrested oocyte is as large as that of the most sensitive oocyte stages in the mouse (the mature and maturing ones), the genetic hazards, under most conditions of human exposure, will be less in the female than in the male. At the same time, there appears to be stronger evidence than before for assuming that the immature arrested oocyte in the human may, like the same stage in the mouse, have a mutational sensitivity which, at most, is much less than that of the male.

352. Abrahamson and Wolff have attempted to fit the data on radiation-induced specific-locus mutation frequencies observed in mice to a simple quadratic equation. For chronic exposures of mature and maturing oocytes, the quadratic fit gave much higher expected frequencies than those actually observed. The authors attributed these poor fits to an admixture of mutationally less sensitive immature oocytes into the sampled population. However, W. L. Russell has shown, in several separate experiments, that the duration of the sensitive stage is longer than that assumed by Abrahamson and Wolff and that, therefore, their argument is not tenable. Thus, the mutational response, even of this sensitive stage, is low under conditions of protracted irradiation, and is not as high as theoretically predicted in the fit of Abrahamson and Wolff. In any case, this stage is not likely to be the best model for assessing the mutational response of the human immature arrested oocyte.

353. The analysis of the nature of specific-locus mutations (spontaneous and radiation-induced) which had earlier been carried out for mutations at the *d-se* region have now been extended to the *c* locus. A majority of the *c* locus mutations derived from x- or gamma-irradiation experiments (spermatogonia) are homozygous viable. Complementation tests have revealed that the lethal mutants group themselves into seven complementation groups.

354. Recent work on mice with spontaneous rate of origin of autosomal recessive lethals and their induction by x irradiation has produced results which confirm those presented in the 1972 report of the Committee: they show that the spontaneous rate is about $0.5\ 10^{-2}$ per gamete and, at the 500-R level, the rate of induction is $0.9\ 10^{-4}\ R^{-1}$ per gamete. In addition, computer-simulation studies designed to investigate the magnitude of temporal variation in the frequencies of heterozygotes for autosomal recessive lethals in inbred strains have shown that the frequencies can vary over an enormous range in the different generations and that the use of an inbred strain does not automatically guarantee genetic homogeneity. Because of this, in experiments aimed at determining spontaneous or induced frequencies of recessive lethals, care must be taken to ensure that the strain (from which the animals are derived for experimentation) is maintained with an adequate number of breeding pairs in every generation and furthermore, the experiments ought to be repeated at different time intervals.

355. Substantial progress has been made in the study of radiation-induced skeletal mutations in the mouse. The data show that the frequency of presumed mutations detected in the earlier work of Ehling is closely paralleled in studies by Selby and Selby in which the

actual inheritance of skeletal abnormalities of a similar type was determined by breeding tests. Another finding of considerable importance is that most of the mutations have pleiotropic effects with various degrees of penetrance.

G. EFFECTS OF INCORPORATED RADIOISOTOPES

356. In its 1966 report (587) the Committee briefly considered the genetic effects of internally deposited radioisotopes such as ^{90}Sr (in mice), ^{14}C and ^{3}H (as tritiated thymidine and tritiated deoxycytidine (in *Drosophila*)), and in the 1969 report (588) some attention was given to the incidence of chromosome aberrations in humans occupationally exposed to radioactive substances. Since then, interest in the study of effects of radioisotopes has increased, in part stimulated by the growth and anticipated expansion of the nuclear power industry. Results of experiments with mice on the genetic and cytogenetic effects of ^{239}Pu and ^{3}H have now become available, and these will be discussed in the following paragraphs.

1. Plutonium-239

(a) *Distribution of ^{239}Pu in the mouse testis*

357. Green *et al.* (199) studied the distribution of intravenously-injected ^{239}Pu (as a solution of the citrate salt) in the mouse testis. Four or 12 weeks after injection (a mean activity of 88 μCi per mouse, 3.2 mCi/kg body mass), the mice were killed and their testes suitably processed for radiochemical measurements and autoradiography. It was found that the amount of ^{239}Pu in the testes was roughly the same with the two intervals used (mean amount, (280 ± 45) pCi/g of testis and (350 ± 38) pCi/gm of testis after 4 and 12 weeks respectively). The results confirmed the earlier findings of Ullberg *et al.* (585) in demonstrating that the distribution of ^{239}Pu in the testis was inhomogeneous: in autoradiographs, about 90 per cent of the plutonium seemed to be deposited within the intertubular spaces and in the peritubular tissues surrounding them. Of the 12 000 alpha tracks counted, 42 per cent lay above the peritubular membrane or adjoining the layer of cells containing the spermatogonial stem cells, 47 per cent lay entirely above the intertubular spaces, and 11 per cent lay entirely within the peritubular membranes, but excluding those in the first group.

358. On the assumption that the testis consists of a closely packed series of cylinders of uniform diameter (the experimentally determined mean diameter was 206 μm), the authors calculated that the 10-μm shell of tissue surrounding each tubule and containing the spermatogonial stem cells constituted 17 per cent of the whole testis mass, yielding an estimated dose-inhomogeneity factor of 42/17 = 2.5, i.e., the radiation dose rate to spermatogonial stem cells is higher by a factor of 2.5 than the average for the testis as a whole, calculated directly from the total amount of ^{239}Pu deposited.

(b) *Induction of post-implantation loss through ^{239}Pu treatment of male mice*

359. Lüning and Frölen (301) and Lüning, Frölen and Nilsson (304) have reported on the induction of dominant lethals in male mice following the injection of plutonium salts. In the first series of experiments (E1 and E2), in which the males received ^{239}Pu nitrate solution (intravenous injection), there was no evidence of any significant increase in intra-uterine mortality over that observed in controls (10 255 and 7216 implants were analysed in the experimental and control groups, respectively). The situation was different however, when a solution of the citrate was used (experiments E3, E4 and E5). In these studies, the effects were examined over the range 0.05-0.5 μCi per mouse. After injection, each male was mated to three females each week for up to 24 weeks. Appropriate controls were run. The pregnant mice were dissected 18 days after the commencement of the matings for examination of their uterine contents. The number of living and dead implants was recorded; the latter group was further divided into early deaths (no foetus discernible) and late deaths (dead foetuses). In certain groups, the pregnant mice were allowed to go to term and the F_1 male progeny were used in dominant lethal and semi-sterility tests.

360. The data on the induction of dominant lethals in plutonium-injected males are given in table 39. The figures given are pooled over successive weeks. It can be seen that in the plutonium-injected groups, there is a significant excess of intra-uterine mortality, and this is unrelated to the amount of injected plutonium per male (range, 0.05-0.5 μCi). When the results of all the plutonium series (E3, E4 and E5) were combined (ignoring the amounts of Pu injected) and the pattern of mortality over the successive weeks was examined (figure 2 in reference 304), it was found that the mortality increased from an initial value of about 9 per cent (first week) to about 12-13 per cent during the fifth week, after which the changes were small and non-significant. The sperm utilized during the first five weeks would have been in post- and peri-meiotic stages at the time of injection while those utilized subsequently would have been in earlier stages. It thus appears that the increased mortality observed during the first five weeks is due to the induction of dominant lethality in post- and peri-meiotic stages of sperm development.

361. Another interesting finding that emerged from the analysis of the weekly records of mortality in the plutonium series was that there was a trend for an increasing proportion of late deaths in later weeks, one which was opposite to that found by Bateman (29) with x irradiation (200 R). The causal basis for the increase in late deaths is unknown at present.

362. Dominant lethal tests performed on F_1 males sired by fathers which received plutonium injection (and derived from matings during the ninth, fourteenth and sixteenth weeks) showed that here again there was (a) an increase in intra-uterine mortality relative to controls and (b) a trend for an increase in the proportion of late deaths.

363. In studies primarily aimed at comparing the cytogenetic effects of protracted exposures to alpha particles from ^{239}Pu and to gamma rays from ^{60}Co, Searle *et al.* (516) collected some data on the induction of dominant lethals. In the plutonium series, male mice were intravenously injected with ^{239}Pu citrate solution (4 μCi/kg body mass, or about 0.1 μCi per mouse) and kept for 21, 28 or 34 weeks. The estimates of the absorbed gonadal dose and dose rates (based on radioactivity counts and testis mass determinations at the end of the exposure periods) were 13 ± 1 rad (0.090 rad/day), 18 ± 2 rad (0.095 rad/day) and 18 ± 1 rad (0.088 rad/day), respectively. The means were 17 ± 1 rad and 0.088 rad/day. (The fraction of injected plutonium actually retained in the testis was unchanged from 21 to 34 weeks at 0.027-0.028 per cent.) In the gamma-ray series, the exposure period was 28 weeks, with an accumulated dose of 1128 rad delivered at a rate of 0.004 rad/min.

364. In both series, the irradiated males were mated to females during the last four weeks of exposure, for dominant lethal tests. In the gamma irradiated series, there was good evidence for the induction of dominant lethality, with a marked increase in both pre- and post-implantation losses, relative to controls. The induced frequency of post-implantation losses was 12.9 per cent. In the plutonium experiments, there was no significant heterogeneity between the amounts of embryonic lethality for the three exposure periods. The pooled data on post-implantation mortality suggested a slight non-significant increase, relative to controls. If the data are taken at their face value, the frequency of induced dominant lethality is 4.6 per cent.

365. Since nearly all the induced dominant lethality in the irradiation series would be expected to arise in the meiotic and post-meiotic stages, the frequencies mentioned above can be divided by the gonadal dose received in this maturation period of 28 days (mean doses of 161 rad in the gamma series and 2.5 rad in the plutonium series). The rates obtained are: $8.6\ 10^{-4}$ rad^{-1} per gamete (gamma) and $1.9\ 10^{-2}$ rad^{-1} per gamete (alpha). At face value, these results thus suggest that irradiation with alpha particles from plutonium is about 22 times as effective as chronic gamma irradiation for the induction of dominant lethals in the germ-cell stages mentioned. It should be noted that the alpha-particle dose of 2.5 rad is that to the testis as a whole. The dose to the germ cells at risk may be different from this because of dose inhomogeneity resulting from preferential deposition of plutonium in the interstitial tissue (199) but cannot be calculated at present.

(c) *Cytogenetic effects of ^{239}Pu in male mice*

366. In their first studies, Beechey *et al.* (33) investigated the cytogenetic effects of intravenously injected ^{239}Pu (10 μCi/kg body mass in 1% trisodium citrate solution, about 0.3 μCi per mouse). The mice were killed 6, 12 and 18 weeks after injection and their testes were used for radiochemical determinations (left testis) and cytogenetic analysis (right testis). Control mice injected with the citrate solution alone were processed likewise.

367. It was found that the fraction of injected plutonium retained in the testes changed very little over the period of the experiment; therefore, the radiation dose was taken to be the product of the assay-derived dose rate (≈0.0002 rad/min) and the time interval between injection and killing. The estimated doses and the results of cytological analysis of the spermatocytes are summarized in table 40. It can be seen that (a) although the frequency of cells with fragments was higher in each experimental group than in the controls, the difference between the overall fragment frequency after treatment, 3.4 ± 0.7 per cent, and the control frequency, 1.3 ± 0.7 per cent, did not quite reach a significant level ($P = 0.07$); (b) there is a significant increase in the frequency of cells with quadrivalent configurations in the plutonium series; while most of these were rings and chains of four arising from the chromosomal reciprocal translocations in spermatogonia (as in earlier low- and high-LET radiation studies), a few were similar to those observed after x irradiation of oocytes in female mice (513) and were considered to be the result of chromatid interchanges in spermatocytes during meiotic prophase; and (c) the yield of quadrivalents declines at the longest exposure period of 18 weeks.

368. The data on reciprocal translocations were used by Beechey *et al.* to obtain a rough estimate of the RBE of alpha particles relative to chronic gamma and neutron irradiation (0.7 MeV). For this purpose, only the data pertaining to the 6- and 12-week periods were used (the 18-week data were excluded since it was thought that the decline in frequency might be connected with preferential killing of the sensitive spermatogonial cells). To estimate the period of spermatogonial exposure, the authors subtracted 13 days from the actual exposure period, the former being the time taken for the germ cells to pass through meiosis to metaphase-I. When the "amended" doses of 10 and 25 rad (instead of 14 rad and 30 rad; see table 40) are used and linearity of response is assumed, the rates of translocation induction at the 6- and 12-week intervals are, respectively, $1.0\ 10^{-3}$ rad^{-1} and $1.9\ 10^{-3}$ rad^{-1}, with a mean of $1.45\ 10^{-3}$ rad^{-1} of the average *testis doses*. However, Green *et al.* (199) found that the alpha particle dose to spermatogonial stem cells was 2-2.5 times that to the testis as a whole in their experiments, after ^{239}Pu injection. Assuming a factor of 2.5 in the present experiments (with similar experimental conditions to those of Green *et al.*), one obtains a rate of induction of $0.58\ 10^{-3}$ rad^{-1} of spermatogonial alpha particle dose. This is very similar to that of $0.53\ 10^{-3}$ translocations per rad found after chronic exposures of mouse spermatogonia to fission neutrons of mean energy 0.7 MeV (518), which suggests that these two high-LET radiations have similar RBEs.

369. In the next set of experiments from the same laboratory (details of which were given in paragraph 363), Searle *et al.* (516) made a direct comparison of cytogenetic damage induced by chronic exposures to ^{239}Pu alpha particles and to gamma rays, with a reduction in the amount of plutonium injected and with longer exposure periods than in the previous study (33). As can be seen from an inspection of table 40, in the plutonium series, there were no significant differences in

frequencies of translocations between 21, 28 and 34 weeks of exposure. The estimated mean spermatogonial dose (not taking into account the inhomogeneous distribution of plutonium in the testis) was 15.5 rad and the corresponding mean frequency of translocations, 0.74 ± 0.18 per cent. With gamma rays (1055 rad to spermatogonia), the frequency was 1.7 ± 0.4 per cent. The induction rates, estimated by subtracting the mean control frequency (for both the plutonium and the gamma series), were $3.4\ 10^{-4}$ rad^{-1} of alpha particles and $1.4\ 10^{-5}$ rad^{-1} of gamma rays (linearity of response assumed). Thus it appears that irradiation with alpha particles from ^{239}Pu is about 24 times as effective as chronic gamma irradiation for translocation induction.

370. Other data reported in the paper of Searle *et al.* (516) pertain to induction of chromosome fragments, the rate for which was estimated as $1.2\ 10^{-2}$ rad^{-1}, which is over twice as high as the rate of $4.6\ 10^{-3}$ rad^{-1} which can be estimated from the results of Beechey *et al.* (33) with shorter exposures. Relative to chronic gamma irradiation, the ratio of effectiveness is about 24. In the same experiment, an alpha-gamma ratio of 22 was found for dominant lethal induction (see paragraph 365). Thus, three indices of cytogenetic damage gave very similar alpha-gamma ratios of 24, 24 and 22, from which a mean value of 23 can be derived. However, the frequencies of sperm-head abnormalities were not significantly different from that in controls in the plutonium series, whereas in the gamma series, they were significantly higher (17.1 per cent compared with 3.9 per cent).

371. The above estimates of relative effectiveness of alpha particles and gamma rays for the induction of cytogenetic damage are based on the average dose to the testis, which is likely to be different from the doses to the germ cells at risk because of the known inhomogeneity in plutonium distribution in the testis (199). The authors felt that it was unwise to try to correct for this because (a) no estimates of the magnitude of the inhomogeneity were available except for spermatogonial stem cells and (b) even for these stem cells, the 2-2.5 correction factor estimated by Green *et al.* (199) might not apply under the much more protracted exposure conditions of the present experiment. In particular, there were signs of aggregation of the plutonium deposit (198), which might decrease the effective alpha particle dose to the stem cells and thus could help to account for the much lower rate of translocation induction by alpha particles found in the present experiment ($3.4\ 10^{-4}$ rad^{-1}) than in the previous one of Beechey *et al.* (33), namely, $1.45\ 10^{-3}$ rad^{-1}. In any event, the present results confirm the high effectiveness of ^{239}Pu alpha particles in inducing translocations, even with very protracted exposures.

2. Tritium (^{3}H)

(a) *Induction of dominant lethals in mice*

372. Carsten and Commerford (81) and Carsten and Cronkite (80) have published the results of their studies on the induction of dominant lethals in mice (random-bred, Hale-Stoner-Brookhaven strain) fed with tritiated water (HTO). The HTO test animals were first-litter mice resulting from breeding of eight-week-old animals that had been maintained on HTO (3 μCi/ml) since weaning at four weeks of age. The control animals were first-litter mice taken from the colony and maintained on tap water. From the second generation animals, four experimental groups were established for dominant lethal tests. Group 1 consisted of animals where both the male and female were on HTO. Group 2 females received HTO, males, tap water. In group 3, the situation was the reverse of that in group 2, and group 4 received only tap water (both males and females). At eight weeks of age, in each group, each male was mated to five females for a 5-day period, and 15 days after the mid-point of this breeding period, the females were killed and their uterine contents examined for assessing dominant lethality.

373. The results, based on 366 pregnant females in the controls, 764 in group 1, 315 in group 2, and 316 in group 3, clearly demonstrated that dominant lethals are induced by HTO in both sexes. Significantly fewer viable embryos were found when either both mating partners or only the female was maintained on the tritium regimen. Similarly, when both the partners were on tritium, the incidence of early death (dark mole) is significantly higher than in the control group. Treatment of the males only gave similar effects, but these were not significant. When post-implantation mortality (early *plus* late deaths in the authors' terminology) is used as the basis for comparison, the increased mortality due to HTO in groups 2 and 3 is of the same magnitude in both sexes, and in group 1 (both sexes on HTO) the effect is nearly twice that in groups 2 or 3. Current experiments are directed at repeating these studies with a lower concentration of 1.0 μCi/ml.

(b) *Induction of specific-locus mutations in male mice*

374. Cumming *et al.* (128) have completed the first series of experiments on ^{3}H-induced specific-locus mutations in mice, providing the only data available on such gene mutations in any mammal. In view of possible levels of tritium release, not only from existing nuclear installations but also from contemplated controlled thermonuclear reactors, these data are of great relevance. A total of 14 groups of males was used. Two groups were injected with 0.75 mCi, and the 12 others with 0.50 mCi, of tritiated water per gram of body weight. The results demonstrate that beta radiation from the decay of tritium can induce specific-locus mutations in spermatogonia as well as in post-meiotic stages: 16 mutations have been recovered among a total of 20 626 offspring derived from germ cells irradiated as spermatogonia and 11 in 7943 offspring from irradiated post-meiotic stages. The mean absorbed dose to the spermatogonial cells has been estimated to be 700 rad and that to post-meiotic cells, 430 rad. These data thus permit mutation-rate estimates of $1.58\ 10^{-7}$ rad^{-1} per locus for spermatogonia and $4.60\ 10^{-7}$ rad^{-1} per locus for the other stages. These rates are within the statistical limits of what would have been expected from a comparable external dose of x or gamma irradiation. The point estimate of the RBE for post-spermatogonial stages is close to 1, with fairly wide confidence intervals;

that for spermatogonia is slightly above 2, with confidence intervals that include 1. There are some indications that the distribution of mutants among the seven loci may differ from that produced by gamma rays: noteworthy is the observation that only one of the mutations was at the *s* locus (the expectation would be about 5 or 6). In more recent studies, currently in progress at Oak Ridge, Cumming and W. L. Russell (129) are engaged in collecting more extensive data on tritium irradiation, focusing attention on the induction of mutations in spermatogonia.

(c) *Induction of chromosome aberrations in human lymphocytes by tritiated water (HTO)*

375. Hori and Nakai (233) and Bocian *et al.* (39) have reported on the induction of chromosome aberrations in human lymphocytes exposed to tritiated water *in vitro*. Exposures were carried out by the addition of whole blood to the culture medium containing tritiated water. In the work of Hori and Nakai, the concentration of tritium ranged from $1\ 10^{-6}$ μCi/ml to $1\ 10^{-2}$ μCi/ml, and the cells were exposed during their entire period in culture (48 h). Bocian *et al.*, used two regimens: in one ("acute exposures" in the authors' terminology), the lymphocytes were exposed for a 2-h period prior to PHA stimulation (range of concentrations, 1.71-14.36 mCi/ml), after which they were washed and cultured (53-h cultures); in the other ("protracted series") the cells were exposed during 53 h (concentration range, 0.063-0.51 mCi/ml).

376. The results indicate that with protracted exposures (48 or 53 h) the aberrations produced were mostly of the chromatid type, such as gaps, deletions and fragments, and there were relatively few chromatid exchanges. In the concentration range used by Hori and Nakai, the dose-effect curve for the number of breaks induced was quite complex at low concentrations. In the work of Bocian *et al.* and with the range of concentrations they used, the frequency of chromatid aberrations increased linearly with dose. A quantitative comparison of the frequencies between the two groups of authors is, however, not possible because each group used only one (but different) fixation time, and in addition, the ranges of concentration were different.

377. In the 2-hour exposure experiments of Bocian *et al.*, chromosome-type aberrations were found to be induced (dicentrics, centric rings, terminal and interstitial deletions). The data for dicentrics plus rings, as well as those on deletions, gave a good fit to a linear plus quadratic model. Using the data obtained in x irradiation experiments (acute doses of 50-300 rad), Bocian *et al.* have estimated that the RBE for the induction of dicentrics plus centric rings is about 1.2.

3. Summary and conclusions

378. During the past few years, there has been a growing interest in the study of the biological effects of radioisotopes, particularly of ^{239}Pu and ^{3}H. A number of genetic and cytogenetic studies that have so far been carried out in mice demonstrate that these isotopes are capable of inducing dominant lethals, chromosome aberrations and point mutations (for the last category, only the effects of ^{3}H have been studied).

379. Autoradiographic studies have shown that in mice, intravenously injected ^{239}Pu (as citrate solution) is inhomogeneously distributed in the testis and is largely localized in the interstitial tissue outside and between the seminiferous tubules. A consequence of this is that the alpha-irradiation dose rate to the spermatogonial stem cells is 2-2.5 times greater than the average for the testis as a whole.

380. When ^{239}Pu-injected males are mated to females, there is a significant excess of intra-uterine mortality relative to controls and the effect persists in matings up to five weeks after injection (post- and peri-meiotic stages sampled). In addition, the effect appears to be unrelated to the amount of ^{239}Pu injected (in the range 0.05-0.5 μCi per mouse).

381. Dominant lethal tests performed on F_1 males sired by fathers which received plutonium injection (and derived from matings during the ninth, fourteenth and sixteenth weeks) showed that here again there was an increase in intra-uterine mortality relative to controls.

382. Relative to chronic gamma irradiation, alpha particles from ^{239}Pu seem to be more than 20 times as effective in inducing dominant lethality (post-implantation) in meiotic and post-meiotic stages.

383. In male mice exposed to alpha particles from ^{239}Pu (intravenously injected citrate solution) for a duration of 6-34 weeks, reciprocal translocations (in spermatogonia) and chromosome fragments (in spermatocytes) are induced. Relative to chronic gamma irradiation, alpha-particle irradiation from ^{239}Pu is more than 20 times as efficient for the induction of these effects. This finding is similar to that recorded for the induction of dominant lethals in meiotic stages. These calculations do not take into account the inhomogeneous distribution of ^{239}Pu in the testis.

384. Male and female mice fed on tritiated water, show, in dominant lethal tests, an increased amount of intra-uterine death.

385. In specific-locus tests, mutations have been found to be induced in male mice fed with tritiated water. The data currently available suggest that the rate of induction per unit dose of irradiation with beta particles from ^{3}H is about the same as that of x irradiation. The estimates are $1.58\ 10^{-7}$ rad^{-1} per locus for spermatogonial mutations and $4.60\ 10^{-7}$ rad^{-1} per locus for post-spermatogonial stages. These estimates have wide confidence limits. There is some evidence that the distribution of mutants among the seven loci may be different from that after x irradiation.

386. In human lymphocytes exposed to tritiated water *in vitro*, both chromosome- and chromatid-type aberrations are induced, depending on the concentration of ^{3}H and the duration of exposure.

H. THE RADIATION RESPONSE OF SPERMATOGONIAL CELLS AND ITS RELEVANCE TO THE INTERPRETATION OF GENETIC EFFECTS OF RADIATION

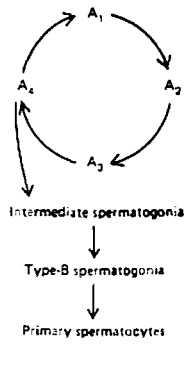
387. In the 1972 report, the model of stem cell renewal proposed by Oakberg (372) for the mouse and by Huckins (239) for the rat and the model of Clermont and Bustos-Obergon (113) for the rat were discussed. According to the Oakberg-Huckins model, stem-cell spermatogonia occur as single isolated cells in contrast to the groups of differentiating spermatogonia which form "chains" of cells connected by cytoplasmic bridges. Renewal of stem cells occurs by the division of some 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, then into A_3 cells, and so on. Division of A_4 cells results only in the formation of cells of the intermediate type. There is no evidence that the A-type spermatogonia of any type are formed from A_4 cells. The model can be diagrammatically shown as follows:

A_s – singles → A-paired (A_{pr}) → A-chains (aligned) (A_{al}) → $A_1 \rightarrow A_2 \rightarrow A_3 \rightarrow A_4$ → intermediate spermatogonia → type-B spermatogonia → primary spermatocytes

388. The A_s spermatogonia are the most resistant of the spermatogonial types; they are the only survivors after x-ray exposures of 150 R or more and are responsible for the repopulation of the germinal epithelium. The studies of Huckins (237, 238, 239), Oakberg (372, 373) and de Rooij (439) have shown that some of the A_s cells have a long cell cycle (about 8.5 days in the mouse (372, 373)); it also seems that most have a much shorter cycle, only slightly longer than 26-28 h characteristic of differentiating spermatogonia (238, 374). The relationship between A_s cells having these two average cycle times has not been explained.

389. On the other hand, the "two-stem-cell" model of Clermont and Bustos-Obergon (113) assumes that there are two populations, one of which is the non-cycling A_0 (reserve stem cells, activated only if the more mature classes of cells become depleted) and the other, a renewing cycling population of A_1-A_4 spermatogonia. The A_4 cells were given the role of pivotal cells, forming either more differentiated *In* (intermediate spermatogonia) or less differentiated A_1 spermatogonia as in the following scheme:

(Activated A_0 in emergency)



390. In recent experiments, Oakberg and Huckins (377) used ^{3}H-thymidine labelling in conjunction with x irradiation to study further the problem of germ-cell repopulation in the testis of the mouse, more specifically, to examine whether the A_4 cells form A_1 cells and intermediate spermatogonia (as the model outlined in the preceding paragraph predicts), or whether confirmatory evidence can be obtained for their earlier findings that A_s cells are the active stem cells of the germinal epithelium. Both sectioned material (as in the earlier work of Oakberg) and whole mounts were used to compare the results of the two techniques. The activity of ^{3}H-thymidine used was 17.5 μCi/0.25 ml (intraperitoneal injections, either one or six at 12-h intervals), and the x-ray exposures were 150 R and 300 R (90 R/min).

391. The prediction was that, if the model of Clermont and Bustos-Obergon were valid, only A_1-A_4 cells should be labelled (and subsequently be killed by irradiation) and the A_0 cells should not be labelled. If the Huckins-Oakberg model were valid, since A_s cells are the only survivors after x-ray exposures of 100 R or more, only these should be labelled. It is worth pointing out that data demonstrating labelled A spermatogonia 5 days after an x-ray exposure as high as 1000 R had been available for some time (370) and preliminary results from long-term labelling studies in control mice revealed heavily labelled cells as long as 10 days (more than one cycle of the seminiferous epithelium) after ^{3}H-thymidine injection. On the basis of these results, it has been suggested that the A_0 spermatogonia are the active stem cells of the seminiferous epithelium (371).

392. The results obtained using both methods (sections and whole mounts) demonstrated the survival of A spermatogonia labelled with ^{3}H-thymidine prior to irradiation, and the survivors were almost exclusively A_s spermatogonia. The data are consistent with the hypothesis that the stem cells are indeed in continuous cycle, and that the total cycle time of some A_s cells is long. There was no evidence for a non-cycling reserve stem cell (A_0 population).

393. De Ruiter-Bootsma *et al.* (447) examined the survival of spermatogonial stem cells in the mouse after irradiation with 1-MeV fission neutrons. Groups of male mice (CBA strain) were irradiated with neutrons with acute doses of 200-410 rad (7 levels at 10 rad/min). For purposes of estimating the RBE, two other groups of mice were exposed to 900 and 1200 rad of 300-kVp x rays at 30 rad/min. The number of surviving cells after irradiation was estimated in histological preparations of the testes of the mice killed 11 weeks after irradiation by determining the fraction of repopulated seminiferous tubules in cross-sections. This fraction, called the "repopulation index" (RI)[17] was assumed to be directly proportional to the number of surviving stem cells.

394. It should be pointed out that the above method of estimating stem cell survival is different from the one

[17] The repopulation index after a given irradiation dose D, is defined as $RI(t) = 100\ Ng(t)/L$ (per cent), where N is the number of surviving stem cells, $g(t)$ represents the growth function (mean clone length as a function of time t), and L is the total length of all seminiferous tubules in a testis.

used by Oakberg and others. Subdividing the morphologically undifferentiated A-type spermatogonia into three classes, A-isolated, A-paired and A-aligned, Oakberg (372) observed that the isolated cells were the least sensitive, their numbers being reduced to about 58 per cent of control value when measured 72 h after irradiation with 100 R of x rays, while the numbers of the other undifferentiated types were drastically reduced. De Ruiter-Bootsma *et al.* point out that by counting the number of cells in cross-sections of the testis (as Oakberg has done), difficulties are encountered in distinguishing the isolated cells and the other spermatogonia. To avoid this complication, the authors use the colony-forming unit[18] as the stem cell and employ RI as a reliable measure of survival of A_s spermatogonia.

395. The results showed that the dose-response curve for stem cell survival had an exponential slope with a D_0 of 85 rad, indicating the rather low radiosensitivity of the stem cells. A comparison of the values of RI from the neutron and the x-ray experiments revealed that under the experimental conditions used, neutrons have an RBE of about 4.4. It also approximates the RBE of 6.5 for fission-neutron (1-2 MeV) doses of 18-101 rad and of 4.7 for 172-258 rad reported by Oakberg (370).

396. In a subsequent paper, de Ruiter-Bootsma *et al.* (448) have reported additional data on the radiation response of the spermatogonial stem cells in the mouse testis. Both neutron irradiation (1 MeV, 50-410 rad, 10 rad/min, single exposures) and x irradiation (200-1200 rad, 30 rad/min, single exposures) were used. The mice were killed at four different intervals after irradiation (3, 5, 8 and 11 weeks) and the numbers of surviving stem cells were determined using the colony method discussed earlier, as well as direct colony counts. The formula for calculating RI was slightly modified as follows: $RI(t, D) = 100\ Ng(t, D)/L(D)$ (per cent). According to this formula, at a given time t, RI is a linear function of N if either both the growth function $g(t)$ and the total tubular length L are independent of the degree of stem cell depletion or they vary in the same proportion.

397. The main results are the following: (a) for all post-irradiation intervals studied, RI is exponentially related to the neutron dose, except when its value exceeds 90 per cent; this has been explained by the authors by assuming that the clones which are distributed at random over the tubules, by growing in length, have an increased chance of meeting each other resulting in clonal growth inhibition; (b) the mean D_0 value of the stem cell survival curves in this period is 81 ± 2 rad, pointing to the existence of a highly radioresistant population of stem cells; (c) there is a good correspondence between the RI values and mean numbers of clones counted per testis; (d) the age of the animals does not influence stem cell sensitivity once the animal is adult, although clones grow at a slower rate in older animals; (e) the x-ray curve for stem cell survival is exponential from 400 rad onwards and the value of D_0 is 242 ± 7 rad; (f) the RBE of fission neutrons is dose-dependent and decreases from 5.5 at a surviving fraction of 0.3, to 4.1 at a fraction of 0.01; (g) their findings permit an estimate of about 1800 stem cells present in the normal unirradiated testis.[19]

[18] Commenting on the technique used by Ruiter-Bootsma *et al.* (447), Oakberg (367) has drawn attention to the possibility that the colony method can lead to errors if surviving stem cells are not randomly distributed, especially if longer intervals are used. His preliminary data suggest that survival at high doses is dependent upon the stage of the cell cycle of the seminiferous epithelium i.e., specific areas of the tubule) and, furthermore, that the number of tubules with more than one survivor per cross-section is greater than would be expected. This could lead to a change in the mechanics of repopulation along the inner wall of a cylinder.

1. Effects of dose fractionation

398. In the paper of de Ruiter-Bootsma *et al.* discussed in paragraph 393, results on the effects of neutron-dose fractionation on stem cell survival are also given. In these studies, male mice received 350 rad of neutrons delivered in two fractions of 150 and 200 rad, in that order, with intervals ranging from 4 to 48 h. The data show that fractionation decreases the number of surviving stem cells; the extent of such a decrease depends on the time interval between the fractions. The radiosensitivity of the stem cells increased sharply between 4 and 8 h after the first fraction. The authors surmise that, if this decrease in cell survival is due to a progressive decrease in D_0 values of the stem cells after irradiation, then the first dose apparently triggers the cells to pass into a very sensitive phase of their cell cycle within a period of 4-8 h. (The rise in translocation frequencies in the short-term fractionation experiments discussed in paragraphs 112-113 may thus be explained on the basis of the increased sensitivity of the stem cells to the induction of reciprocal translocations.)

399. Rudnicki and Trojczuk (445) and Rudnicki and Kochmánska-Twardowska (446) investigated the effects of x-ray dose fractionation (8 X 25 rad compared with 1 X 200 rad, 8 X 50 rad compared with 1 X 400 rad, 2 X 50 rad, 5 X 20 rad and 9 X 10.1 rad compared with 1 X 100 rad) on survival and repopulation kinetics of the different spermatogonial cell types. The interval between the dose fractions was 24, 72 or 96 h. The findings are that (a) the different cell types have differential sensitivity to killing, (b) dose fractionation leads to a detectable delay in the rate of repopulation of germinal epithelium and (c) the survival is approximately equal after single and fractionated irradiation and appears to be independent of the number of fractions and the interval between them.

2. Effects of dose rate

400. To evaluate the possible role of spermatogonial cell survival on mutation frequencies at different dose rates, Oakberg and Palatinus (378) irradiated male mice with 300 R of x rays (at 94 R/min) or with 300 R of ^{137}Cs gamma rays at rates of 0.0056 and 0.0007 R/min. One group of mice was killed immediately after

[19] From the relationship $\ln N_D = \ln N_0 - (D/D_0)$ where N_D is the number of clones after a dose D, N_0 is the number of cells in an unirradiated testis, and D_0 is the 37% survival dose.

removal from the gamma field; a second group was killed 24 h later in order to observe initiation of cell repopulation. Cell counts for these intervals were the same for gamma-irradiated mice, and the data were pooled. Mice exposed to x rays were killed five days later when cell survival is minimal. The testes were then processed for making histological preparations.

401. The results showed that (a) after 300 R of acute x rays, only 16 per cent of the stem cells (relative to controls) and only a few differentiating spermatogonia survived; (b) after gamma irradiation at 0.0056 R/min, the survival was 37 per cent for A_s and 24 per cent for A_1 cells; the remaining spermatogonial types showed a depletion to about 5 per cent of control, indicating a markedly higher radiosensitivity of these stages; and (c) at 0.0007 R/min, the number of stem cells was unaffected with survival values for A_1-A_4 spermatogonia ranging from 81 to 97 per cent.

402. On the basis of these data, the authors conclude that (a) the mutation frequency at 0.0007 R/min is measured on a cell population which is maintaining itself at normal levels; (b) after 300 R at 0.0056 R/min (which reduces the A_s spermatogonia to 37 per cent of control), the mutation frequency appears to be the same as after 0.0007 R/min (para. 284); and (c) after 300 R of x rays at 94 R/min (where cell survival is only 16 per cent), the mutation frequency is the highest of all (1972 report, Annex E, table 14); this result is the reverse of that expected if there is a positive correlation between sensitivity to cell killing and sensitivity to mutation induction under the radiation conditions investigated. Other mechanisms, such as repair, are indicated as the primary factors in the reduced frequency of mutations observed at low dose rates.

403. Hsu and Fabrikant (235) conducted a study to examine the cellular response and cell population kinetics during spermatogonial cell renewal in the mouse testis exposed to continuous gamma irradiation (^{137}Cs) at 1.8 rad/day (=0.00125 rad/min) and at 45 rad/day (=0.031 rad/min). Labelling with ^{3}H-thymidine was used for the analysis of cell-population kinetics. Mice were killed at different times during irradiation and their testes were processed for histological examination and autoradiography. In addition, the pattern of post-radiation recovery of the seminiferous epithelium was investigated in mice which were exposed to irradiation at 45 rad/day for two weeks (accumulated dose, 630 rad). The main results are given in the following paragraphs.

404. Depending on the dose rate, continuous irradiation alters the cell-population kinetics in the seminiferous epithelium and more damage occurs at the higher dose rate. At 1.8 rad/day, the type-A population considered as a whole (A_s and A_1-A_4) shows an initial decrease within the first two days of irradiation followed by a new level of steady-state growth at about 80 per cent of the control level. This is essentially a reflection of the decrease in the A_1-A_4 cells, since the A_s cells remain at near-control levels during the entire seven weeks of continuous irradiation. Among the A_1-A_4 spermatogonia there is a gradient in radiosensitivity, the reduction in cell numbers increasing from A_1 through A_4.

405. In the 45-rad/day group, there is a marked decrease in the numbers of type-A spermatogonia (to about 50 per cent) in the first three days (accumulated dose, 135 rad) followed by an increase to normal values by the second week, then a fall and a rise again (60-70 per cent) in about three to five weeks. The A_s cells alone undergo a reduction in numbers to nearly 50 per cent of controls during the first three days of continuous radiation; from day 4 up to about 10, this figure is about 80 per cent of controls and from then on, up to week 3 (when the studies were terminated) control levels are reached. The proliferating type-A cells, depending upon their individual radiosensitivities, reach steady-state levels at various levels below controls.

406. At the lower dose rate, in the *In* cell population, there is an initial (compensatory) rise in cell numbers on day 1, after which normal levels are attained for two weeks, followed by a slight decline to approximately 95 per cent of control values. At the higher dose rate, there is a steep decline in cell numbers and no cells are found in the seminiferous epithelium at the end of the first week of exposure.

407. At the lower dose rate, there is also a decrease in the type-B population, which reaches steady-state levels at about 80 per cent of controls by the third week of radiation exposure. At higher dose rates, the decline is steeper, so that by day 5 of exposure, this compartment is completely depleted.

408. Examination of the recovery pattern *after* irradiation (45 rad/day for two weeks; accumulated dose, 630 rad) shows that the repopulation of the seminiferous epithelium commences with increased cell proliferation in the A_s cells and is supported by the finding of an increase in the A_s mitotic index; the wave of renewed cell proliferation moves from A_s cells through to the A_4 cells. By day 14 after irradiation, *In* and type-B cells reappear, and their cell numbers reach a compensatory peak between 25 and 50 per cent above normal cell numbers, but subsequently stabilize at normal levels. By eight weeks, the curves showing the percentage of labelled mitoses for type-A, *In* and type-B cells show cell-cycle parameters similar to those for unirradiated controls.

409. All these findings of Hsu and Fabrikant (235) are in very good agreement with those of Oakberg (243, 370, 372), Oakberg and Clark (375), Oakberg and Palatinus (378), and Oakberg and Huckins (377). They demonstrate that at a dose rate of 0.00125 rad/min, which is twice the lowest rate employed by Oakberg and Palatinus, the A_s spermatogonia maintain themselves at control levels and that the temporal sequence of recovery commences with the A_s cells.

3. Stem-cell killing and sterile period

(a) *Irradiation of adult male mice*

410. It is known that when adult male mice are irradiated, a period of sterility develops several weeks after treatment and that it is a direct consequence of spermatogonial-cell killing. The length of the sterile

period therefore provides a good indirect measure of cell killing. A relationship between sterile period and dose was indicated in several studies carried out in the 1930s, but until the recent studies of Sheridan (533) with fractionated exposures, no precise measurements had been reported.

411. Sheridan irradiated male mice of the CBA strain with either single x-ray exposures of 600 or 800 R or with fractionated exposures of 300 + 300, 400 + 400 and 500 + 500 R, separated by time intervals of 24, 48, 72 or 144 h. he found that the mean number of days between irradiation and return of fertility was prolonged after fractionation (24, 48 and 72 h) relative to single treatments or with an interval of 144 h. He interpreted this observation as indicating that the shorter intervals used (24, 48 and 72 h) lead to a greater amount of cell killing.

412. Cattanach and Moseley (92) and Cattanach (90) made a thorough study using the length of the sterile period as a measure of spermatogonial killing by irradiating F_1 hybrid (C3H/HeH X 101H) mice with a wide range of single and fractionated x-ray exposures. It was found that the sterile periods were much shorter than those reported by Sheridan at equivalent exposures, even allowing for the fact that Sheridan reported his data in terms of the mean, rather than the median, as Cattanach did.

413. Cattanach (90) found (table 41) that after single x-ray exposures, the dose response curve for the recovery of fertility was irregular; the median day of return to fertility increased little from 300 to 400 R; there was then a slight increase to 500 R, but from 500 to 600 R, there was a dramatic increase in response. Beyond this level, there was little change up to 800 R, but the 900-R and 1000-R treatments gave rapidly increasing lengths of the sterile periods. Quite clearly, the exceptional point is that for the 600-R level: were it not for this, a simple exponential relationship would be obtained. However, five replicate experiments were carried out at this level, and all the results were consistent; consequently Cattanach feels that there is little doubt that the plateau is a true phenomenon. The fact that at 600-800 R, a plateau is obtained is not without significance: it is at these exposure levels that the yields of translocations and specific-locus mutations begin to decline and then fall giving humped dose-effect curves. The coincidence between the hump in the dose-effect curve for genetic damage and the plateau in the sterile period supports the conclusion that throughout the exposure range investigated, the sterile period is a direct consequence of the stem-cell killing.

414. Cattanach believes that the most readily acceptable explanation for the shape of the cell-killing curve is that it has two components, these representing two cell populations with different sensitivities to radiation killing. Since on the basis of Oakberg's data (372), all spermatogonial cells other than the stem cells should be eliminated by even the lowest dose (300 R) employed here, it may be concluded that the postulated sensitive and resistant cells must exist within the spermatogonial stem cell population itself. The two populations could represent two distinct stem cell types and this would find support in the histological observation that within the stem cell population, there are cells with quite different cell cycle times. Alternatively, the two populations could represent stages in the cycle of a single stem cell type. By either interpretation, the existence of two cell populations of different radiation sensitivities could provide the basis of the heterogeneity of the spermatogonial stem cell that has been proposed as an explanation for the humped dose-effect curves for genetic damage (190, 382, 470). Thus, if the genetic damage stems primarily from the population more sensitive to killing, then a drop in yield would be expected when killing increases beyond a certain point and at the highest exposures, genetic damage may only be recoverable from the more resistant cell population.

415. There is doubt on the extent to which the 600-800 R plateau in the length of the sterile period (para. 413) is really connected with stem-cell killing as Cattanach (90) has suggested. Oakberg's data (370) on survival of type-A spermatogonia gave a good fit to a simple exponential curve, as did the stem cell data of de Ruiter-Bootsma *et al.* in the 400-1200 rad range, as discussed earlier (paras. 396-397). Factors other than stem-cell killing, such as the mechanics of stem-cell repopulation, may well influence the shape of the dose-effect curve, for return to fertility, although it is difficult to see why they would modify the time recovery of fertility so specifically over a narrow range of doses.

416. The data obtained from the fractionation experiments (24-h interval between fractions) show that the effect on the sterile period varies according to the total exposure (table 41). Thus at the 400-R level, fractionation results in a sterile period not different from that with the single exposure, but at 600-R, fractionation reduces the sterile period irrespective of the relative magnitude of the two fractions. The opposite situation was then found at the 700-R level where fractionation enhanced the sterile period above that for the single exposure; at 1000 R, fractionation gave either the same response as the single exposure or caused some enhancement. The uncertainty about the effect of fractionation at the 1000-R level derives from the great variability in the response: while this may be characteristic only of high exposures, there is reason to suspect that the relative magnitude and the order of fractions may be important. When 100 R follows 900 R, the median day of return to fertility (108 days) is not very different from the 1000-R single exposure level (102 days) but when the fractions were given in the reverse order, this number is 135.

417. In spite of these complexities, two general conclusions can be made: (a) fractionation at high exposures causes more, rather than less, stem-cell killing than does single treatments. This demonstrates that the decline in yield of genetic damage with higher single doses does not derive from extensive cell killing *per se* and supports the idea that with this form of treatment, genetic damage and cell killing tend to occur in the same cells so that at higher doses, more cells with genetic damage are killed (382). The corollary of this is that, with fractionation, the cell killing brought about by the second of two fractions must tend to occur in different

cells from those in which the genetic damage had been induced by the first fraction: (b) the cell killing response with fractionated treatments rises regularly with increasing exposures, as does genetic damage, so that the irregularity of the single-exposure curve which indicated the existence of two cell populations is eliminated. This would again suggest that whether considering genetic damage or cell killing, the two fractions tend to hit different cells. Further interpretation of the data is not practicable, but it is probable that the diverse response obtained with fractionation, relative to that with the single treatments, stems from an alteration of the relative sensitivities of the two stem-cell populations. The results of Oakberg (372) show that differential cell survival occurs within the long-cycling A_s population.

418. In a subsequent paper, Cattanach and Moseley (92) explored the effects of 500 + 300 R, 500 + 500 R and 300 + 300 R separated by 2 h to 12 days (800-R series), 4 and 7 days (1000-R series), and 24 h, 3, 7 and 10 days (600-R series) between the two fractions (x irradiation). In some experiments, the yields of translocations and/or specific-locus mutations were also measured in addition to the determination of the lengths of the sterile periods.

419. In the 800-R series, with intervals of 16 h to 4 days, fractionation resulted in an increase in the sterile period, well beyond that of the acute exposure (from a median of 71 to one of 81 days); with longer intervals, there was a gradual decrease, reaching a minimum with a 10-day interval (median, about 55 days). In the 1000-R series, the 4-day interval gave a sterile period somewhat shorter (95 days) than that of the 1000-R acute (102 days); the 7-day interval led to a drop (about 82 days), but the results of the individual tests overlapped. In the 600-R series, fractionation reduced the sterile period from about 67 days (acute) to about 52 days (fractionated), and there was very little change with fractionation intervals longer than 24 h.

420. Although the translocation and specific-locus data collected in the fractionation experiments of Cattanach and Moseley (92) are discussed in other sections, it is worth pointing out here that there is no correlation between genetic damage and cell killing (as measured by the length of the sterile period) with different fractionation intervals. However, the translocation and specific-locus mutation experiments give, qualitatively if not quantitatively, a similar variation in response to variation in the treatments. Both indicate a peculiar state of cells surviving the first fraction, such that they may be particularly sensitive to genetic damage 24 h later. The translocation data suggest that this sensitive state may last up to two days and both types of experiment demonstrate that this state does not exist beyond this time.

(b) *Irradiation of young mice*

421. In a recent paper on x-ray induction of translocations in young male mice, Cattanach, Murray and Tracey (94) have presented some results on the incidence of sterile period. In the first two series involving whole-body exposures of 300 and 500 R (acute), 5-day-old, 10-day-old and adult mice were used. The younger age-groups were selected on the basis of Selby's results (527), which suggested a change from an immature to an adult response by the eighth day. In the third series, the exposure was 1000 R (part-body), and in this the response of 3-day-old mice was also studied. The results are summarized in table 41.

422. It can be seen that with 300- and 500-R treatments, the 5-day-old mice did not exhibit any obvious sterile period. As a group, they could be considered to become fertile as they reached maturity, and the fertility remained until the time of sacrifice; it was not of short duration. With adult mice irradiated with 300 and 500 R, the results are in good agreement with all previous tests (median day of return to fertility, 51 and 53, respectively). The response of 10-day-old mice is similar to that of adults.

423. After 1000 R, the response was different: in the 5-day-old group, 1 out of the 10 became fertile on reaching maturity, 5 showed some clear indication of a sterile period and the remaining 4 appeared to be permanently sterilized. The irradiation of 3-day-old mice gave similar results. Fertility in both groups, when regained, was highly variable; litter-size was often quite small, litter production was irregular and 4 of the 14 "fertile" males became sterile after producing only a few litters. All these findings contrast sharply with the response following adult irradiation. The response of animals treated with 1000 R when 10 days old tended to be intermediate between those of the younger and adult groups.

424. The lack of any clear sterile period following the irradiation of 3- or 5-day-old mice might suggest that their germ cells are perhaps more resistant to radiation killing than those of the adult. However, histological studies with rats irradiated when 4-5 days old have shown that the germ cells of the immature animal are highly sensitive to radiation killing (241, 287, 532). The difference from the adult lies in the fact that the surviving cells can still proceed directly into spermatogenesis and lead to the appearance of sperm at sexual maturity (241, 532). It is likely that a similar situation is obtained in the mouse, and this provides an explanation for the lack of clear sterile periods following irradiation of immature animals and points to oligospermia being the cause of the variable fertility observed with the most severe treatment.

(c) *Other species*

425. In studies on the induction of translocations in the spermatogonia of the golden hamster, guinea-pig and the rabbit, Lyon and Cox (308, 309) also examined the length of the sterile periods after different x-radiation doses delivered either singly or in two or more fractions with various time intervals between the fractions. A comparison of the results obtained with those collected by Cattanach (90) for the mouse clearly show that after single exposures the golden hamster, guinea-pig and rabbit took longer to return to fertility than the mouse; among the three, the golden hamsters were the most sensitive. After 600 rad. the guinea-pig appeared to have a somewhat shorter sterile period than the golden

hamster, but the confidence limits were wide. Since in the golden hamster the duration of spermatogenesis is 35 days, as in the mouse, and since the sterile period includes a component due to multiplication of surviving stem cell spermatogonia and another for the duration of spermatogenesis (after adequate spermatogonial repopulation has occurred) the longer sterile period in this species must indicate a longer period spent in spermatogonial repopulation. This might mean that either the spermatogonial killing is more severe in the golden hamster or the dynamics of their recovery is very different.

426. In the guinea-pig and rabbit, spermatogenesis lasts longer than in the mouse, durations of 49 days in the guinea-pig and 52-60 days in the rabbit being estimated for spermatogenesis plus epididymal transport. Therefore, for equal killing, longer sterile periods would be expected than in the mouse, but only 1-2 weeks longer (since spermatogenesis plus epididymal transport in the mouse takes about 42 days). In fact the sterile periods were more than 1-2 weeks longer and hence, again there is a suggestion either for more severe spermatogonial killing or for slower rates of recovery in the guinea-pig and hamster than in the mouse. Thus, although differences in recovery cannot be ruled out, it seems probable that spermatogonial killing is more severe in the guinea-pig and hamster than in the mouse and is most severe in the hamster.

427. In the golden hamster, with a 2 X 200 rad (24-h interval), the sterile period was longer by about a month relative to that after the acute dose (4.5 ± 0.5 months compared with 3.5 ± 0.5 months); in the guinea-pig treated with a 2 X 300 rad (24 h) the sterile period was much longer, and at the time cytological examinations were made, the testes were very small (one third normal size) and suggested that the animals were unlikely to recover fertility; in contrast, after a 600-rad single exposure, fertility was invariably recovered after 4.5 ± 1.5 months. Thus the increased length of the sterile periods in both the species indicate that spermatogonial killing was severe after the fractionated exposures. As will be recalled, in the mouse, at these dose levels, there was either no change in the length of the sterile period (relative to that after acute exposures) or a slight lowering with fractionation. The data with eight-week fractionation also suggest that in both the species, the spermatogonial population did not return to normal at the end of the second fraction. With multiple fractions, the sterile periods were slightly shorter than after single doses, but owing to repopulation of the testes during the course of the treatment, it is not possible to interpret this in terms of spermatogonial-cell killing.

4. A model for stem cell heterogeneity in the adult and immature mouse testis

428. The existence of heterogeneity in radiosensitivity among spermatogonial cells to the mutagenic and killing effects of ionizing radiation had long been inferred from genetic and cytongenetic studies and direct evidence had been provided by histological studies of the irradiated testis. It is also known that the A_s spermatogonia are the most resistant of the spermatogonial cell types (and the only survivors after x-ray exposures of 150 R or more) and are responsible for the repopulation of the germinal epithelium. Although the exact basis of stem cell heterogeneity is not fully resolved, two current ideas are the following: (1) two or more distinct stem cell types with differing radiosensitivities exist (90, 92, 93), and some basis for this is provided by the histological evidence from both rat and mouse that stem cells with different cell cycle times are present in the testis (see paragraph 388); (2) heterogeneity simply reflects cell stage sensitivity differences within a single cell type (90, 92, 93, 420). The model of Cattanach *et al.* (94), which in turn is based on the model of cell cycle devised by Smith and Martin (541) argues that the heterogeneity in radiosensitivity stems from variations in cell cycle times that normally occur in the stem-cell population (see also reference 96).

429. The model of Smith and Martin divides the cell cycle into two parts. One, termed the B phase, is regarded as a deterministic and highly co-ordinated process; it corresponds to S, G_2, M and part of G_1. The other part, contained within G_1 is called the A phase and is thought of as an indeterminate, static condition in which the cell is not actually progressing through the cell cycle. Cells may remain in the A phase for any length of time, but leave and enter the B phase exponentially with respect to the time they have spent in the A phase in much the same way as radioactive material will decay at a rate determined by its half-life. The model also predicts that the intermitotic times of sibling cells should also be exponentially distributed. It might be noted that a long A phase together with the deterministic part of G_1 constitutes the so-called G_0 period for slowly dividing or non-dividing cells.

430. Application of this model to the spermatogonial stem-cell populations of the adult mouse suggests that the existence of long (8.5-day) and short ($\approx$ 28-h) cycling cells (374) may perhaps be an over-simplification. A range of cell cycle times should be expected and these would not be characteristic of different cell types, but represent only cell-to-cell and generation-to-generation variations in the time a single cell type spends in the A phase. On the basis of this, the heterogeneity in radiosensitivity of the spermatogonial stem cells is a reflection of the existence of the A phase in addition to the stages of the B phase of the cell cycle. Cells in a long A phase might be most resistant to radiation damage since, like the non-dividing gonocytes (primordial cells) of the new-born mouse, they would have a great time available for mutational/lethal repair before the onset of S. Cells in a short A phase, leaving an A phase of any length or in the B phase might be more sensitive to radiation damage and yield higher levels of genetic damage at doses compatible with significant levels of survival. (However, this interpretation seems to be at variance with the observations of Huckins (237), which suggest that fast-cycling A_s spermatogonia are probably already differentiated.)

431. The above line of reasoning applies to spermatogonial stem-cell populations under normal conditions.

However, within a few days of germinal depletion by irradiation exposure, a quite different situation may exist. Genetic and sterile period data indicate that the surviving, formerly more resistant cells are stimulated into proliferating activity, this being maintained until stem-cell repopulation is essentially complete (90, 92, 93, 420). During this period, cell cycle times must be much shorter than under normal conditions and, on the model of Smith and Martin, this should be achieved by shortening or eliminating the A phase, and this might be expected to lead to a greater radiosensitivity. This may be true for cell killing (92, 93), but the yield of genetic damage from proliferating cells is actually reduced (92, 93, 420).

432. Evidence against the hypothesis of Cattanach *et al.* outlined in the preceding paragraphs has recently been presented by Oakberg (367a, 377a), and the relevant data are summarized in table 42. This shows no evidence for alterations in the cell-cycle properties of A_s spermatogonia labelled prior to irradiation with exposures of 100, 300, 500 and 600 R and scored 207 h after irradiation, even though survival was reduced to 9 per cent of control by 600 R. Differential cell survival was observed after a single 1000-R exposure and after two 500-R fractions given 24 h apart, yet no apparent effect on cycling properties occurred since relative positions of the different exposure groups (in per cent labelled cells) was maintained over the 207-414 h interval. (The lower value for controls at 414 h arises from differentiation into A_{pr} and A_{al} cell types.)

433. Turning now to the response of the testis of young mice, the observations of Cattanach *et al.* (94) are: (a) the lack of any clear sterile period following the irradiation of 3- or 5-day-old mice; (b) their reduced adult testis weight; (c) lower translocation yields than in adults with a decline in yield at the highest exposure of 1000 R, which is more pronounced than in adults (about 1 per cent in 3-day-old mice, 0.3 per cent in 5-day-old mice, and 4.2 per cent in adults). A consideration of the developmental processes that occur in the testis within the first few days of birth suggest an explanation for the different response of the immature mouse. Histological studies with rat and mouse testis (32, 240, 241, 341) have established that only non-dividing gonocytes are present at birth, but within the next 2-3 days, large division figures may be seen and the first generation of spermatogonia appear. These lie centrally within the sex-cords, but, at about 6.5-7.5 days in the rat (240) and evidently much earlier in the mouse (95), these move towards the limiting membrane, where proliferative activity is initiated. It is not known however, whether these mitotically active spermatogonia are stem cells or the more differentiated types that appear during spermatogenesis. Quite probably, both are represented.

434. Thus, it is clear that spermatogenesis must be underway in the 5-day-old mouse, for the animal is fertile by at most six weeks of age (the time taken for the duration of spermatogenesis, including sperm transport to the ejaculate). In addition, there may be a build-up of stem cell numbers over the first week or two of life. Proliferative activity by the stem cells could alone account for the low translocation yields. The situation may be considered analogous to that of the adult testis several days after an initial radiation exposure (92, 93, 420). In both situations, most spermatogonial stem cells may have a short cell cycle and yield low levels of genetic damage. It might also be expected that under these conditions, not all cells would be proliferating. Some would be longer cycling and thus, liable to be in the relatively radioresistant A phase. The presence of such cells in the generally proliferating stem-cell population of the immature testis would account for the heterogeneity in radiosensitivity indicated by the decline in yield of genetic damage at the highest exposure. Alternatively, this decline of genetic damage at the highest doses could be attributed to differential survival of stages in the mitotic cycle, just as in the adult.

435. Finally, it bears mentioning here that for the adult testis, the model of stem cell heterogeneity proposed by Cattanach *et al.* (94) is, in several conceptual aspects, strikingly similar to that independently advanced by Preston and Brewen (420) although the latter authors did not, at least explicitly, relate their model to that of Smith and Martin.

5. Summary and conclusions

436. Recent histological and labelling studies carried out with x-irradiated mouse testes have provided confirmatory evidence for the conclusion that the A_s spermatogonia are the active stem cells of the seminiferous epithelium and that they are in continuous cycle. There is no evidence for a non-cycling reserve stem cell.

437. After acute irradiation with fission neutrons, the dose-effect relationship for spermatogonial stem cell survival (50-410 rad) has an exponential character with a D_0 value of around 80 rad; after acute x-irradiation, the curve is also exponential from 400 to 1200 rad, with $D_0 = 242$ rad.

438. The RBE of fission neutrons relative to x rays for stem cell survival (acute irradiation) decreases from 5.5 at a surviving fraction of 0.3, to 4.1 at a fraction of 0.01.

439. Fractionation of a neutron dose of 350 rad into two fractions (150 + 200) with intervals ranging from 4 to 48 h leads to decreased survival of spermatogonial stem cells; the radiosensitivity of the latter increases sharply between 4 and 8 h after the first fraction.

440. After an x-ray exposure of 300 R delivered at a rate of 94 R/min, the survival of A_s spermatogonia is reduced to 16 per cent of that in controls; with gamma-ray exposures of 300 R delivered at rates of 0.0056 and 0.0007 R/min, the relative survival of the A_s spermatogonia was reduced to 37 per cent with the first régime and unaffected with the second. The mutation frequencies (specific-locus mutations) under the first régime (acute x rays) is higher than those under the latter two. These observations suggest that under these conditions, there is no correlation between spermatogonial stem-cell killing and mutation rate.

441. Under continuous gamma-irradiation conditions at a dose rate of 1.8 rad/day for seven days, the A_s spermatogonial cells show no reduction in number; when the dose rate is increased to 45 rad/day, the number of A_s cells is reduced to 50 per cent of that of controls during the first three days of continuous irradiation; from day 4 to 10, this figure is 80 per cent, and from then on, control levels are reached.

442. Examination of the recovery pattern after continuous irradiation with gamma rays at 45 rad/day for two weeks shows that the repopulation of the seminiferous epithelium commences with increased cell proliferation in the A_s cells and is supported by the finding of an increase in the A_s mitotic index.

443. The duration of the sterile period in irradiated male mice is a good but approximate measure of the amount of spermatogonial stem-cell killing. With acute x-ray exposures ranging from 300 to 1000 R, the relationship between exposure and the median day of return to fertility gives a good fit to an exponential relationship if the data obtained at levels of 600-800 R (where a plateau is observed) are excluded.

444. The length of the sterile period following fractionated x-ray exposures varies with the total exposure, the size and sequence of the fractions, and the time interval between the fractions. Thus, for instance, fractionation at high exposures (with 24-h intervals) causes more, rather than less, stem-cell killing than do single treatments. When 100 R follows 900 R, the median day of return to fertility (108 days) is not very different from the 1000-R single exposure (102 days), but when the fractions are given in the reverse order, this number is 135. In experiments where the fractionation interval varied from 2 h to 12 days, the length of the sterile period also varied; for instance, in the 800-R series (500 + 300 R), with intervals of 16 h to 4 days, fractionation led to an increase in the length of the sterile period (relative to that acute exposure), and with longer intervals there was a gradual decrease reaching a minimum with a 10-day interval.

445. X irradiation of 3- and 5-day-old male mice (300 and 500 R) did not lead to any clear sterile period; the adult testis weight of these mice was reduced and lower translocation yields were obtained (relative to adults); with a higher exposure of 1000 R, the response was variable: some mice became permanently sterilized, some showed clear indications of sterile period, and some became fertile (but in these, litter size, litter production etc., were quite variable). the translocation yields were also severely reduced.

446. After single x-ray exposures to males, the golden hamster, quinea-pig and rabbit took longer to return to fertility than the mouse; of the three, the golden hamsters were the most sensitive. With fractionated exposures, the sterile period in the golden hamster (2 X 200 rad, 24-h interval) was about a month longer than after acute exposure; in the guinea-pig (2 X 300 rad, 24 h interval), the situation was qualitatively the same. The data after multiple small fractions showed that the length of the sterile period was slightly shorter.

447. A model to account for spermatogonial stem-cell heterogeneity in the mouse has been proposed by Cattanach and co-workers, which in several respects is similar to that proposed by Preston and Brewen. The heterogeneity is attributed to a variation of cell cycle times within a single stem cell type. Those in the long G_1 (A phase) would be the most radioresistant, having greater time available for repair before the onset of S; those with a shorter A phase, leaving the A phase or in other stages of the cell cycle (S, G_2 and M) would be more radiosensitive and contribute most to the total yield of genetic damage at lower x-ray exposures (<600 R). Under normal conditions most cells will be in G_1, and after acute irradiation most of the genetic damage will be recovered from these stem cells. However, under fractionated irradiation conditions, for instance when cell proliferation takes place (depending on the interval), such cells must spend a much higher proportion of their time in S and G_2, and the recovery of both translocations and point mutations from these stages should approximate only one half of that from G_1 due to chromatid segregation. Likewise, yields from short-cycling cells such as those in the immature testis will be lower.

448. An alternative explanation has been proposed by Oakberg on the basis of the data of Huckins, which suggest that the short-cycling A_s spermatogonia are already differentiated. Furthermore, it appears that only the long-cycling A cells survive exposures of 300 R or more. As repopulation begins, short-cycling cells reappear, but they all will have been derived from survivors of the long-cycling A_s spermatogonia. The heterogeneity therefore arises from differences attributable to a stage in the mitotic cycle of the long-cycling stem cells.

I. TIMING OF OOCYTE DEVELOPMENT IN THE ADULT MOUSE

449. The timing of oocyte development in the adult mouse is an essential prerequisite to our understanding of the dramatic change in radiation-induced mutation rate; in addition, such knowledge is very important to relate specific oocyte (and follicular) stages to specific post-irradiation litters. With this objective in mind, Oakberg and Tyrell (379) have initiated an autoradiographic study in which adult eight-week-old female mice are given two injections of 50 μCi of N-(acetyl-^{3}H)-D-glucosamine 16 h apart (a total of 100 μCi per female). Mice are killed at intervals ranging from 1 h to 10 weeks after irradiation and the ovaries appropriately processed. The progressive appearance of unlabelled oocytes (unlabelled in the zona pellucida) and concurrently developing follicles was used to obtain an estimate of about 35 days for a follicle of 20-30 cells to reach maturity and be ovulated; this period of time is not long enough to account for the number of litters conceived after 50 R. However, more recent work (367) suggests that 45-46 days are required for all stage *3b* follicles to reach ovulation. Stages *2* and *3a* appear to grow quite slowly, and this may explain the fertility response after acute exposure of adult female mice to 50 R of x rays.

J. THE INDUCTION OF CHROMOSOME ABERRATIONS IN SOMATIC CELLS, THE ARM NUMBER HYPOTHESIS, AND ITS RELEVANCE TO THE EVALUATION OF HAZARDS FROM THE INDUCTION OF RECIPROCAL TRANSLOCATIONS

1. Introduction: the arm number hypothesis

450. The literature on the induction of chromosme aberrations in somatic cells, mainly in human peripheral blood lymphocytes, was last reviewed comprehensively in the Committee's 1969 report (588). In 1972, the Commitee had at its disposal some new data that had just become available from the comparative study of Brewen and colleagues[20] on the x-ray induction of asymmetrical interchanges (dicentrics) in lymphocytes of six different mammalian species (man, marmoset, wallaby, pig, Chinese hamster and mouse). The data showed that (a) in all the species except the wallaby, in the range of exposures up to 400 R, the exposure-frequency relationship for dicentric induction gave a good fit to the model

$$Y_j = b_j D + c_j D^2$$

where Y_j is the yield in the jth species, b_j and c_j, the linear and quadratic coefficients, and D, the radiation exposure. In the wallaby, the best fit was to the model $Y = cD^2$; (b) the combined dicentric data of all the species gave a satisfactory fit to the model

$$Y_j = (N_j - 1)(bD + cD^2)$$

where N_j is the effective chromosome arm number of the species and the other symbols are defined in (a); (c) the above models accounted for a large amount of variation in the frequencies among the species, but not all of it; (d) man, with an effective chromosome arm number of 81, was found to be twice as sensitive as the mouse, with an arm number of 40; and (e) with regard to the production of deletions, which was also studied, the arm number did not seem to influence the yields, and man and the mouse had approximately equal sensitivity.

451. Assuming that the probability of forming a reciprocal translocation is the same as that of forming a dicentric (as shown by Heddle's results in *Vicia faba* (224)), Brewen *et al.* concluded that "dicentric production in these organisms is, at best, a direct measure of reciprocal translocation production and that man is twice as sensitive as the mouse in the dose range measured". Both UNSCEAR and BEIR (34, 589) considered the above line of reasoning valid and assumed that human spermatogonia are twice as sensitive as those of the mouse for the induction of reciprocal translocations. Their risk estimates for this class of genetic damage were based on this assumption.

2. Relationships between the frequencies of dicentrics in lymphocytes, reciprocal translocations in spermatocytes, and heritable translocations in F_1 progeny

452. Subsequently, Brewen and Preston (52, 54) made a direct comparison of the yields of dicentrics after irradiation of lymphocytes and of reciprocal translocations in spermatocytes after spermatogonial irradiation in the mouse, Chinese hamster, marmoset and guinea-pig. The results showed that the model of a linear relationship between the dicentric yield and the effective chromosome arm number (which has come to be called the "arm number hypothesis") is applicable to translocation induction in spermatogonia as well; however, the absolute frequencies of reciprocal translocations in spermatocytes were roughly one fourth of those of dicentrics in lymphocytes.

453. The earlier published data of Ford *et al.* (170) had shown that, in mice, the frequencies of heritable translocations recovered in the F_1 progeny of irradiated fathers (spermatogonial irradiation) were about one eighth of those expected on the basis of direct counts in spermatocytes of the irradiated males themselves (also spermatogonial irradiation). Considering all these results together, Brewen and Preston (52, 54) proposed that (a) a method is now at hand to make at least a preliminary estimate of germ-cell radiosensitivity from an extrapolation of that of somatic cells and (b) the frequencies of heritable translocations after spermatogonial irradiation are predictable from those of dicentrics in lymphocytes (i.e., frequency in F_1 progeny = dicentric frequency in lymphocytes X 1/4 X 1/8 = 1/32).

3. Further work on the arm number hypothesis

454. Since the hypothesis looked plausible and, if valid, would permit a meaningful use of data collected with lymphocytes in the context of hazard evaluations, the work of Brewen and colleagues catalysed research. The work that has been carried out during the last few years in this area (see reference 494 for a recent review) bear on the different steps in the extrapolation sequence, dicentrics in somatic cells → reciprocal translocations in somatic cell → reciprocal translocations in spermatocytes → reciprocal translocations in the F_1 progeny. The evidence thus far accumulated suggests that the arm number hypothesis is now less strong than it was at the time it was formulated and the procedure of extrapolating from the sensitivity of somatic cells to that of germ cells in fraught with uncertainty.

(a) *The relationship between dicentrics and reciprocal translocations in lymphocytes*

455. Until recently, most of the data on the induction of aberrations in human peripheral blood lymphocytes were collected from experiments that were designed for purposes other than for testing whether dicentrics and reciprocal translocations are induced at equal frequencies (88, 231, 497, 504). There is a suggestion of an excess of dicentrics in these studies. The recent work of Buckton (66), in which she used G- and R-banding to identify the break-points in human lymphocyte chromosomes, shows, however, that after an x-ray dose of 200 rad, the frequencies of dicentrics are equal to that of reciprocal translocations involving two chromosomes (G-banding: 100 cells, 29 dicentrics and 31 trans-

[20] The results were published in 1973 (55).

locations: R banding: 100 cells, 26 dicentrics and 27 translocations: conventional staining: 100 cells, 33 dicentrics and 26 translocations).

(b) *Induction of dicentrics in lymphocytes of different mammalian species*

456. Extensive data that have recently become available on the radiation-induction of dicentrics in peripheral blood lymphocytes of species other than those investigated by Brewen and colleagues[21] have shown that the arm number hypothesis is by no means valid for all the species; exceptions have been found in several primate species and two carnivores (the beagle dog and the cat). In addition, the comparative study of de Boer *et al.* (41) involving, among others, mouse and human lymphocytes has shown that at x-ray dose levels of 100 and 200 rad (100 rad/min), the frequencies of dicentrics are equal in both species:

	Dose level (rad)			
	100		*200*	
	Mouse	*Man*	*Mouse*	*Man*
Cells scored	800	200	500	200
Dicentric frequency (%)	12.3	10	39.6	34

The major technical difference with respect to the mouse work is that de Boer *et al.* found that, 36 h after the initiation of the cultures, the irradiated-stimulated lymphocytes were in the first division and used this as their harvesting time. In the work of Brewen *et al.*, however, longer culture times (60-63 h) were used. The sensitivity of the lymphocytes of the tobacco mouse, which was also investigated in the study of de Boer *et al.* at the 100-rad level, was found to be the same as that of normal mouse lymphocytes.

457. In the rhesus monkey (*Macaca mulatta,* arm number 84), van Buul (70) noted that the frequencies were lower than those in man at x-ray dose levels of 100, 200 and 300 rad, although the difference reached statistical significance only at the highest dose (26.6 per cent in the monkey compared with 39.0 per cent in man). However, in this study, the absolute frequencies of dicentrics in man were much lower than those obtained in other work (see Brewen *et al.* (55), for example).

458. The results of Muramatsu and Matsuoka (352) with lymphocytes of the cynomolgus monkey (*Macaca fascicularis,* arm number 83) showed that their sensitivity relative to sensitivity of human lymphocytes was 0.79. Hirai and Nakai (229) noted that in the cynomolgus monkey, the squirrel monkey (*Saimiri sciureus,* arm number 77) and the slow loris (*Necticebus cougang,* arm number 99), the frequencies of dicentrics after 100, 200 and 300 rad of gamma rays were roughly similar. The lymphocytes of the two carnivorous species, the beagle dog (*Canis familiaris,* arm number 79) and the domestic cat (*Felix catus,* arm number 71), studied by Muramatsu and Matsuoka (352) manifested much lower sensitivities than human lymphocytes (16 and 22 per cent, respectively, of human lymphocytes). To this list may be added the work of Sasaki *et al.* (501) with human lymphocytes. These investigators found that, at a dose of 160 rad, approximately twice as many exchange aberrations (dicentrics plus rings) were induced in the lymphocytes of individuals who had trisomic constitutions (trisomy 21, trisomy 18, trisomy D etc.) as in those of normal individuals.

459. In contrast to the above findings, the work of Scott and Bigger (502) with potoroo lymphocytes (arm number 24) has shown that the frequencies of dicentrics observed were in agreement with the expectation based on the arm number hypothesis. The data of Léonard *et al.* (286) for the lymphocytes of the goat (*Capra hircus,* arm number 60), the sheep (*Ovis aries,* arm number 60) and the pig (*Sus scrofa,* arm number 64) showed that, up to 300 R (x rays), there were no striking differences between the dicentric frequencies of these three species. After 400 R however, higher yields were obtained with the pig and goat lymphocytes (76.3 ± 4.4 and 88.5 ± 4.5 per cent) than in those of sheep (57.5 ± 3.7 per cent). Although the sensitivity of the lymphocytes of the cow (*Bos taurus,* arm number 64) was also assessed in the work of Léonard *et al.*, the frequencies between replicates were so widely different that a firm conclusion cannot be made. The *average* frequencies recorded up to 300 R were not very different from those in the other three species, and at 400 R they were similar to those in sheep.

460. With rabbit lymphocytes, the reported results are contradictory: Scott and Bigger (502) found that after x- or neutron-irradiation, the observed frequencies of dicentrics were less than one half of those in human lymphocytes. The x-ray data of Muramatsu and Matsuoka (352) and the gamma-irradiation data of Sasaki (498) for the rabbit are in line with those of Scott and Bigger. In contrast, Bajerska and Liniecki (20) found that the frequencies of dicentrics in rabbit lymphocytes were similar to those recorded for human lymphocytes in an earlier study (291). There were several technical differences between the experiments of Bajerska and Liniecki and those of the others, so that here again, no firm conclusion can be reached.

(c) *Induction of dicentrics in mouse and human fibroblasts*

461. In assessing the radiosensitivity of another type of somatic cells, namely fibroblasts, Sasaki (499) found that (a) mouse embryonic fibroblasts irradiated *in vitro* at the fourth and sixth subculture generations manifested the same sensitivity to the induction of dicentrics as comparable human material irradiated under similar conditions. In addition, the frequency of dicentrics recorded were very similar to those in an earlier study of Sasaki and Tonomura (500) at 100-500 R with human lymphocytes; (b) fibroblasts from mice irradiated as embryos or as new-borns were less than one half as sensitive as those mentioned under (a), but the frequency increased to about 60 per cent for

[21] Brewen *et al.* had observed that in the marmoset, the dicentric yields were consistently higher than expected on the basis of relative arm numbers.

4-day-old mice and to 90 per cent for 11-day-old mice; and (c) human embryonic fibroblasts (from embryos 9-11 weeks old) had the same sensitivity as that of fibroblasts in advanced *in vitro* passages (see (a) above).

462. In other experiments briefly mentioned in his paper, Sasaki (499) noted that in the cultured fibroblasts of the rat (62 arms) and the African green monkey (*Cercopithecus aethiops*, arm number 120) similar yields of dicentrics were obtained, despite the wide difference in the chromosome arm numbers.

(d) *Induction of reciprocal translocations in spermatogonia of different mammalian species*

463. Work on translocation induction in spermatogonia of rats, guinea-pigs and rabbits have revealed no apparent correlation of the relative yields with arm numbers. Thus, after 300 R of x irradiation, the frequencies of translocations in the rat and the mouse were the same (191). The findings of Lyon and Cox (309) show that in the range of 100-300 rad of x rays, the mouse, with 40 chromosome arms, is the most sensitive, followed by the rabbit (80 arms), guinea-pig (64 arms) and golden hamster (80 arms). Essentially similar results were obtained with post-meiotic germ cells of the above four species. In the rhesus monkey, van Buul (70) and Lyon *et al.* (314) found that reciprocal translocations were induced at much lower frequencies than in the mouse.

(e) *Relationship between dicentrics (lymphocytes) and translocations (spermatogonia)*

464. It may be recalled (paras. 452-453) that the results of Brewen and Preston (54) in the Chinese hamster, mouse, marmoset and guinea-pig[22] suggested that there appeared to be a definite quantitative relationship between the frequencies of dicentrics in lymphocytes and those of reciprocal translocations in spermatocytes, namely, that the latter were about one fourth of the former. The complete data recorded in the paper of Brewen, Preston and Gengozian (57) for the marmoset and man[23] now raise questions about the validity of this relationship, as can be seen from an inspection of table 43. In this table data for the rhesus monkey and the rabbit are also given. It will be clear that the relationship between the dicentric frequencies (in lymphocytes) and reciprocal translocations (in spermatocytes) is dose- and species-dependent. Also worth noting is the finding that the radiosensitivity of human spermatogonia is more similar to that of the marmoset than to that of the rhesus monkey.

(f) *The relationship between the frequency of translocations observed in spermatocytes and in the* F_1 *progeny*

465. The new data of Generoso *et al.* (186) and of Brewen *et al.* (56) on the relationship between the frequency of translocations recorded in spermatocytes (after spermatogonial x irradiation) and that in the F_1 progeny have raised the possibility that the latter may be one fourth of the yield in spermatocytes at an exposure level of 150 R, one that is consistent with theoretical expectations. This is in contrast to the observations of the above authors at 300, 600 and 1200 R and the earlier ones of Ford *et al.* at 1200 R, where the ratio was one eighth. The limited data of Pomerantzeva *et al.* (416) (see paragraph 132) suggest that at a gamma-ray exposure level of 900 R (delivered in three equal fractions at 28-day intervals), the above ratio may be one fourth, although not significantly different from one eighth.

4. Problems, perspectives, summary and conclusions

466. The information presented in the preceding paragraphs supports the view that the arm number relationship is not adequately documented to be used at present to predict from lymphocyte data of one species the expected frequencies of dicentrics in lymphocytes of another, nor can it be reliably used to estimate the frequencies of translocations in spermatocytes of one species from those of another. Furthermore, the two-fold higher sensitivity of human lymphocytes to the induction of dicentrics (relative to the mouse) found by Brewen *et al.* could not be confirmed. The available human data (testicular irradiation), although consistent with a two-fold higher sensitivity of the human testis to translocation induction relative to mouse, do not rule out a difference as high as four-fold. Besides, the primate data considered as a whole show striking species differences.

467. The research interest generated by the work of Brewen and colleagues and the work that has followed have exposed several interesting facets of the problem of the induction of exchange-type aberrations, whether it be in somatic or germinal cells. Among these may be mentioned (a) the variations in aberration yields depending on fixation times arising as a consequence of the heterogeneity of the radiosensitivity of the lymphocytes even though they are at the same stage of the cell cycle; (b) differential spermatogonial killing in the different species, the nature and kinds of reciprocal translocations induced in spermatogonia and their differential effects (leading to different degrees of underscoring in spermatocytes, for instance); (c) differences in radiosensitivity between chromosomes and between parts of chromosomes and differences in the capacity for accurate DNA repair; and (d) the extent to which aberration induction is influenced by the amount and/or distribution of heterochromatin and the organization of the chromosomes and heterochromatin in the nuclei of cells or cell stages irradiated.

468. In their human lymphocyte experiments, Searbright (504), Holmberg and Jonasson (231), Caspersson *et al.* (88) and Aymé *et al.* (16) found that chromosome breakage was non-random and that the break-points were located preferentially in the so-called R bands (bands of weak fluorescence). In contrast, Buckton's results (66), while not contradicting the findings of non-random breakage of the chromosomes, show that chromosomes rich in R-band material are not preferentially damaged by irradiation at all and that

[22] The guinea-pig and marmoset results were incomplete at the time the paper (54) was published.

[23] Preliminary data on human testicular irradiation given in reference 51 were not inconsistent with a two-fold higher sensitivity of our species relative to the mouse for the induction of translocations.

about 30 per cent of the breaks that are induced occur neither in a G-positive nor in a negative band, but on the interface between them (which certainly does not constitute a third of the chromosome material). She argues that most of the investigators have used either G or Q bands for identification purposes and the majority of identifying features on the chromosomes is constituted by the positively stained bands (389). Because of this, if one positively stained band is determined to be present and the next positive band to be absent, there will tend to be a bias for locating the break-point in the associated negative band. Her technique in which the same cells are G-banded followed by R-banding appears to permit greater resolution of the location of break-points. In any case, the observations that breakage is non-random are valid, although the mechanism underlying it is not known. This non-randomness may have important implications for the formation of chromosome aberrations.

469. Conclusive cytogenetic evidence on the radiosensitivity of our species for translocation induction (at least in males) will have to come from irradiated human material. In the meantime, interspecific cytogenetic studies with several experimental mammals (including primate species) will continue to serve a useful purpose in providing us with insights into the kinds of problems that we are likely to encounter at the human level, so that the right questions can be asked.

III. NATURE OF RADIATION-INDUCED DAMAGE AND THE PHENOMENON OF REPAIR

1. Introduction

470. The 1972 report of the Committee considered in some detail the developments in the molecular and cellular radiobiology of damage to DNA induced by uv light and ionizing radiations and the processes of repair associated with it. The past few years have witnessed considerable progress in the study of uv damage in DNA and its repair in prokaryotic systems and an extension and application of this knowledge and know-how to eukaryotic systems and to damage induced by ionizing radiation and a number of chemicals. A comprehensive and up-to-date appraisal of the current state of the art in this area is given in the proceedings of the recent Squaw Valley Conference (343). Much of the progress made stems from the continued refinements of the existing methods and the development of more powerful and more sensitive ones; these have led to the isolation and characterization of a number of enzymes involved in the repair of DNA damage and the exploitation of the unique properties of such purified specific lesion-recognizing enzymes in further studies (392).

2. Progress in the enzymology of repair after uv irradiation

471. Ultraviolet and chemical treatment, particularly with prokaryotes, has led to the elucidation of major enzymatic reactions underlying both photoenzymatic and excision repair. An *E. coli* photoreactivating enzyme has been purified and characterized (551). A number of specific lesion-recognizing enzymes involved in excision repair (after uv or chemical treatment) have been purified and characterized including a further delineation of their roles (49, 101, 102, 103, 194, 207, 227, 392, 423, 590). Among these may be mentioned endonucleases specific for pyrimidine dimers, apurinic sites, uracil residues etc. (see references 207 and 392 for recent reviews). Advances in the study of post-replication repair in bacteria have demonstrated, among other things, that uv-induced pyrimidine dimers remain in high molecular weight DNA after DNA replication (181, 182, 449) and that they are transferred (by a process which may involve genetic exchange) into all daughter strands during post-replication repair (182). Evidence is rapidly accumulating for the existence of still another type of repair process in *E. coli*—called the "SOS repair"—which is induced or activated following damage to DNA, one which is "error-prone" ("mutation-prone") (424, 603, 615).

472. The continued efforts to elucidate repair pathways in *E. coli* and other prokaryotes have greatly aided in the search for similar ones in a number of eukaryotes. The existence of three repair mechanisms—photoenzymatic, excision and post-replication repair—is now documented in several higher organisms (213), including mammalian cells cultured *in vitro* (388). Attempts to isolate and characterize the photoreactivating enzyme from human leucocytes (550, 552) and from murine and human fibroblasts (553) have been fruitful. Sutherland *et al.* (554) have recently demonstrated that fibroblasts from patients with xeroderma pigmentosum contain low levels of the photoreactivating enzyme (0-50 per cent of normal in the four different strains tested). With the exception of *E. coli,* the excision repair process has perhaps been most extensively investigated in human diploid fibroblasts. The impetus came from the discovery of Cleaver in 1968 (110) that the molecular basis for one of the xeroderma pigmentosum syndromes (XP) in man is a defect in an early step in excision repair. Subsequent research revealed that XP is much more complicated than was originally imagined. (See reference 112 for a recent review.)

473. Employing somatic cell hybridization techniques, it has been possible to demonstrate the presence of five different complementation groups (A to E) associated with defects in excision repair in XP cells (43, 138, 139, 140, 258, 435). There is also evidence for the involvement of defects in post-replication repair in XP (276). Tanaka *et al.* (568a) found that when uv-irradiated XP cells of complementation groups A to E are incubated with endonuclease V of bacteriophage T4, the ability to perform unscheduled DNA synthesis was restored in all of them. These results suggest that those XP cells might have a common defect in the first step of excision repair. Motelmans *et al.* (347) recently showed that crude extracts of normal human diploid fibroblasts and of human peripheral blood lymphocytes excise thymine dimers from purified uv-irradiated *E. coli* DNA or from the DNA presumably present as chromatin in cell-free preparations of cells that has been labelled with ^{3}H-thymidine. Extracts of XP cells from complementation groups A, C and D also excise thymine dimers from

purified DNA, but extracts of group-A cells do not excise dimers from the DNA radioactively labelled, unfractionated cell-free preparations. These findings suggest a biochemical defect in cell-free extracts that mimics a defect known to exist in intact XP cells.

474. Buhl and Regan (67) reported the presence of sites sensitive to dimer-recognizing endonuclease from *Micrococcus luteus* in newly made DNA from uv-irradiated XP cells; this finding and one reported by Meneghini and Hanawalt (333) are similar to those recorded by Ganesan (182) in bacteria, suggest that DNA synthesis after uv-irradiation does not necessarily leave gaps opposite the dimers, raise the possibility of a recombination mechanism in which segments of dimers containing parental strands are inserted in daughter stands. However, in work with uv-irradiated XP cells, a Chinese hamster cell line, and chick embryonic cells (all of these are defective in the excision of dimers), using a protocol similar to that of Buhl and Regan (67), Paterson, Lohman and Sluyter (396) obtained no indication of endonuclease-susceptible sites in the daughter DNA. The technique employed by Paterson *et al.* would have detected the presence of as few as 15 per cent of the dimers initially induced in the parental DNA.

475. Work with XP prompted the search for similar repair defects in other human diseases but thus far has met only with limited success (see also paragraph 502). Thus, for example, in fibroblasts from a patient with Fanconi's anaemia (characterized by a progressive aplastic anaemia, increased frequencies of spontaneous and radiation-induced chromosome aberrations and a high incidence of cancer) normal amounts of unscheduled DNA synthesis were observed at early times after uv-irradiation; however, the excision of dimers assayed at 24 h was depressed compared to normal cells at doses over 15 J/m^2 (254-nm radiation). Excision seemed normal at early times and, at low doses, at late times (418). It may be that at high doses the basic defect is not in the repair system but in other cellular properties that result in the turn-off of the repair system at high doses and long times (530).

476. Some success has been achieved in showing the presence of putative dimer-specific endonuclease and a dimer-excising exonuclease in extracts of human cells (18, 142). Work in this area of enzymology of repair in mammalian cells has just begun.

477. Cell-fusion techniques have also been successfully used at the interspecific level: the experiments of Paterson *et al.* (397, 398, 399) and Paterson and Lohman (306) focused attention on a further characterization of repair mechanisms present in human and chick fibroblasts and an elucidation of the efficiency with which repair enzymes unique to each species can operate on uv-damaged foreign DNA in multinucleate human-chick heterokaryons artificially derived from both fibroblastic lines. Advantage was taken of the fact that primary human cells lack photoenzymatic repair but possess excision repair machinery, whereas chick fibroblasts possess photoenzymatic repair but not excision repair, to cope with uv-induced pyrimidine dimers. The results provide good evidence that "after artificial fusion of human and chick fibroblasts, DNA repair enzymes peculiar to each species not only remain functional in the resulting multinucleate heterokaryons, but are free to act on pyrimidine dimer-containing sites in foreign DNA contributed by the fusion partner ... with absolute impartiality" (393).

478. The use of uv-irradiated human viruses as a sensitive test to detect the various defects in DNA repair have already yielded interesting results (131, 425). For instance, uv-irradiated adenovirus-2 assay procedures have revealed that every xeroderma pigmentosum strain tested has a DNA repair defect, including those previously judged by other methods to have normal DNA repair.

3. Ionizing radiation: nature of the lesion produced and its repair

479. In contrast to the great strides made with the uv work, a brief overview of which was given in the preceding paragraphs, the progress in our understanding of the precise nature of the lesions induced by ionizing radiations and the repair processes operating on this damage must be considered modest. Ionizing radiations induce single- and double-strand breaks in the DNA, cross links, apyrimidinic and apurinic sites, as well as damage to the base and sugar moieties. The elucidation of the relationship between changes induced in the biological activity of DNA by ionizing radiation and specific structural modifications or lesions has therefore been difficult. Most of the available information is concerned with the production of single- and double-strand breaks, their yield, rejoining and biological significance; some of it was reviewed in the 1972 report of the Committee.

480. The physical reconstruction of radiation-induced single-strand breaks has been shown to occur in a number of organisms and systems investigated in this respect. In *E. coli*, this process of repair is mediated by at least two distinct mechanisms: rapid repair involving DNA polymerase I and a slower, presumable recombination, repair involving the *recA* gene product (574, 575). In the radioresistant bacterium *Micrococcus radiodurans*, radiation-induced double-strand breaks also are repaired (132, 257), and evidence for such repair is accumulating in other systems as well (116, 242, 269, 432). Recent studies on a radiosensitive mutant of *Micrococcus radiodurans* have shown that it was deficient in the initial fast-rejoining reaction and the second phase proceeded more slowly, concomitantly with greater degradation of the DNA (42). The work of Gentner (187) has provided evidence for the presence of a level of Mg-dependent DNA polymerase in the mutant that was only 2 per cent of normal. A good coverage of the efficiency of the production of double-strand breaks and the dynamics of repair of these lesions in *Micrococcus radiodurans* is given in Burrell and Dean (69).

481. It has been known that extracts of *Micrococcus luteus* (394, 610), *E. coli* (568) and human cells (50) contain endonuclease activity which selectively attacks defects other than strand breaks in gamma-irradiated DNA (the so-called "gamma lesions"). Using crude

protein extracts of 12-day-old chick embryos as the source of the test endonuclease in *in vitro* assays, Paterson and Smith (395) recently identified gamma lesions in the DNA of human diploid fibroblasts. Under anoxic conditions, the incidence of gamma lesions approximates 1.2 times that of single-strand breaks concomitantly produced. Substitution of the chick extract with a crude extract of *Micrococcus luteus* yields the same number of lesions, suggesting that the endonuclease activities involved have identical, or at least closely overlapping, substrate specificities. Although there is some indication that both the type of gamma lesions and the yield depend upon the irradiation conditions (563), the chemical identity of the gamma lesions is not known with certainty. Two possibilities exist, namely apurinic sites and ring-saturated radiolysis residues of pyrimidines (see below). Since neither the calf thymus endonuclease (Bacchetti and Benne, 17) nor *E. coli* extracts act on apurinic sites, ring-saturated pyrimidine residues are the more likely. In fact, the work of Hariharan, Cerutti and colleagues strongly supports this conclusion (reviewed by Cerutti (96)).

482. Early evidence for the contribution of DNA base damage to the lethal effects of ionizing radiation came from work with bacteriophages in which the formation of single- and double-strand breaks as a function of dose and radiation conditions could be accurately measured and related to the loss of infectivity. The involvement of some form of base damage in the inactivation process was inferred when less than one double-strand break was introduced per genome per biological hit (177, 178, 597). While a considerable amount of work has been done on the radiation chemistry of free nucleic acid bases, especially of thymine, not much is known as to whether or not the major reactions observed in these model systems under non-physiological conditions are representative of reactions occurring *in situ* in the living cell. The recent experimental demonstration in Cerutti's laboratory of the formation of thymine damage following irradiation with ionizing radiation (gamma rays) in both bacterial and mammalian systems and the progress made in the enzymology of repair of these lesions therefore represent a very significant forward step in molecular radiobiology with ionizing radiations. While the assay procedures used permit the identification only of damage to thymine, additional damage undoubtedly occurs at adenine, guanine and cytosine moieties, and consequently it has been concluded that base damage is a major type of damage induced by ionizing radiation in bacterial and mammalian cells (96).

483. Hariharan and Cerutti (214, 215) have developed two highly sensitive radiochemical procedures for the determination of gamma-ray-induced thymine damage in DNA both *in vitro* and *in vivo*. The first assay determines the radiochemical reactivity of the thymine methyl group and the second, ring-saturated radiation products of thymine of the 5-hydroxy-6-hydroperoxydihydrothymine type (the latter will hereafter be abbreviated to t′). In a series of papers, evidence was presented showing that (a) thymine damage is one of the major types of gamma-ray-induced damage in bacterial (214, 215, 556, 557) and mammalian cells *in situ* (219, 440); (b) excision repair of such products occurs in bacterial and mammalian cells (97, 215, 219); (c) crude concentrated extracts of *E. coli* (216, 218) and isolated mammalian nuclei (219, 326, 441) are able to selectively remove the t′ residue from gamma-irradiated poly [d(A-T)] or OsO_4-oxidized poly [d(A-T)] or bacteriophage PM2 DNA; (d) although the identity of the endonuclease recognizing t′ products in *E. coli* is not fully established, possible candidates appear to be endonuclease II (196) and the endonuclease recognizing depurinated DNA (598); (e) the second step in the excision repair of t′ (namely the exonucleolytic degradation of the damaged DNA region) is accomplished by $5' \rightarrow 3'$ exonuclease associated with the polymerase I of *E. coli* (247); (f) the last step in excision repair (strand resealing) is accomplished by ligase (216); and (g) the excision of gamma-ray-induced thymine damage in mammalian cells used (CHO, HeLa S3) is considerably faster—between 25 and 35 per cent of the t′ removed within 60 minutes of incubation at 37°C (219)—than that of cyclobutane-type photodimers, which has been shown to continue for several hours in human cells (see Cleaver (111) for a review of the latter).

484. In the context of the excision repair of gamma lesions discussed in the preceding paragraph, it is worth noting that Paterson *et al.* (400) observed a decreased ability of some ataxia telangiectasia (AT) strains to undergo repair replication following exposure to gamma rays delivered under anoxic conditions. This finding implicated defective DNA repair in the aetiology of radiosensitivity and predisposition to neoplastic transformation, two clinical features of the syndrome. In leucocytes from these patients, the radiation-induced chromosome aberrations were found to be enhanced (228, 426). In fibroblasts, the rates of rejoining of single and double-strand breaks appear to be similar to those in normal cells (571).

485. The results of Remsen and Cerutti (430), on the other hand, show no *in vitro* deficiencies for the excision of t′ products from exogenous DNA-chromatin substrates (from sonicates of three AT cell lines tested, including two studies by Paterson *et al.* (400)) which had been gamma irradiated under aerobic conditions. Remsen and Cerutti point out that differences in experimental design (in particular the irradiation conditions) between their work and that of Paterson *et al.* do not permit a straightforward comparison of the results. In addition, Remsen and Cerutti used unirradiated cell preparations as the enzyme source whereas supra-lethally irradiated cells were used by the other investigators. Different lesions are produced in DNA under aerobic and anoxic conditions, which may be processed by different repair pathways. Support for this notion comes from work with XP cells, where ionizing radiation induced normal levels of repair replication under aerobic but not anoxic conditions (386).

486. The role of DNA repair in the progeria syndrome (a human genetic disease characterized by accelerated ageing) is not clear and the reports that have appeared are contradictory. Epstein *et al.* (158) reported that progeroid fibroblasts seemed defective in strand-break repair, but Regan and Setlow (429) were unable to repeat this observation and suggested that the basic defect is not one of repair enzymes, but more likely one

of permeability or fragility of progeroid cells in culture. From the findings that several progeric strains manifest differing and unusual responses to ionizing radiation, a finite repair capacity in repair-deficient strains and that normal repair apparently returns after SV40 transformation, Little *et al.* (292) suggest that the control of the genetic expression of a repair system may be involved.

4. DNA repair in mouse germ cells

487. In spite of a number of DNA repair studies carried out with different mammalian somatic cell systems, not much similar work has been carried out with germ cells until recently. The report of Sega (522) in 1974 demonstrating unscheduled DNA synthesis in the germ cells of male mice exposed *in vivo* to alkylating chemical mutagen EMS,[24] therefore, is a very significant contribution. Using the well studied sequence of events occurring during spermatogenesis in particular and administering (by testicular injections) ^{3}H-dT, Sega obtained clear evidence for unscheduled DNA synthesis; the germ cell stages showing it ranged from early to middle meiotic prophase stages through early to middle spermatids. The initiation of this synthesis (taken to be evidence for repair of chemically-damaged DNA in these germ-cell stages) is rapid, beginning within one hour after injection of EMS. Unscheduled DNA synthesis was not detected in the most mature germ-cell stages, which are responsive to the induction of dominant lethals, nor was it found to occur in stages where protamine replaces the chromosomal proteins (during spermiogenesis).

488. Subsequently, Sega *et al.* (524) focused attention on early spermatids and extended the findings with EMS to three chemical analogues: MMS, PMS and IMS. The results indicate that (a) with all four there is a linear increase in DNA repair in early spermatids with increasing dose of the chemical; only at the highest dose of MMS (100 mg/kg) is the repair response observed higher than expected on linearity; and (b) with equimolar doses, the most effective chemical in inducing DNA repair is MMS followed by EMS, IMS, and PMS.

489. In a further study, Sega (523) has extended the finding of unscheduled DNA synthesis (with chemicals) to x-ray damage. The stages showing the repair of x-ray damage are the same as those found undergoing repair of EMS-induced damage except that the level of unscheduled incorporation of ^{3}H dT after x-ray treatment is much less, in spite of the fact that the EMS dose used (250 mg/kg) gives a roughly comparable yield of dominant lethals to that given by 600 R of x rays (185). Secondly, the level of DNA repair occurring in early spermatids has been found to increase linearly with increasing x-ray exposures from 50 to 600 R, and at 1200 R there is evidence for an elevated level of repair. Thirdly, unscheduled DNA synthesis (after 600 R) is highest immediately after irradiation and continually decreases during the subsequent 4 h although some repair is still measurable at this time.

[24] EMS = ethylmethane sulphonate, MMS = methylmethane sulphonate, PMS = propylmethane sulphonate, IMS = isopropylmethane sulphonate.

490. Ono and Okada (386) irradiated male mice 10-14 weeks old (gamma irradiation, 10-40 krad, 3300 rad/min) and used the testes from these animals to study single-strand breaks in the DNA and their rejoining using alkaline density-gradient centrifugation methods. Microscopic examination of cell suspensions from the testes revealed that the population was made up of about 3 per cent spermatogonia, 12 per cent spermatocytes, 70 per cent spermatids, 6 per cent spermatozoa and 9 per cent unknown. The conclusions drawn therefore apply primarily to DNA from spermatid-rich populations. It was found that the number of single-strand breaks was 0.22 per 10^{12} daltons of DNA per rad, one which was significantly lower than the rate of 0.6 to 0.7 per 10^{12} daltons per rad for mouse liver and thymus DNA found in an earlier investigation (385). The authors obtained evidence to show that this difference is primarily due to hypoxic conditions of the testicular cells. A majority of the breaks were found to rejoin quickly, the time necessary for rejoining of 50 per cent of the breaks being about 18 min at 10 krad and 50 min at 40 krad. The remainder of the breaks did not seem to rejoin until about 6 h after irradiation. Ono and Okada suggest that these latter breaks might be of the slow and/or non-reparable DNA scissions, since even under "low" dose-rate conditions (40 rad/min compared with 3300 rad/min) a similar situation was obtained.

491. In a subsequent study, Ono and Okada (387) refined their methods and were able to study single-strand breaks and their rejoining in spermatogonia-rich and spermatozoa-rich populations. In spermatogonia, single-strand breaks were induced at a rate of 0.42 10^{12} daltons of DNA per rad under oxic (aerated) conditions, decreasing to 0.24 per 10^{12} daltons per rad under hypoxic conditions. In spermatozoa, the break-efficiency was 0.22 breaks per 10^{12} daltons per rad under oxic conditions and changed little under anoxic conditions. The breaks were efficiently repaired in spermatogonia, 50 per cent of the breaks rejoining within about 10 min after 10 krad and within about 30 min after 40 krad. The slow or non-rejoining fraction for these doses was 40-60 per cent. In contrast, the breaks in spermatozoa were not rejoined at all even two days post-irradiation.

492. Masui and Pedersen (325) examined the repair capacity of full-grown mouse oocytes after exposure to uv-irradiation. Fully-grown oocytes were aseptically isolated from the ovaries of adult mice and cultured in chemically-defined medium. Irradiations were carried out 2-18 h after isolation and the oocytes were then immediately incubated with ^{3}H-dT. Many of the oocytes underwent germinal vesicle (GV) breakdown within 3 h after isolation and metaphase I (MI) was reached 4-8 h later. Oocytes cultured for 16-18 h reached metaphase II (MII) as evidenced by the appearance of the first polar body (PBI). Autoradiography showed that grain counts over the GVs or the chromosome set were significantly higher after uv irradiation than in controls, regardless of the stage of meiotic maturation at which they were irradiated. The grain count increased with dose from 30 to 60 J/m^{2}, but except for the MI oocytes, there was no further increase at 120 J/m^{2}. The numbers of grains over the GVs were approximately an order of magnitude higher than over the MI and MII chromosomes and those

over the PBI were much less (relative to MI and MII). It is thus clear that mouse oocytes can carry out unscheduled DNA synthesis after uv irradiation and thus seem capable of excision repair of DNA damage in these stages.

5. DNA repair in *Drosophila*

493. In recent years, an increasing interest has been manifest in studies on DNA repair in *Drosophila*. In 1969, Valencia and Plaut (594) demonstrated unscheduled DNA synthesis in the salivary glands of x-irradiated larvae. Trosko and Wilder (577) showed that thymine-containing dimers are excised from the DNA of uv-irradiated tissue culture cells. Boyd and Presley (47) showed that repair replication of DNA (in first instar larvae) was stimulated by treatment with uv, x rays, MMS and EMS; MMS was found to be ten times as potent as EMS. With uv, a dose of 20 J/m^2 largely saturates the level of repair replication; the latter falls off after x-ray exposures of 80 kR. The authors also studied photo repair (as a reduction in repair synthesis resulting from post-irradiation exposure to photo-reactivating light) and found that this occurs after uv irradiation but not after x irradiation.

494. Other lines of work have focused attention on the isolation of mutagen-sensitive mutants as experimental material for the systematic analysis of genetic controls of DNA replication, DNA repair and recombination (542, 543). A number of mutants which were selected on the basis of hypersensitivity to larval killing by MMS have now been assigned to seven complementation groups on the X-chromosome (48, 543), and mutants at one locus have been clearly identified as alleles of a previously described meiotic mutant *mei-41* (23), thereby confirming that mutagen sensitivity can be employed as an effective method for the selection of recombination-defective mutants in this organism. In addition, mutants belonging to certain complementation groups are sensitive to nitrogen mustard (HN_2) and current evidence (48) indicates that there is a correlation between sensitivity to HN_2, to 2-acetylaminofluorene and a deficiency in post-replication repair. Several of the mutants exhibit sensitivity to gamma rays, although only the *mei-41* mutants are hypersensitive to uv irradiation. Nguyen and Boyd (362), who studied the *mei-9*^{b} mutant (mutant alleles at the *mei-9* locus which reduce meiotic recombination in homozygous females (77)) using the technique employed previously by Boyd and Presley (47), found that it was deficient in repair replication after uv or x irradiation. This result is complementary to their observations that homozygous larvae of *mei-9* alleles are hypersensitive to killing by x-rays as well as uv irradiation.

6. Relationships between the radiation-induced lesion in DNA, its repair, and the production of mutations and chromosome aberrations

495. Turning now to the question of how the nature of the uv- or x-ray-induced lesions in the DNA and the mechanisms by which they are repaired can account for the production of mutations or chromosome aberrations, it can be stated that we have relatively more information available for uv damage. In prokaryotes where this problem has been intensively studied, there is no doubt that pyrimidine dimers are an important source of mutations, although the role of other photoproducts is less clear. In the yeast *Saccharomyces cerevisiae*, there is evidence that pyrimidine dimers are responsible for most of the revertants of *cyc1-9* in *rad+*, *rad 1* and *rad 6* strains (271) (see also paragraph 498). If pyrimidine dimers or the other photoproducts were the direct cause of mutations, every kind of repair that eliminates these products should reduce the frequency of mutations. This however is not the case. Convincing evidence for the possibility that under certain circumstances repair may produce mutations rather than erase them was provided in bacteria by Witkin (613). Her distinction between error-proof (mutation-proof) and error-prone (mutation-prone) repair has been generally accepted. Both types of repair remove lethal lesions and increase survival, but while the former restores the original nucleotide sequence and thus removes potential mutations, the latter tends to insert a wrong base sequence and thus produces mutations.

496. Photoenzymatic repair is error-proof; the bulk of excision repair, too, is error-proof and reduces lethality as well as mutation frequency. Only a small fraction of mutations induced by uv arise from errors in pre-replicative repair while the bulk are produced by an error-prone post-replicative process. Recently, Witkin and others (188, 614, 616) have adduced evidence that in bacteria the error-prone mechanism requires an inducible enzyme for its action.

497. There is much less information on the relationship between uv damage and chromosome aberrations. Griggs and Bender (202) demonstrated that all the lesions which lead to uv-induced chromosome aberrations in *Xenopus* cells disappear on photoreactivation. This is perhaps the only experiment which directly demonstrates a relationship between DNA repair and chromosome aberrations. Following uv irradiation of XP cells, Parrington *et al.* (391) demonstrated that there was a six- to seven-fold increase in the frequency of chromosome aberrations (relative to normal cells). However, the data are only preliminary, with only one fixation time, namely 30 h, and the increase could be due to other factors as well (357).

498. The possible role of single-strand breaks and base changes in generating x-ray-induced mutations was considered in the 1972 report of the Committee (Annex E, paras. 516-525). The recent experimental demonstration that base damage is a predominant component of x-ray damage in the DNA (*in vivo* and *in vitro*) and that it is repairable will undoubtedly intensify the search for correlations between this and mutational damage in prokaryotic and eukaryotic systems. Whatever the nature of the primary lesion, its final fate depends largely on the type of repair to which it is exposed. It is clear that repair mechanisms—some of them identical with those acting on uv lesions—enter in similar ways into x-ray mutagenesis. In bacteria, the functions of two repair genes (*recA* and *exrA*) that are essential for uv

mutagenesis also play a major role in the production of mutations by x rays (234). A similar situation is obtained in the yeast *Saccharomyces cerevisiae:* Lawrence *et al.* (271) have shown that the highly specific uv-inducible reversion of *cyc1-9* (an ochre mutant (UAA codon) of the structural gene for iso-1-cytochrome c) depends largely on the action of the *rad6* locus, which appears to be involved in the repair of damage induced by uv gamma rays and all chemicals tested. *Rad18,* a gene concerned in the same pathway as *rad6,* also has some influence on the specificity, though the evidence is less decisive in this case. *Rad1,* a gene that functions in ecxision repair, has no effect on this specificity.

499. In a subsequent paper (271a), Lawrence and Christensen have presented data which suggest that in *Saccharomyces cerevisiae* there is a single error-prone repair pathway involving the function of at least seven genes (*rad6, rad9, rad18, rev1, rev3* and possibly *rev2* and *rad8*). The function of two of these (*rad6, rev3*) would seem to be essential for uv mutagenesis at all sites and of all types, while the others appear to act principally or exclusively during the production of certain mutational events. In contrast, all mutations blocking excision repair exhibit enhanced mutability per unit dose, a result compatible with the idea that this process is largely error-free.

500. Double-strand breaks in the DNA are presumably responsible for the production of chromosome aberrations. The possibility of single-strand breaks being converted into double-strand breaks during repair by a single-strand nuclease has been proposed by Bender *et al.* (35) in their general model for chromosome aberration production by diverse agents. Currently available information on the sensitivity of cells from patients with different inherited disorders to the induction of chromosome aberrations by different mutagens (including x rays) and the known defects in DNA repair mechanisms in these cells has been summarized by Natarajan (357). In the case of AT cells discussed earlier (para. 484) there is evidence for an increased sensitivity to chromosome aberration induction by x rays in leucocytes, and as may be recalled, these cells may be deficient in excision repair of base damage under certain conditions. XP cells and cells from patients with Fanconi's anaemia and progeria do not appear to show any increased sensitivity to x-ray induction of aberrations although they are known to be deficient in one or another step in DNA repair.

501. Scott, Fox and Fox (503) studied the relationship between chromosomal aberrations, survival and DNA repair in tumour cell lines of differential sensitivity to sulphur mustard (SM) and x rays. In the first comparison with a pair of rat lymphosarcoma cell lines (Yoshida) with a pronounced differential sensitivity to killing by SM (but with the same sensitivity to x rays), the resistant line suffered much less chromosome damage after SM treatment than the sensitive cells in spite of equal alkylation of DNA, RNA and protein in both; the amount of x-ray-induced chromosomal damage was similar in both. In the second comparison with a pair of mouse lymphoma cells (one resistant and the other sensitive to killing by x rays), much less chromosomal damage was observed in the resistant line, although there were no differences between the two lines in their capacities for repair replication either after SM or x-ray treatment. As Natarajan (357) has pointed out, the type of measurement used for repair replication, i.e. unscheduled DNA synthesis, gives information only about the whole repair process which may not all be related to the induction of chromosome aberrations.

7. Conventional (non-molecular approach) repair studies in *Drosophila*

502. Several aspects of radiosensitivity and repair phenomena studied in *Drosophila* germ-cell stages were reviewed in the 1972 report of the Committee. A comprehensive treatment of the subject has since appeared (496), besides others which focus attention on the radiosensitivity of embryonic stages (623) and on aberration induction and segregation in female germ-cell stages (390). In what follows, some of the more recent work on repair of genetic radiation damage in *Drosophila* and that which has a bearing on the restitution of chromosome breaks induced in mature spermatozoa will be considered.

503. It has long been known that chromosome breaks induced in *Drosophila* spermatozoa do not rejoin before fertilization (256, 350). More recently, Leigh and Sobels (277) showed that compound chromosomes (autosomes) homozygous for markers on one or the other of the chromosome arms could be recovered after x irradiation of post-meiotic male germ-cell stages. The interpretation of this finding was that these chromosomes originate by a chromatid type of exchange occurring after the post-zygotic chromosome replication, the implication being that the chromosome breaks induced in these germ-cell stages stay open until this time. Further evidence substantiating this thesis came from exposure fractionation studies which showed interaction of breaks produced in spermatids in male flies (544) (or in male pupae, 48 h old (545)) with those produced in mature spermatozoa (in inseminated females). There are other findings which strongly suggest that the repair machinery in the oocytes plays a vital role in the formation of induced chromosome aberrations in male germ-cell stages (see paras. 504-505). It should therefore be possible, by suitably modifying the genotype of the females and/or physiological environment of the oocytes to influence the magnitude of the genetic damage induced in the male germ cells. The work that has been carried out during the past few years in the laboratories of Sobels and of Würgler adequately support this line of reasoning.

504. In a series of studies, Würgler and his collaborators (68, 622, 625) have collected data which indicate that (a) the rate of recoverable ring-X chromosome losses in mature sperm is dependent on the genotype of the females used; (b) such "maternal effects" are demonstrable for radiation-induced dominant lethals, loss of rod-X chromosomes and translocations; and (c) for all the female stocks, the rate of recoverable XO males falls drastically to nearly one-half after the first day. The authors concluded that (a) some factors which are under the control of the maternal genome influence the

x-ray-induced lesions in the sperm chromosomes after insemination of the egg, (b) a change in the lesions in the stored sperm occurs during the first 24 h of storage in the females, and (c) sperm inseminating old, stored stage-14 oocytes (present in aged virgin females) are influenced by a maternal factor in a different way or to a different degree than sperm inseminating newly produced non-stored oocytes. The observation of Würgler and Maier (622) that the frequency of sex-chromosome losses induced in spermatozoa is dependent on the genotype of the females used for the matings and that of Würgler, Bürki and Bürki (624) and Bürki (68) that it is differentially affected in stored and non-stored oocytes in the females have been extended by Clark (109).

505. Following the earlier work of Proust (421) and of Proust, Sankaranarayanan and Sobels (422), which showed that mating of irradiated males to females injected with actinomycin D led to an increase in the frequencies of dominant lethals and a decrease in those of translocations and recessive lethals, Mendelson (330, 331) and Mendelson and Sobels (332) extended this line of inquiry to the effects of caffeine treatment of females. It was found that (a) caffeine treatment of females led to an increase in the frequencies of dominant lethals and sex-chromosome losses and a decrease in those of translocations induced by x irradiation of mature sperm; (b) different strains of females used manifested different levels of repair of radiation-induced breaks in males, with one strain very deficient in this repair machinery; and (c) the repair system specific for the repair of chromosome damage induced in the paternal genome is susceptible to blocking by metabolic inhibitors such as caffeine.

506. In a very recent study, Maddern and Leigh (320) have obtained convincing evidence for the thesis that a high proportion (as much as 75 per cent) of the "total" and "partial" sex-chromosome loss induced by x irradiation of mature *Drosophila* sperm is a consequence of chromatid rearrangements arising from chromosome breaks which stay open until they are replicated. This finding provides a fresh insight into our thinking on the mechanism of chromosome loss.

8. Summary and conclusions

507. The past few years have witnessed the accumulation of a substantial amount of information on DNA damage induced by mutagenic agents and on repair processes in both prokaryotic and eukaryotic systems.

508. A number of specific lesion-recognizing enzymes involved in excision repair have been purified and characterized. Evidence for the presence of photoreactivating enzymes in human leucocytes, human and murine fibroblasts have also been adduced.

509. With the exception of *E. coli*, the excision repair process is perhaps most extensively investigated in human fibroblasts. There is evidence that there are at least five complementation groups associated with defects in excision repair in xeroderma pigmentosum cells. Search for similar repair defects in other human diseases has met with only limited success.

510. By means of cell-fusion techniques (human and chick fibroblasts) it has been possible to show that the repair enzymes peculiar to each species remain functional in the resulting multinucleate heterokaryons and act on pyrimidine dimer-containing sites in foreign DNA contributed by the fusion partner.

511. The most significant advance in ionizing radiation studies is the identification of base (thymine) damage and the development of sensitive procedures (radiochemical) for detecting it in microorganisms as well as in mammalian cells. These gamma lesions (as they are called) are subject to excision repair and an endonuclease acting specifically on such lesions has been found.

512. Some ataxia telangiectasia cell strains appear to be defective in the excision repair of gamma lesions following gamma-ray exposures under anoxic conditions; after irradiation and under aerobic conditions, they are excision-repair proficient.

513. The induction of single-strand breaks in mouse germ cells has been investigated. The results show that they are induced at rates of 0.42 per 10^{12} daltons of DNA per rad in spermatogonia, 0.22 per 10^{12} per rad in spermatids, and 0.22 per 10^{12} per rad in spermatozoa (under aerated conditions). A majority of the single-strand breaks induced in spermatogonia and spermatids undergo rapid rejoining (50 per cent of the breaks rejoining in 10-18 minutes after 10 krad), while the remainder were of the slow or non-rejoining type. In contrast, in spermatozoa, the breaks induced do not undergo rejoining even two days post-irradiation.

514. Unscheduled DNA synthesis has been demonstrated to occur following treatment with alkylating agents and with x rays in meiotic prophase stages and in spermatids in the mouse; such synthesis also takes place in full-grown mouse oocytes after uv irradiation.

515. There is evidence for the occurrence of unscheduled DNA synthesis in the salivary glands of x-irradiated *Drosophila* larvae and for the excision of thymine-containing dimers in tissue culture cells. In first instar larvae, repair replication is stimulated by treatment with uv, x rays, MMS and EMS.

516. In *Drosophila* as in prokaryotes, sensitivity to mutagens can be employed as an effective method for the selection of recombination-deficient mutants. Mutants selected on the basis of hypersensitivity to larval killing by MMS have been assigned to seven complementation groups. Mutants belonging to certain complementation groups are also sensitive to nitrogen mustard, 2-acetylaminofluorene and appear to be deficient in post-replication repair.

517. The relationships between lesions induced in DNA, repair processes and mutations have been worked out in prokaryotes and some simple eukaryotes; after uv irradiation, there is no doubt that pyrimidine dimers are an important source of mutations, but there is evidence that certain repair processes may be error-prone. Photoenzymatic repair is error-proof; most

excision repair is also error-proof. The bulk of mutations are produced by an error-prone post-replicative repair process (after uv irradiation).

518. In higher eukaryotic systems (mammalian cells, for instance) there is no direct evidence on the relationship between uv lesions and mutation induction. In *Xenopus*, there is some evidence for the involvement of photoreactivable uv damage in chromosome aberrations.

519. The recent experimental demonstration that base damage is a major component of damage induced by ionizing radiation in prokaryotic as well as eukaryotic systems and that it is subject to excision repair will intensify the search for correlations between these and mutational events.

520. Double-strand breaks induced in DNA are presumably responsible for the production of chromosome aberrations, but the exact relationship between these and the known DNA repair processes is not clear.

521. Studies on the repair of x-ray-induced genetic damage in male germ-cell stages of *Drosophila* (non-molecular approach) have shown that chromosome breaks induced in spermatids of pupae 48 h old remain open and are available for interaction with breaks produced in spermatozoa.

522. A considerable amount of evidence has been obtained in *Drosophila* which strongly suggests that the repair machinery in females plays a vital role in the formation of induced chromosome aberrations in male germ-cell stages. This has been demonstrated by suitably modifying the genotype of the females and by physiological alteration of the oocyte environment with metabolic inhibitors.

523. In *Drosophila*, recent results indicate that a high proportion of the total and partial sex-chromosome losses induced by x rays in spermatozoa is a consequence of chromatid rearrangement arising from breaks which stay open until they are replicated (after sperm entry into the egg).

IV. INTERSPECIFIC COMPARISON OF MUTATION RATE ESTIMATES

1. Introduction

524. Recently, Abrahamson *et al.* (4) re-examined the available data on forward mutations at specific loci induced by acute, low-LET irradiation for a wide variety of organisms from bacteria to mammals and noted that the rates per locus per rad varied over three orders of magnitude; however, when these rates were adjusted for the amount of DNA per nucleus, the mutation rates obtained were all essentially the same, varying by a mere factor of 3 instead of 1000. The authors speculated that "the consistency that obtains when the data are adjusted for the amount of DNA per nucleus for each species indicates either that it is the nucleus and not the locus that determines the target size or that, on the average, the size of a (radiation-mutable) locus is proportional to the total genome (DNA content) for the species . . . it suggests that extrapolation directly from experimental organisms to man can be done with confidence."

525. In a subsequent paper, Conger (118) compiled and analysed the data on the amount of chromosomal proteins and RNA in relation to the amount of DNA in different species. He found that the total mass of chromatin per unit mass of DNA is not very different over the span from prokaryotes to higher eukaryotes, i.e., the total chromosomal mass and expected target size is proportional to DNA content over this span of species. That permitted him to conclude that "the correlation of induced mutation rate with DNA content per cell over a wide range of species cannot be used to establish that DNA alone is the target for induction of mutations by ionizing radiation. The target could be DNA alone; but equally well, it could be total chromatin mass." The *Ad Hoc* Panel on Research Needs for Estimating the Biological Hazards of Low Doses of Ionizing Radiations of the United States National Research Council–National Academy of Sciences (431) expressed this opinion: "We believe this normalizing of mutation rates by the amounts of DNA per nucleus is a valid genetic bridge between lower organisms and man" (see also Wolff (617)).

526. Kohn (265) made estimates of gene sizes in different species based on target theory considerations and found that (a) in the mouse, the estimates from the data on the seven-specific-loci tests (300 R, acute x irradiation, spermatogonia) are consistent with expectations, whereas those from the histocompatibility loci are not; and (b) most of the genes (or groups of genes) tested in different organisms for forward mutation with low-LET irradiation tend to behave more like the histocompatibility loci than the seven loci in the mouse (see also paragraph 275).

527. If the hypothesis of Abrahamson *et al.* (4) is valid, it will have far-reaching implications, especially for the evaluation of mutational radiation hazards in man. Schalet and Sankaranarayanan (562), who made a critical analysis of the data used by Abrahamson *et al.* (and of others not cited by these authors but which bear on the issue in question), concluded that (a) the notion of simple proportionality between DNA content and mutation rate cannot be substantiated and (b) the generalization that we can now extrapolate from lower organisms to man with greater confidence must be viewed with extreme reservations. Since all the arguments have been given in detail in the above paper, the following paragraphs will be devoted to summarizing the main points only. The papers of Conger (118) and of Kohn (265) will not be further considered in this review since, for the former, the basic premise (the existence of a linear relationship between mutation rate and DNA content) is weakened (562) and, for the latter, the estimates of gene size based on target theory considerations are likely to be incorrect.

2. General considerations

528. One very important consideration that should be borne in mind in normalizing mutation rates in different species to DNA content is whether the mutation rates

themselves relate to a common base line, namely, to changes within a "locus" or gene to the extent to which we can define them with precision at present. Furthermore, for most of the organisms, owing to the lack of biochemical and genetic analysis, there is the uncertainty as to whether a locus consists of one gene or more than one gene, although the terms "locus" and gene are used interchangeably in the absence of a better alternative.

529. A second important consideration is whether, with the array of mutation screening techniques used in different organisms, the same proportion of all the induced mutations is detected. The question itself is not a new one and has been raised earlier (see, for example, reference 358). In fact, the particular technique used for detecting mutations can be a major determinant of the mutation rate estimate. Two examples will suffice to illustrate this point. First, in *Drosophila, rosy* locus mutants are detected as brownish eye-colour variants by conventional tests; in addition to eye-colour variants, there are, at this locus, several classes of mutations which present a normal eye colour and that can be detected by other techniques (105). Secondly, there are other findings, such as those in *Saccharomyces cerevisiae,* which show that, at the molecular level, different forward mutation systems vary widely in their ability to detect the broad spectrum of intragenic mutational events (539) and consequently, the comparison of mutation rates even in one and the same organism is complex, not to mention interspecific comparisons.

530. Thirdly, and this is a relatively minor point, some of the DNA values cited in the paper of Abrahamson *et al.* refer to total cellular DNA. Therefore, these values include different, but for the present purpose generally insignificant, proportions of non-nuclear DNA. However, it is clear that Abrahamson *et al.* erred in using a DNA content for *E. coli* which is about three times the widely accepted value (0.004 pg) (75, 119). Furthermore, a very recent estimate of the nuclear DNA content for *Saccharomyces cerevisiae* has raised the distinct possibility that the value used by Abrahamson *et al.* may also be overestimated by a factor of three (270).

3. Examination of the data used by Abrahamson *et al.*

531. The data used by Abrahamson *et al.* are derived from experiments and with test systems which differ widely in their ability to discriminate between changes within genes as opposed to those involving several genes or loci and those which can include gross chromosomal aberrations, such as losses of chromosome arms or even entire chromosomes. That means that the effective radiation-mutable target is not necessarily the same in the different organisms whose mutational response is compared, making the grounds for comparison insecure, if not totally invalid.

532. The rate for the induction of *waxy* mutations given by Abrahamson *et al.* is not a forward mutation rate but a back mutation rate, does not pertain to three loci but to a single locus, and does not relate to acute irradiation but to chronic irradiation with ^{90}Sr. Arguments have been presented in the literature (159, 290) for believing that radiation-induced *waxy* mutations are in fact chromosomal aberrations. Likewise, the data for tomato used by Abrahamson *et al.* pertain to chromosomal deletions (60, 61, 434) and some of the mutations may represent loss of chromosome arms or even whole chromosomes.

533. The *E. coli* data of Demerec and Latarjet (137) on the resistance to T1 phage used by Abrahamson *et al.* were collected at a time when much was not known about the nature of changes involved in phage resistance in *E. coli.* Several lines of evidence accumulated since then testify to the complexity of this phenomenon. Phage resistance in *E. coli* can originate in two unlinked regions designated as *tonA* and *tonB*, the latter of which is a classical deletion system: *E. coli* can tolerate extensive deletions of *tonB* including the neighbouring tryptophan operon, and such deletions exhibit the *tonB* phenotype. Mutations to T1 resistance having an additional auxotrophic phenotype of many types other than tryptophan have been reported (64, 65). Some of these may have been deficiencies involving the *tonA* region while others seem to involve nutritional deficiencies that are not linked to either *tonA* or *tonB*. *TonB* mutants and deletions require exogenous iron for their growth and are inhibited by chromium (210); surprisingly high concentrations of chromium have been found in commercial agar preparations commonly used to prepare solid media (120). Consequently, the recovery of phage-resistant mutants on solid media containing chromium in sufficient quantity to inhibit growth of resistant (but not of sensitive) cells may well have contributed to the low mutation rate reported by Demerec and Latarjet (137).

534. In the other organisms–*Neurospora, Drosophila,* and the mouse–(the data for which were used by Abrahamson *et al.*), the level of genetic analysis used to detect and characterize the events scored as specific-locus mutations varies a great deal. Only in *Neurospora* can one be certain that the data pertain to intragenic changes, since here the distinction between intragenic and extragenic changes has been made by intensive and rigorous complementation tests. In *Drosophila,* this distinction is made on the basis of cytological analysis of the mutants (salivary gland chromosomes), and the absence of a detectable aberration (within the limits of resolution possible) is considered presumptive evidence that one is most likely dealing with a change at the level of the gene. While the viability of homozygotes for the mutation (or of hemizygotes in the case of sex-linked ones) can be considered good evidence for intragenic change, its lack does not necessarily demonstrate the presence of an aberration, since lethal intralocus changes are well documented in *Drosophila* (604). In the mouse, transmission tests (to determine whether one is dealing with a mutation) and homozygous viability tests are used to characterize the nature of changes. Here again, while homozygous viability can be construed as good evidence for an intralocus change, its lack does not necessarily demonstrate the presence of an aberration. For instance, most of the *d* locus mutations recovered from irradiated (x or gamma) spermatogonia have been found to be homozygous lethal, but the evidence from complementation tests suggests that a majority of these involve intragenic lethal changes at the *d* locus (453).

535. The estimate of $1.28\ 10^{-8}$ rad^{-1} per locus of Alexander (9) used by Abrahamson *et al.* represents the best presumptive intralocus mutation rate for *Drosophila* spermatogonia. For mouse spermatogonia, the rate of $1.7\ 10^{-7}$ rad^{-1} per locus (used in the 1972 report of the Committee) has been estimated from the results obtained in experiments involving two sets of loci (seven and six respectively, with one locus being common to both sets) giving equal weight to each locus. It should be remembered that the overall response of the two sets of loci differ by a factor of three and within the seven loci (for which the data are extensive) the difference between the lowest rate (*a* and *se* loci) and the highest rate (*s* locus) is more than 30-fold (488). Schalet and Sankaranarayanan (562) used the data presented by W. L. Russell and L. B. Russell (488) and L. B. Russell and W. L. Russell (459), in conjunction with those given by L. B. Russell (453) to estimate a probable intralocus rate for mouse spermatogonial specific-locus mutations. The assumptions here are that (a) all homozygous viable mutations are intralocus changes and (b) most of the *d* locus lethals are intralocus events. From these they concluded that for the seven loci, about 37 per cent of all spermatogonial mutations are likely to be intralocus changes. This gives a figure of $0.81\ 10^{-7}$ rad^{-1} per locus (i.e., 37 per cent of $2.2\ 10^{-7}$).

4. Other relevant data

536. An examination of other relevant mutation rate data published in the literature suggests, not unexpectedly, that the average mutation rate is a function of a given cell stage, a given set of loci, or a given set of experimental conditions; there is therefore no sound reason to use one or the other alone as typical of the response of the species under consideration. This point can be illustrated by the following examples.

537. In *Neurospora*, de Serres *et al.* (529) reported an intralocus rate of (5-6) 10^{-9} rad^{-1} per locus (*ad-3A* and *ad-3B* loci) under slightly different experimental conditions than the ones used in the work of Webber and de Serres (602). (In the latter work, the rate was estimated as $2.7\ 10^{-9}$ rad^{-1} per locus.) Again in *Neurospora*, Hoffmann and Malling (230) found that the rate of x-ray induction of forward mutations at the *aza* locus was higher than in the *ad-3* system, being $3.1\ 10^{-8}$ rad^{-1}. Although the isolated mutants were tested for transmissibility, no analysis was made to discriminate between intralocus and extralocus events. If the *aza-3* locus were to show a proportion of intralocus mutations similar to that in the *ad-3* system (in the latter, at 10 kR, roughly two thirds were intralocus events), then the mutation rate becomes $2.0\ 10^{-8}$ rad^{-1}. The above data plus those for the *ad-3* loci permit an overall estimate of $8.7\ 10^{-9}$ or $1.0\ 10^{-8}$ rad^{-1} per locus, depending on whether the *ad-3* data used are those of Webber and de Serres or those of de Serres *et al.* (See also the arguments presented in Hoffmann and Malling (230) for believing that the *aza-3* frequency and possibly even the *ad-3* frequencies were obtained under conditions of suboptimal mutant expression.) At face value, either of the two rates above is not far removed from the rate for *Drosophila* spermatogonia ($1.28\ 10^{-8}$ R^{-1} per locus). The *Drosophila* haploid DNA content is about 4.5 times higher than that of *Neurospora*.

538. In *Saccharomyces cerevisiae*, Skobkin (540) studied the gamma-ray induction of canavanine resistance and noted that the maximal recovery rate was $1.0\ 10^{-10}$ rad^{-1} per locus or about one fifteenth of that given by Abrahamson *et al.* Skobkin found that the selection of mutants, among other things, was so strongly dependent on the initial number of cells inoculated into the medium and the concentration of canavanine used that, under certain conditions, no mutants were recovered. (For details of other complexities encountered in this system, see also references 201 and 637.) Schwaier (564) who studied the x-ray induction of forward mutations at the adenine loci in the same organism, reported a rate of $6.0\ 10^{-9}$ rad^{-1} per locus. In a similar system studied in *Schizosaccharomyces pombe*, the data of Nasim (356) and of Loprieno *et al.* (293) permit estimates ranging from $0.4\ 10^{-9}$ to $1.1\ 10^{-9}$ rad^{-1} per locus per haploid genome.

539. In barley, the available data for the x- or gamma-ray induction of eceriferum, erectoides and chloroplast mutations show that there is no discernible relationship between the mutation rates and DNA content; in *Arabidopsis thaliana* the mutation rate to thiamine auxotrophy is $(1.1\text{-}1.5)\ 10^{-9}$ rad^{-1} per locus per haploid genome, which is an order of magnitude *lower* than that for *Drosophila* spermatogonia, although the haploid content of *Arabidopsis* is 1.5 times that in *Drosophila*.

540. Data collected by W. L. Russell (475, 478, 480) in female mice (see reference 506 for a recent review) show that the mutation rate at the seven specific loci (acute x irradiation) for maturing oocytes varies with the dose; in immature oocytes virtually no mutations have been recovered. These observations are out of line with the predictions of Abrahamson *et al.* on the relationship between DNA content and mutation rate. The near absence of x-ray-induction of mutations at the histocompatibility loci (paras. 272-274) in the mouse provides an additional case in point.

541. Table 44 summarizes the data (some of which were discussed in the preceding paragraphs) that are worth examining in the context of interspecific comparisons, although it should be realized that here, too, there are several uncertainties that stem from the lack of detailed genetic analysis, resolution power of the system and the paucity of loci examined for some organisms. Inspection of the table will reveal that an increase in DNA content is not always accompanied by a proportional increase in mutation rate (compare, for example, the entries for barley with those for *Arabidopsis* or *Neurospora*). Thus the data do not bear out the reasoning that higher DNA content and higher mutation rates go hand in hand; it is true of some cases, but by no means all, and consequently no unifying interpretation is possible.

542. To explain the "uniformity" of mutation rates among different species that they observed, Abrahamson *et al.* suggested as one possibility that, on the average,

the size of a radiation-mutable locus is proportional to the total genome (DNA content) for the species. A simpler way of stating this is that bacteria and higher organisms contain the same number of genes, but in the latter they are larger, with the implication that the larger the gene, the higher the mutation rate. As the authors themselves point out, although the inference of larger genes "is contrary to the usual genetic expectation that the structural gene should be of the same size in bacteria as in man, we note that the functional genetic unit in *Drosophila* corresponds to a band in the salivary gland polytene chromosomes. This indicates that complementation groups in higher forms contain much more DNA than is necessary for the specification of a protein. It has been postulated that the extra DNA has a regulatory function and that mutations in any one of the components of the functional genetic unit would fail to complement mutations in any of the other components."

543. Recent studies of DNA organization in higher organisms indicate that *Drosophila* DNA exhibits a quite different pattern of sequence organization as compared with many other eukaryotes including the rat, which have higher DNA contents (130, 323). Thus, for *Drosophila*, there appears to be much greater average length for the single copy sequence DNA (about 13 000 nucleotides compared with 800 to several thousand nucleotides), which is interspersed with relatively larger middle repetitive sequences (5600 nucleotides average length compared with about 300 nucleotides). It would therefore appear that the *Drosophila* pattern may not serve as the best model to speculate about the size of the radiation-mutable target.

544. In *Drosophila* there are some data that permit one to examine whether there is a positive correlation between salivary band size and mutation rate as might be expected if the total amount of DNA associated with a particular band played a major role in determining the mutation rate. The basic premise here is that a functional genetic unit corresponds to a salivary chromosome band (252). Inspection of table 45, in which the data that bear on this point are summarized, will reveal that there is no simple correlation between band size and mutation rate. For example, loci associated with bands of the smallest size (*g, ct* and *ras*) span the greater-than-one-order-of-magnitude range of mutation rates indicated by the loci listed in the table; when the male-lethal *ct* and *ras* mutations are included as "gene mutations", the rates for these loci are among the highest.

545. The inability to detect a predictable relationship between band size and mutation rates is not surprising in view of the detailed cytological analysis of the *white (w)* locus, which indicates that, for this locus at least, the radiation-mutable part occupies at most only a small fraction of the DNA present in the band with which it has been thought to be associated (546). Evidence from yet another *Drosophila* system documents a similar conclusion: Mutations at the *rosy (ry)* locus in chromosome III can be detected as eye-colour mutants. The cytological position of the locus has only been delimited to a region containing no more than five bands (*86D8-12*). In Lefevre's view (272), whichever of the bands *ry* is associated with, it would be of below average dimensions. (Gelbart *et al.* (183) believe that the bands are of average dimensions.) Although the kind of cytogenetic analysis performed at the *w* locus has not been done here, the *ry* locus has the advantage in that it is known to code for the structural gene of the enzyme xanthine dehydrogenase (XDH). Of primary importance is the fact that spontaneously occurring electrophoretic variants of the enzyme are known, and the mutant sites responsible for electrophoretic differences in XDH have been located on the known genetic map of x-ray-induced null-enzyme eye-colour mutants.

546. Gelbert *et al.* (184), in a very recent paper, have presented more decisive evidence than that presented earlier (183) and have concluded that "every rosy eye-colour mutant (null XDH) is now included within the structural element" and that there are "no mutant sites, either radiation- or EMS-induced, which fall outside of this region". From the work of Rudkin (443, 444), it is known that the amount of DNA in the average band, $3.5\ 10^4$ base pairs, is sufficient to code for approximately 30 average-size proteins. The XDH polypeptide requires a coding sequence of (3-4) 10^3 base pairs (183). The available mutation rate data for intralocus null-enzyme *ry* mutants (23 mutants in 170 000 flies after about 4700 R exposure of spermatozoa) give a rate of $2.9\ 10^{-8}\ R^{-1}$ (526, 561), which falls close to the upper limit of the range of mutation rates given in table 45. One is thus left with the firm conclusion that, at the *ry* locus, an average or above-average mutation rate has been elicited from changes restricted to the structural portion of the locus, i.e., the DNA which codes for XDH and represents a target size of about one tenth of the amount of DNA in the average size salivary gland chromosome band.

547. In summary, an examination of mutation rates for a number of loci associated with bands of different dimensions reveals that the relative size of the bands is *not* quantitatively correlated with the intralocus mutation rate. There are therefore *no* firm grounds for believing that the higher average mutation rate in *Drosophila* as compared with some lower forms may be due to the fact that on the average, the amount of DNA associated with complementation groups in *Drosophila* is far in excess of that required to code for single average-size proteins.

5. Summary and conclusions

548. On the basis of an examination of some data on forward mutations at specific loci induced by acute low-LET irradiation in different organisms, Abrahamson *et al.* proposed that there is a linear relationship between DNA content and mutation rate and that this relationship could be used to extrapolate directly from lower organisms to man with confidence and to predict the mutational response of human genes to irradiation.

549. The data used by Abrahamson *et al.* come from experiments and with test systems which differ widely in their ability to discriminate between changes within genes as opposed to changes involving several genes or loci and those which can include gross chromosomal

aberrations such as losses of whole chromosome arms or even entire chromosomes. The effective radiation-mutable target is therefore not the same in the different organisms, and consequently the grounds for comparison are insecure, if not invalid.

550. A consideration of data other than those cited by Abrahamson *et al.* does not lend credence to the line of reasoning pursued by them. There are several notable exceptions, such as the response of the immature oocytes of the mouse and that of the histocompatibility loci.

551. In *Drosophila*, the available evidence indicates that there is no simple quantitative correlation between the size of the band in the salivary gland chromosome and the mutation rates of loci located in them. For instance, at the *rosy* locus which codes for the enzyme xanthine dehydrogenase, every null-enzyme mutant (whether x-ray or EMS-induced) is located within the structural portion of the gene, which is no more than half (and perhaps as low as 10 per cent) of the DNA in the band with which *rosy* is associated. Yet, the intralocus mutation rate for null-enzyme mutants at this locus (after x irradiation) is close to the upper limit of intralocus mutation rates in this organism.

552. In conclusion, the reanalysis of the data on mutation rates has made it clear that the hypothesis of a simple linear relationship between DNA content and the radiation-induced forward mutation rate in living organisms as a whole cannot be substantiated. Even within the different germ-cell stages of a single species such as the mouse, there is extreme variation in radiosensitivity with no variation in DNA content. In view of this, the belief that DNA content can be used as a valid genetic bridge to extrapolate from micro-organisms through higher species to man is unwarranted. The diverse processes, including repair, which intervene in mammals between the induction of mutation and its expression would, in any event, preclude such an extrapolation.

V. SOMATIC CELL GENETICS[25]

A. INTRODUCTION

553. The 1972 report of the Committee (589) considered the data then available from studies on mutation induction in mammalian somatic cells. The events studied are biochemical ones capable of expression at the cellular level under appropriate conditions. Most of the investigations reviewed in the report had been carried out with an aneuploid (near-diploid) Chinese hamster cell line (V 79) and studies with human diploid fibroblasts were just beginning. During the last few years, the scope of somatic cell genetics has expanded: new cell systems and selection schemes have come into use. While the roles of some of the factors that affect the recovery of the "mutants" are now better understood, several new ones have been uncovered and their effects are actively pursued at present.

[25] In this section, the terms "mutant" and "variant" will be used interchangeably in view of the fact that although there is good evidence for mutational origin of the events studied, it is not unequivocally proven.

B. HYPOXANTHINE-GUANINE PHOSPHORIBOSYL TRANSFERASE (HG-PRT)

1. Gene localization

554. The discovery of a severe deficiency of hypoxanthine-guanine phosphoribosyl transferase (HG-PRT) (521) in patients affected with the Lesch-Nyhan syndrome (288) catalysed interest in studies on purine metabolism in man. The Lesch-Nyhan syndrome is characterized clinically by symptoms of excessive production of uric acid and certain neurological abnormalities, including mental retardation. Studies of pedigrees affected with this syndrome indicated that the locus for HG-PRT is on the X-chromosome and that the mode of transmission is X-linked recessive (63, 226, 366, 629). With *in vitro* methods (somatic cell hybridization) it was demonstrated that in human fibroblasts, the loci for HG-PRT, G-6-PD and 3-phosphoglycerate kinase (PKG) are linked (329); current evidence indicates that HG-PRT and G-6-PD genes are localized on the distal half of the long arm of the X-chromosome and with the following sequence: centromere, PGK, HG-PRT and G-6-PD (433, 533). A major review article on HG-PRT deficiency in man has recently been published (62).

555. Chu (106, 107) advanced the hypothesis that in Chinese hamsters and perhaps in other mammals as well, the gene controlling HG-PRT activity is also X-linked. This hypothesis has now been confirmed for the Chinese hamster by Westerveld *et al.* (606), who demonstrated linkage of loci for PGK, HG-PRT and G-6-PD, and for the mouse by Epstein (156, 157), Shows, Brown and Chapman (536), and Hashmi and Miller (223).

2. Screening methods and origin of HG-PRT deficiency

556. Normal substrates for HG-PRT 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-azaguanine (8-AG), 6-mercaptopurine and 6-thioguanine to their respective nucleotides, the incorporation of which results in growth inhibition or death, indicating that normal cells are sensitive to these metabolites. The screening systems employed to detect the HG-PRT variants therefore use resistance to these purine analogues as markers. It has been found that some cell variants that are resistant to 8-AG have either a defective HG-PRT protein, caused by a mutation in the structural genes (31), or a variant enzyme with altered kinetic constants and heat sensitivity (531).

557. In his review, De Mars (135) considered in detail the general question of resistance of cultured human fibroblasts and other cells to purine and pyrimidine analogues in relation to mutagenesis; he pointed out that

(a) genetic changes have been, and may still be, regarded as reasonable explanations for the origin of base-analogue-resistant variants although definitive proof for the mutational origin of these is lacking; (b) arguments favouring explanations invoking epigenetic mechanisms of resistance to base-analogues (such as those of Harris (220, 221) and of Mezger-Freed, (336)) are based on inconclusive evidence; and (c) emphasis must be placed on rigorous studies aimed at examining the nature of events scored for which several possibilities can be envisaged (mutations in genes determining the structure of the enzyme, mutations in genes regulating the expression of the structural genes, mutations in genes that have epistatic functions modifying the expression of the enzyme phenotypes, karyotype changes, mitotic recombination, extra-nuclear genetic changes, etc.).

3. Factors affecting the recovery of spontaneous and induced mutants

558. Several factors, such as the analogue used, its concentration, cell-seeding density, time of addition of the analogue to the mutagen-treated cells (expression time), the source of serum used in the selective medium, interclone metabolic co-operation etc., have been found to influence the recovery and type of mutants obtained (2, 7, 86, 87, 105, 135, 173, 380, 407, 538, 575, 630, 633, 634, 635). Table 46 summarizes the role of some of these factors in the different cell systems currently in use. While most of the studies referred to above have employed 8-AG as the selective agent, in recent years, a preference for the use of 6-thioguanine (6-TG) has been expressed (125, 126, 175, 259, 632). Human HG-PRT has a much greater affinity for 6-TG than for 8-AG (267).

559. Nikaido and Fox (364), who compared the relative effectiveness of both these selective agents in two V 79 Chinese hamster cell lines, (V 79A and V 79S) found that in both cell lines (a) the frequency of surviving colonies exposed to either agent was dependent on the initial plating density; (b) different degrees of metabolic co-operation were manifest and the loss of both spontaneous and added mutants (the latter in reconstruction experiments) occurred at lower cell-density with 6-TG; (c) both analogues were degraded on the incubation in medium plus serum in the absence of added cells; and (d) variations in serum batch had little effect on the rate of degradation or on the frequency of colonies recovered after treatment with 8-AG. They concluded that, at least in V 79 cell lines, (a) cell killing by 8-AG is less dependent on cell density than that by 6-TC, (b) 6-TG is apparently not more efficient in selecting for HG-PRT-mutants (although 6-TG is effective in cell killing at much lower doses), and (c) since 6-TG has been shown to be incorporated into DNA (155) (in contrast to 8-AG which is incorporated into RNA (342), 6-TG itself may be mutagenic, and therefore 8-AG is preferable.

560. Thacker *et al.* (573) however, found that the use of 8-AG as the selective agent yielded highly variable results with the Chinese hamster cell line V-79-4 and that different batches of sera in the growth medium could affect the response considerably. Although dialysis of the serum eliminated some of the variation, it also reduced the plating efficiency of the cells. More consistent results were obtained with 6-TG, although, the "mutation fraction" was not completely resistant to the analogue. The authors noted that the mutants fell into two classes: one class is HG-PRT$^-$ (less than 2 per cent wild-type activity), resistant to 5 μg/ml of thioguanine and sensitive to azaserine[26] while the other shows about 15 per cent of wild-type HG-PRT activity, resistance to 1 μg/ml of thioguanine and some growth in azaserine medium. The two classes of mutants differed little in ability to withstand suppression of their growth on dishes by wild-type cells and performed similarly in competition experiments where mixtures of mutant and wild-type cells were kept in exponential growth over several population doublings. Only one of these types (HG-PRT$^-$) increased in frequency with radiation dose. The authors suggested that these results can account for many of the discrepancies encountered in previous studies and stressed that characterization of the mutants is essential to establish the true frequency of resistant mutants. The work of Cox and Masson (125) provides additional evidence that in human diploid fibroblasts, too, 6-TG is a better selective agent.

561. Van Zeeland and Simons (631) studied the problem of the x-ray induction of 8-AG mutants in two sets of diploid and tetraploid Chinese hamster cells (DON and V 79) and found that the induced mutation frequencies in both kinds of cells were of the same order of magnitude and that all mutants showed an almost complete loss of HG-PRT activity, except that in the tetraploid V 79 cells, 50-100 per cent activity was retained. Measurements of G-6-PD activity in these cells revealed that the spontaneous and induced mutants from the diploid V 79 and DON, as well as the tetraploid DON cells, retained the G-6-PD activity of the parental cells whereas the induced mutants from the tetraploid V 79 cells had about 35 per cent of the parental G-6-PD activity. Using 6-TG as a selective agent, the authors found that the induced "mutant" frequencies in diploid and tetraploid DON cells and in diploid V 79 cells were in the same order of magnitude, but no mutants could be recovered from tetraploid V 79 cells in a single step. Thioguanine-resistant tetraploid V 79 cells could only be obtained from the AG-resistant mutants and some of them showed an increase in G-6-PD activity. All these results taken together led the authors to conclude that the induction of HG-PRT deficiency may not be a single-step event and that it may be the result of a two-step process, as has been suggested by them for the human diploid fibroblasts (635) and by Freed and Mezger-Freed (176) for BUdR resistance in haploid frog cells.

562. Van Zeeland *et al.* (635) tested the validity of the assumption implicit in selecting for purine-analogue-resistant variants in somatic cells (AG-resistance in this case), namely, that their origin is independent of the selective agent. Should this be true, then the proportion of mutants with no or very little HG-PRT activity will be

[26] Azaserine is known to inhibit *de novo* synthesis of purines in cultured human cells and cells dependent for growth on *de novo* purine synthesis (as a result of defective purine salvage enzymes). Impaired growth in azaserine medium might be expected (180).

the same at different selective AG concentrations. DeMars and Held (136) reported that in diploid human fibroblasts, 90 per cent of the variants selected at 1.2 μg/ml AG concentration had residual enzyme activities; in contrast, the experiments of van Zeeland and colleagues showed that mutants selected at 5 or 10 μg/ml AG were totally deficient in HG-PRT activity. Further experiments were therefore aimed at resolving this contradiction.

563. Using human fibroblasts, van Zeeland *et al.* (635) selected for spontaneous mutants at 1.2 and 5 μg/ml AG concentrations. It was found that the total mutation frequencies were the same at both concentrations; however, the mutants selected at the low concentration (the A mutants) were qualitatively different from those selected at the high concentration (the B mutants): among the A mutants, the proportion that had very little or zero enzyme activity was small (3 out of 28) whereas in the other group, a vast majority fell in this class (20 out of 25). This finding strengthened the results obtained from the direct HG-PRT assays of the mutants and suggested that AG is not acting merely as a selective agent. To test this, mutants recovered from both groups were examined for their stability in the presence or absence of AG (1.2μg/ml). It was found that 5 mutants without residual HG-PRT activity (4 A and 1 B) remained stable over a period of 30-50 days; 7 with residual activity (5 As and 2 Bs) showed varying degrees of *increase* of activity indicating that stability of the mutant phenotype in these was dependent on the presence of AG. On the basis of this and other lines of evidence collected, the authors proposed that AG resistance may occur through a two-step process; the first step, which is rare and occurs at random, is AG-independent. In it wild-type cells are predisposed to mutation in a second step, which is AG-dependent. While the validity of this theoretical model remains to be proved, it nonetheless suggests that the property of AG-resistance may not have a simple basis.

564. Until recently, in most of the mutation experiments, the induction of and selection for AG-resistant phenotypes had been carried out within the same dish of cultured cells, as described by Chu and Malling (108). With this technique, as the expression time is increased, the number of presumptive mutant colonies characteristically rises to an optimum and then sharply falls. Thus, for instance, Chu and Malling observed a maximum frequency of AG-resistant colonies at 42 h expression time following treatment of V 79 cells with 10^{-2} M EMS. The usual explanation for the sharp decline in mutant colony frequencies is the metabolic co-operation between wild-type and mutant colonies, causing a shift to the HG-PRT$^+$ phenotype (547, 634). This process limits the time available for expression of the HG-PRT$^-$ phenotype and may in fact cause a spurious reduction in the maximum observable mutation frequency. Myhr and DiPaolo (355), who investigated the reliability of this assay procedure demonstrated this to be true and showed, with three different chemical mutagens, that it is necessary to respread the cells after a suitable expression time before adding the selection medium to obtain maximum frequency of colonies resistant to 8-AG. (The maximum frequencies following MMS or MNNG[27] treatments were about 10-fold greater than those obtained when induction and selection of AG-resistant mutants were performed in the same culture dish.)

565. Fox (173) compared the efficiency of the two assay procedures (*in situ* and replating techniques) for the selection of 8-AG mutants in V 79 Chinese hamster cells (after x-ray, MMS, EMS and uv treatment) and showed that a significantly higher frequency of mutants was obtained in replated experiments as compared with the *in situ* situation. The experiments of Abbondandolo *et al.* (2) adduce further evidence that the identification of a plateau in expression time by the replating techniques is important for an accurate quantitative evaluation of mutation rates. Similar conclusions were reached by Carver *et al.* (86).

4. Dose-effect relationships

566. In the 1972 report, it was pointed out that in V 79 Chinese hamster cells, the dose-effect relationship for the induction of AG-resistant mutations was non-linear and that there were dose-rate and dose-fractionation effects (100-1200 R) (12, 59, 107). The concentration of AG used for selection varied from 7.5 μg/ml in the experiments of Bridges and Huckle (59) and those of Arlett and Potter (12) to 30 μg/ml in those of Chu (107). The results of Albertini and De Mars with diploid human fibroblasts (unpublished at that time) also showed that the dose-effect relationship for AG-resistant mutants was non-linear. Albertini and De Mars (7) have now published their complete results, which show that (a) the average spontaneous rate is about $4.5\ 10^{-6}$ per cell generation ($1.9\ 10^{-6}$ if one large-yield experiment is excluded) and (b) the minimum estimates of the induced rates at different dose levels are

Induced rate (R^{-1} per cell)	*Exposure (R)*
$1.13\ 10^{-9}$	75
$7.14\ 10^{-8}$	125
$6.85\ 10^{-8}$	150
$2.16\ 10^{-7}$	250

indicating, as in Chinese hamster cells, a dose dependence of the mutation rate.

567. Van Zeeland and Simons (632) used the replating method for selecting for induced 6-TG mutants in V 79 cells and showed that the optimal expression time is about 7 days, beyond which the induced mutant frequency stays constant (in contrast to a dropping-off of the yield in earlier studies). The exposure-frequency relationship for x-ray-induced mutants is consistent with linearity in the range up to 800 R, the mutation rate being $1.35\ 10^{-7}\ R^{-1}$ per cell. Thus, with the improved method, there appears to be no dose dependence of the mutation rate.

568. Cox and Masson (126) investigated the x-ray induction of 6-TG mutations in human diploid fibroblasts. They found that the maximum yield was observed when the cells surviving the irradiation had completed 3-4 doublings (6-7 days growth) in a

[27] MNNG = N-methyl-N'-nitro-N-nitrosoguanidine.

non-selective medium and that under these conditions, in the range 50-200 rad, the mutation frequency was linearly related to dose, the rate being 3.1 10^{-7} rad^{-1} per cell.

569. Knaap and Simons (259) have succeeded in developing the mouse lymphoma cell system (L5178Y) as a useful and reliable test system for studying induction of mutations to HG-PRT deficiency (this system differs from others, such as the fibroblasts, in that the growth of the cells takes place in suspension culture). The selective agent used was 6-TG. The results show that (a) the selection of the mutants is not influenced by the concentration of the selective agent (1 or 5 μg/ml); (b) all of the mutants selected (spontaneous as well as induced ones) show a complete loss of HG-PRT activity; (c) the optimal expression time for the mutants is 6-7 days and appears to be dose-independent; and (d) over the range of exposures from 200 to 400 R, the dose-response curve is approximately linear with a rate of (1-1.8) 10^{-7} R^{-1} per cell. After 500 and 600 R, the induction rate is slightly higher. In agreement with the results of Clive *et al.* (114), Knaap and Simons (259) also found that 8-AG was unsuitable as a selective agent in the mouse lymphoma system, owing to inactivation of 8-AG by a serum factor (630).

C. THYMIDINE KINASE (TK)

570. Another locus that is currently used in mutation studies is the thymidine kinase locus (TK) in mouse lymphoma cells. It is known that in man, the gene coding for this enzyme is located on an autosome (chromosome 17) (337, 339, 442), and it is presumed that the situation is similar in the mouse. Like HG-PRT, TK provides a "salvage pathway" which can circumvent a folate-dependent step, i.e., thymidylate synthetase in this particular case. Thus the same principle employed in the HG-PRT system applies to the use of selective media in the TK system. Forward mutations at the TK locus are detected through resistance to BUdR, IUdR or TdR, depending on the cell strain used.

571. In the earlier work, Fox (171) used P388 cells (a mouse lymphoma cell line) and studied IUdR resistance after x irradiation. The selective concentrations of IUdR used in experiments on dose-effect relationships ranged from 10 μg/ml to 100 μg/ml. Surviving colonies, both in control and irradiated populations (scored after 10 days of incubation) were tested for heritable resistance to IUdR. The results showed that (a) after selective doses of 10 μg/ml or less, no heritable resistance could be demonstrated; when higher selective concentrations (20 μg/ml and 100 μg/ml) were used, the number of variant colonies increased, although there appeared to be no consistent increase in efficiency of mutation induction with increasing x-ray dose; and (b) there was a linear relationship between inoculum size and the frequency of IUdR-resistant variants and greater numbers of resistant colonies were recovered when cells were incubated for 24 h before plating on the selective medium.

572. Fox and Anderson (174) isolated IUdR-resistant and TdR-resistant clones from x-irradiation and chemical-treatment experiments with the P388 cell line and conducted TK assays on them. All were found to show significantly reduced enzyme activity relative to wild-type levels, but none showed zero levels. Fourteen of these clones were tested for thymidine uptake and all showed a marked reduction in the rate of ^{3}H-TdR incorporation into acid-soluble fractions and into DNA. Measurements of TK activity in 8 TdR-resistant clones showed that the initial rate of thymidine phosphorylation was not significantly altered in 5 of them; however, significantly lower amounts of phosphorylated products were observed in 6 of the 8 clones. Incorporation of ^{3}H-dTR was reduced in 9 of the 12 clones tested, and 2 of them showed no corresponding reduction in TK activity.

573. In another set of experiments, Fox (172) compared the x-ray induction of IUdR-resistant and TdR-resistant variants in P388 cells and found that the dose-response curves for both of these showed linear and quadratic terms and were roughly similar. In the same study, Fox also compared the x-ray induction of TdR-resistant variants in four L5178Y cell lines, LS (the most sensitive), AII, AIII and AIV, which differed in radiosensitivity to killing. When induction frequencies were compared, either at the same dose or at the same survival levels, the most radiosensitive line (LS) was more mutable than the others, including P388. It may be pointed out that the expression times for both IUdR- and TdR-resistant variants were similar, the maximum frequencies being reached at 48 h followed by a slight falling-off at the longest expression time (96 h) tested.

574. In limited radiation studies with L5178Y, Clive *et al.* (114) recorded an induced mutation rate of 5.0 10^{-7} R^{-1} using BUdR as the selective agent (600-R level). Clive *et al.* as well as Fox and Anderson (174) have discussed the possible genotypic constitution of the respective cell strains used in their mutation assay systems. The former authors showed that the original L5178 Y cell strain is TK^+/TK^+ with spontaneous mutation rate to BUdR-resistance of 5 10^{-11} per generation (based on one recovered mutant); they recovered revertant cell lines from the single TK^-/TK^- originally isolated, and a study of their enzyme levels and mutability indicated that some of these were TK^+/TK^-. One such line was used for determining the x-ray mutation rate given earlier.

575. Fox and Anderson (174) favour the view that the P388 and L5178 cells that they used are TK^+/TK^- and TK^+/TK^+, respectively. The reasoning is based on the following: (a) L5178YS cells have much higher TK levels, possibly a reflection of a gene-dosage effect, as demonstrated by Clive *et al.* (114); (b) L5178YS cells have a very low frequency of IUdR-resistant colonies (no mutants recovered from 10^8 plated cells), which also suggests that they may be TK^+/TK^+; (c) if P388 cells are TK^+/TK^+, it is difficult to explain the large difference in the frequency of spontaneously occurring IUdR-resistant colonies in the two cell lines; this is not the case when other mutagens (chemicals) are used (172, 174).

576. The nature of the events involved in IUdR resistance in the P388 line was examined by Fox and Anderson (174) in revertability tests; it was found that five out of the seven tested reverted at measurable rates

either spontaneously or after mutagenic treatment (HN_2, MNU, MMS etc.), suggesting that the IUdR resistant mutants tested could have been the result of gene mutations at the TK locus rather than deletion-type events.

D. OUABAIN RESISTANCE

577. Arlett *et al.* (13) have recently compared the mutagenic effects of gamma rays, uv light and several chemical mutagens using AG-resistance and systems in Chinese hamster cells resistant to ouabain (Oua). Resistance to Oua is different from that to AG and is probably the result of an alteration of the membrane ATP-ase involved in Na^+/K^+ transport such that it is no longer sensitive to Oua (322). The resistance alleles behave codominantly in somatic hybridization experiments (24). The results of Arlett *et al.* showed that there was a broad agreement between the AG and Oua systems for each mutagen tested, EMS being the most effective mutagen. Surprisingly however, Oua-resistance could not be induced by gamma rays, and the authors have as yet no ready explanation for this negative result.

E. FRUCTOSE UTILIZATION

578. Cox and Masson (124) reported the development of another selective system for mutation studies in human fibroblasts: this is concerned with the utilization of fructose instead of glucose as the major energy and carbon source. HF 10 diploid human fibroblast cultures were initiated from a male embryonic limb, and cells from early passage (6-25 generations in culture) were utilized for the x-ray experiments. Cells were irradiated in suspension and immediately afterwards plated on fructose medium. It was found that the frequency of Fruct clones (uncorrected for cloning efficiency) increased approximately exponentially with radiation dose, the rates per survivor being $4\ 10^{-8}$ (0-100 rad range), $1.24\ 10^{-7}$ (0-200 rad), and $2.10\ 10^{-7}$ (0-343 rad). The genetic basis of $Fruc^+$ variants observed is however, not yet worked out.

F. BACK MUTATIONS

579. Susuki and Okada (549) studied the reversion from alanine auxotrophy to prototrophy in mouse lymphoma cells (L5178Y) both *in vitro* and *in vivo*, following gamma irradiation (100-800 rad). (In the *in vivo* experiments, the alanine-requiring cells were injected into mice (intraperitoneal injections); the mice were irradiated on the eleventh day after inoculation, following which the irradiated cells were removed and assayed for reverse mutations.) The expression time was chosen to be two days for cells grown *in vitro* and five days for those grown *in vivo*. It was found that (a) the cells grown *in vitro* were more sensitive, both to killing and to mutation induction and (b) the dose-effect relationship was linear for cells irradiated *in vivo* and appeared faster than linear for the other. The rate of induction under *in vivo* conditions was approximately $1\ 10^{-7}$ rad^{-1}, for the *in vitro* irradiation, it was higher by factors of 2.5 to about 4.

580. Morrow *et al.* (346) studied the effect of EMS, NG and quinacrine hydrochloride on the induction of reverse mutations from asparagine auxotrophy to prototrophy in rat sarcoma cells; EMS was most effective. No x-irradiation experiments have so far been carried out.

G. GENE TRANSFER THROUGH PURIFIED METAPHASE CHROMOSOMES

581. Following the initial demonstration by McBride and Ozer (327) that incubation of $HG\text{-}PRT^-$ mouse cells with purified metaphase chromosomes from $HG\text{-}PRT^+$ Chinese hamster cells resulted in the appearance of $HG\text{-}PRT^+$ mouse cells in which the Chinese hamster enzyme was expressed, a number of investigators have carried out similar experiments. Willecke and Ruddle (611) extended this work by successfully transferring the human HG-PRT gene (via isolated metaphase chromosomes from He La S3 cells) into murine A9 cells which lacked functional murine HG-PRT activity. Twenty human isozymes other than HG-PRT, whose genes have been assigned to 14 human chromosomes, were found to be absent in the transformed clones. In addition, the human isozymes of G-6-PD and PGK (whose genes are linked to that of HG-PRT) were also absent. In subsequent work, Willecke *et al.* (612) succeeded in transferring two linked human genes which code for the expression of cytosol thymidine kinase and galactokinase to mouse L cells.

582. Wullems *et al.* (619, 620) and Degnen *et al.* (134) published the results of experiments in which both intraspecific and interspecific gene transfers have been accomplished. In the experiments of Wullems *et al.* (619) for instance, the mutant cell line Wg 3-h (derived from the Chinese hamster DON cell line by stepwise selection with increasing concentration of 8-AG and propagated in the presence of 6 μg/ml AG) with HG-PRT activity of about 2 per cent (compared to the DON cells) served as the recipient in all experiments; the donor chromosomes were from the DON line (intraspecies) or from the human He La S3 cell line and the human T cell line (interspecies).

583. The results show that (a) incubation of $HG\text{-}PRT^-$ Chinese hamster cells with chromosomes isolated from $HG\text{-}PRT^+$ Chinese hamster or human cells resulted in the appearance of $HG\text{-}PRT^+$ cells; (b) in the hamster-hamster situation, the frequency of $HG\text{-}PRT^+$ clones was found to be dependent on the number of chromosomes added ($5\ 10^{-5}$ and $16.6\ 10^{-5}$ following incubation with 10^7 and 10^8 chromosomes, respectively). In the hamster-human situation (after incubation with 10^7 human chromosomes) the frequency of $HG\text{-}PRT^+$ cells was $6\ 10^{-6}$, showing that intraspecies transfer may be more effective. Furthermore, in the first situation, the HG-PRT activity of the cells approached the level found in DON cells whereas treatment with human chromosomes resulted in 20-40 per cent of the activity in the Chinese hamster and 15-25 per cent of that in human T cell line; and (c) two clones isolated after incubation of Chinese hamster cells with human chromosomes, which were tested electrophoretically, showed the pattern of the human HG-PRT. However, the human X-linked

genes codes for G-6-PD, phosphoglycerate kinase and α-galactosidase) were not expressed in those clones, suggesting, as in the work of Willecke and Ruddle (611), that perhaps only a small fragment of the X-chromosome was incorporated into the Chinese hamster cells, a finding similar to that of Willecke and Ruddle (611).

584. In their very recent paper, Wullems *et al.* (621) presented evidence for the uptake of the human X-chromosome by human-Chinese hamster cell hybrids, which lack HG-PRT activity, following incubation with isolated human chromosomes from He La S3 cells. The activity of X-linked genes other than that for HG-PRT, such as PGK, alpha-Gal A, G-6-PD, was also found to be present, as determined by electrophoresis (the recipient cells gave no evidence of containing human X-chromosomes). These results therefore suggest that incorporation and expression of He La X-chromosome is accomplished in human-Chinese hamster cell hybrids which lack X-chromosomes. This is in marked contrast to the results obtained with other experimental systems discussed earlier.

H. SUMMARY AND CONCLUSIONS

585. Since the publication of the 1972 report of the Committee, substantial progress has been made in the field of somatic cell genetics. A number of selection systems have come into general use and the role of several factors affecting the recovery of mutants is now better understood.

586. Most of the data have come from the work on the induction of forward mutations leading to a deficiency of hypoxanthine-guanine phosphoribosyl transferase ($HG\text{-}PRT^-$) in Chinese hamster cells, mouse lymphoma cells and human fibroblasts. While the purine analogue 8-azaguanine (8AG) continues to be used as the selective agent, in recent years, there has been a preference to use 6-thioguanine (6-TG) instead; the use of the latter has provided more reproducible results.

587. One of the major technical advances concerns the delineation of the "expression time", the time needed for the mutants to fully express themselves following their induction, in contrast to earlier studies with Chinese hamster and human cell lines where the induction of and selection for mutant phenotypes were carried out in the same culture dishes, under which conditions the frequency of mutant colonies rose to a maximum and then fell. The current use of the replating technique (where the treated cells are respread on to new plates for optimal mutant expression) has facilitated the recovery of mutants. With the replating technique, it has been found, for instance, that the optimal expression time for $HG\text{-}PRT^-$ mutants x-ray or EMS-induced in V 79 Chinese hamster cells is about 7 days, beyond which the induced mutant frequency stays constant. The same is true for the induction of such mutants in human fibroblasts.

588. Using the replating technique, it has been shown that the dose-effect relationship for the x-ray induction of $HG\text{-}PRT^-$ mutants is linear in Chinese hamster cells as well as in human fibroblasts. The rates are, respectively, $1.35\ 10^{-7}\ R^{-1}$ per cell and $3.1\ 10^{-7}\ rad^{-1}$ per cell.

589. In mouse lymphoma cells (L5178Y), 6-TG-resistant mutants have an expression time of 6-7 days, and here too, the exposure frequency relationship is approximately linear, being $(1\text{-}1.8)\ 10^{-7}\ R^{-1}$ (200-400 R); at higher exposures (500 and 600 R) the rate tends to be slightly higher.

590. Another locus at which mutation induction in somatic cells is being studied is the thymidine kinase locus (TK); all the studies so far have been carried out with mouse lymphoma cells. Forward mutations at this locus are detected through resistance to BUdR, IUdR or TdR, depending on the cell strain used. For BUdR resistance in L5178Y cells, the x-ray induction rate is $5.0\ 10^{-7}\ R^{-1}$ per cell (600-R level).

591. Resistance to ouabain (result of alteration of the membrane ATP-ase) is another potentially useful forward mutation system in Chinese hamster cells; x rays have been found to be ineffective in inducing ouabain-resistant mutants, although several chemical mutagens are capable of inducing such mutants.

592. Attempts to induce back mutations from alanine auxotrophy to prototrophy in mouse lymphoma cells and from asparagine requirement to prototrophy in rat sarcoma cells have met with success.

593. Techniques are now available for effecting gene transfer in somatic cells using purified preparations of metaphase chromosomes. Both intraspecific transfer (Chinese hamster) and interspecific transfer (mouse-Chinese hamster, human-mouse, human-Chinese hamster) of HG-PRT genes have been demonstrated. Donor genes can be selected for in the recipient cell population by independent electrophoretic, chromatographic or immunological criteria. The frequency of chromosomal gene transfer is about an order of magnitude higher than the rate of reversion of recipient cells but can also lie within the same range. Evidence for the presence of donor chromosomes was negative in these studies, suggesting that the transferred chromosomal segment must be very small.

594. In contrast, in more recent experiments success has been obtained in transferring a presumably whole human X-chromosome using metaphase chromosomes, from human He La cells as the donor to human-Chinese hamster cell hybrids as the recipient. These studies offer interesting possibilities for examining the problem of gene regulation in somatic cells.

VI. EVALUATION OF GENETIC RADIATION HAZARDS IN MAN

A. A COMPARISON OF THE MAIN CONCLUSIONS OF THE UNSCEAR AND BEIR REPORTS

595. The UNSCEAR (589) and BEIR (34) reports brought into focus the status of knowledge in the area of radiation genetics and, using this knowledge as a basis, made quantitative evaluations of genetic radiation hazards to man. Tables 47 and 48, which are reproduced

from these reports, summarize these estimates. Some of the major conclusions reached in these reports have since been reviewed and compared (360, 383, 495, 508, 509).

596. The general approach to the problem of hazard evaluation has been essentially the same in both the reports; they have the following aspects in common:

(a) The germ cell stages most relevant are spermatogonia in males and oocytes in females;

(b) Due to the paucity of direct human data, the results obtained with experimental mammals, primarily the mouse, form the main basis for hazard evaluations;

(c) Since most of the genetically significant radiation dose received by the human population is delivered either as small doses at high dose rates or as greatly protracted exposures, these are the radiation conditions under which the response of the human germ cells have been assessed;

(d) The rates of induction of different kinds of genetic damage used for hazard evaluation purposes are all based on the response of the mouse germ cells under the radiation conditions stated in (c) above. Appropriate corrections, if any, are made to apply them to man. The rates of induction are assumed to be linear with dose. For the induction of one kind of chromosomal damage, namely that of reciprocal translocations, human germ cells were assumed to be twice as sensitive as those of the mouse.

597. The two reports differ from one another in certain respects. The UNSCEAR report predominantly used the direct method to express risks, namely in terms of the amount of damage expected per gamete per unit dose of radiation; however, some limited attention was paid to quantify risks in relation to the natural incidence of hereditary diseases in man (the doubling dose method). The BEIR report mainly used the doubling dose method to quantify risks, although it stressed the fact that "there are so many facets to the problem that several ways of estimating the risk are more useful than any single one in arriving at the best policy decisions".

598. Although in both reports the increases in the amount of hereditary diseases in man (with the doubling dose method) were estimated using the spontaneous incidence figures as given in the 1962 and 1966 UNSCEAR reports (587, 596) (which in turn were modified from the original ones given by Stevenson (566)), the actual doubling doses were arrived at in different ways. The 100-rad doubling dose used by UNSCEAR was based on those estimated by Lüning and Searle (302). These authors showed that, for different kinds of genetic damage induced in mouse spermatogonia by high acute x-ray doses, the point estimates for the doubling doses ranged from 16 to 51 rad, averaging about 30 rad; with chronic exposures or with acute x irradiation at very low doses, it was inferred (based on different lines of evidence) that the rate of induction will be reduced by a factor of 3-4 and hence the doubling dose under these conditions was estimated as approximately 100 rad for males. Lüning and Searle (302) gave no doubling dose estimates for oocytes since very little information on spontaneous rates in females has been obtained.

599. BEIR, on the other hand, used a range of 20-200 rad as the doubling dose, calculated in the following way: the average rate of induction of recessive specific-locus mutations in the mouse (unweighted average of the rates for mouse spermatogonia and oocytes) under conditions of low-dose, low dose-rate irradiation was taken as $0.25\ 10^{-7}\ rad^{-1}$ per locus. The spontaneous rate of mutations for human recessive genes was taken to be in the range of $(0.5\text{-}5)\ 10^{-6}$ per locus. Dividing the spontaneous rate by the rate of induction, one gets a doubling dose range of 20-200 rad. This range, which, as just mentioned was estimated from spontaneous rates of human recessive gene mutations and induced rates of recessive specific-locus mutations in the mouse was assumed to be applicable to the different categories of genetic disease in man. BEIR considered that (a) the mouse induced mutation rate used above might be on the high side because these loci were to some extent preselected for mutability; (b) the rate of induction of dominant visible mutations in the mouse was at least an order of magnitude lower than that for recessive specific locus mutations; and (c) the mortality data pertaining to the children of Hiroshima and Nagasaki offered strong evidence for believing that the doubling dose is unlikely to be as low as 20 rad of chronic irradiation.

600. The UNSCEAR report gave two estimates for the risk of induction of recessive point mutations per genome using the direct method, namely, one based on the specific-locus mutation rate in mouse spermatogonia ($0.5\ 10^{-7}\ rad^{-1}$ per locus) multiplied by the assumed number of "genes" that make up the human genome (30 000), and another based on data on the induction of autosomal recessive lethals in mouse spermatogonia (since a majority of specific-locus mutations behave as recessive lethals). There was roughly a 40-fold difference between the two estimates (1500 mutations per 10^6 gametes following 1 rad of paternal irradiation (specific-locus data used) versus 36 recessive lethals per 10^6 gametes per rad (recessive lethal data used)).

601. The estimated size of the human genome (30 000 genes) was based on that of the mouse genome (25 000 genes); the latter figure was indirectly derived from results on complementation analysis of a very short segment of the mouse genome (*d-se* region, 0.16 map unit, 1/7500 of the mouse genome) and in fact referred to "functional units" rather than genes or loci. Since there is evidence from complementation tests that some of the events scored as specific-locus mutations involve more than one functional unit, and since specific-locus mutations are scored after birth at weaning age and autosomal recessive lethals, pre-natally, it was thought that these factors could explain the discrepancy between the two estimates (1500 point mutations per 10^6 gametes per rad versus 36 recessive lethals per 10^6 gametes per rad).

602. The UNSCEAR report used, again, a multiplicative method to estimate the rate of dominant mutations per genome (the assumed per-locus rate times the assumed number of loci that determine dominant traits in man). The BEIR report used, as mentioned earlier, the doubling dose method (20-200 rad) to estimate the expected increases in both recessive and dominant genetic diseases.

603. The UNSCEAR report gave no quantitiative estimates for expected increases in congenital anomalies, constitutional and degenerative diseases due to radiation exposures. The reason was that with the direct method employed (where mouse rates were used), such calculations are not possible. Although the calculations can be done with the doubling dose method, the Committee refrained from giving quantitative estimates because of uncertainties regarding the genetic basis of such diseases. The BEIR report, on the other hand, did provide such figures and stressed the uncertainties involved.

604. The UNSCEAR report gave separate estimates of risk for irradiation of males and females and pointed out that in females, because of inadequate or no information, no estimates could be given for recessive lethals, dominant visibles and skeletal mutations. The BEIR report estimates are for irradiation of both sexes; the rates used (in conjunction with the doubling dose range of 20-200 rad) were either unweighted averages of the rates in males and females (when known, as in the case of specific-locus mutation and X-chromosome losses) or were based on the conservative assumption that the rate in females is the same as in males (as is the case with translocations).

605. Estimates of hazards given in the UNSCEAR report related to the induction of the different kinds of genetic damage per rad of parental exposure, and the expected effects in F_1 per 10^6 conceptions. The BEIR report figures relate to the estimated increase among 10^6 live-born individuals whose ancestors had received radiation at the rate of 0.17 rad/year (5 rad per 30-year reproductive generation). The first-generation effects were arbitrarily estimated as one fifth of that expected at equilibrium (for dominant diseases) or as one tenth of that at equilibrium (for congenital anomalies etc.). For chromosomal and recessive diseases the effects were found to be negligible.

B. CURRENT STATUS

1. Introduction

606. The new human and mammalian data reviewed in the preceding sections permit a revision of some of the estimates of hazards arrived at by the Committee in its 1972 report (589). Lüning (295) has drawn attention to some of the main problems in risk assessment that still exist, and Lüning and Eiche (303), Oftedal (383), Newcombe (360), Sankaranarayanan (494, 495), Schalet and Sankaranarayanan (562), Searle (508, 509, 510), and Selby and Selby (528) have considered the extent to which some of the new data bear relevance to human hazard evaluations. In addition, a task group of the ICRP is currently engaged in preparing a document on the impact of a radiation-induced increase in mutation rate on the frequency of serious genetic disease in man (381). In what follows, an attempt will be made first to summarize the pertinent new information and then inquire whether (or not) and how it can be used to make genetic risk estimates. As in the 1972 report, such evaluations will be made using both the direct and the doubling dose methods.

2. Direct method

(a) *Recessive mutations*

607. It may be recalled that from a reanalysis of dose-rate data (gamma irradiation) for the induction of specific-locus mutations in mouse spermatogonia (paras. 276-280), Lyon *et al.* (318) suggested that at very low dose rates, the risk of induction of these mutational events may be higher than hitherto assumed and that this might be due to very low or no spermatogonial cell killing (relative to that which occurs at rates such as 0.009 R/min or higher). However, the available data also fit the concept of no change in mutation rate at exposure rates below 0.8 R/min, and besides, there are now two new lines of evidence. Firstly, the data currently available from the continuing work of W. L. Russell and Kelly (486, 487) (paras. 282-286) show that when a 300-R gamma-ray exposure is delivered to mouse spermatogonia at rates of 0.0056 R/min and 0.0007 R/min, the mutation frequencies are practically identical but significantly above controls. Secondly, in parallel experiments, also at 300 R, Oakberg and Palatinus (378) (paras. 285, 400-402) have demonstrated that the survival of stem-cell spermatogonia under x irradiation is unaffected at a rate of 0.0007 R/min, reduced to 37 per cent of controls at a rate of 0.0056 R/min, and to 16 per cent of controls after 94 R/min. Yet, the mutation rates obtained at the two low rates are identical and highest after high exposure-rate x-irradiation, showing no correlation between sensitivity to cell killing and to mutation induction. It therefore does not seem justified to alter the earlier premise used in hazard evaluations, namely that at low doses and dose rates, the rates of induction of point mutations in spermatogonia is about one third of that observed at high doses and dose rates.

608. As was fully discussed in the 1972 report, the two major uncertainties in the estimate by the multiplicative method of the rate of induction per genome of recessive point mutations (i.e., specific-locus rate multiplied by the assumed number of loci that make up the genome) relate to (a) whether the average specific-locus rate based on the 12 loci is representative of the mutational sensitivity of the genome in general and (b) the number of loci in the genome capable of mutating to recessive visibles. For spontaneous recessive mutations, it is known that the forward mutation rate at unselected loci is at least an order of magnitude lower than that at specific loci. The situation with respect to the radiation-induced rate for recessive visibles at unselected loci is not entirely known, although such data on dominant visibles indicate a very much lower rate relative to that for recessive specific loci (506, 589). Thus, the problem of selected versus unselected loci and the differences in their mutational potentialities will always remain. Since there is no easy way to get a precise estimate of the number of loci in the genome, the reliability of the genome estimate arrived at by the multiplicative method will continue to remain questionable.

609. Autosomal recessive lethals which represent mutations at unselected loci have been found to be induced at a much lower rate per gamete than would have been expected on the basis of the specific-locus

experiments. If for the sake of argument; one divides the per gamete induction rate for recessive lethals ($0.9\ 10^{-4}$ rad^{-1}) by the per-locus specific-locus rate ($1.7\ 10^{-7}$ rad^{-1}), one gets an improbably low figure of about 500 as the total number of loci capable of mutating to recessive lethals.

610. In view of these considerations, it would appear preferable to use the direct estimate based on recessive lethals in hazard evaluations. The value of the specific-locus data in this context lies in the information they provide on the effects of various physical and biological variables on mutation induction. If this basic conceptual change is accepted as a more reliable way to express risks, then the rate of induction of recessive lethals can be used as a meaningful starting point.

611. As was pointed out earlier, the estimated rate of $0.9\ 10^{-4}$ rad^{-1} per gamete (high dose, high dose-rate irradiation) is for autosomal recessive lethals acting *in utero*. The unpublished observations of W. L. Russell referred to in the 1972 report have shown that the proportion of pre-natal lethals averaged over the loci (at specific loci) is less than one half of the total number of mutations. If true in general, then the rate of induction of recessive mutations is likely to be at least $2 \times 0.9\ 10^{-4} = 1.8\ 10^{-4}$ rad^{-1} per gamete. Dividing this by 3 (to correct for expected effects at low dose-rate, low-dose conditions applicable to man) one gets a figure of $0.6\ 10^{-4}$ rad^{-1} per gamete for spermatogonial irradiation.

612. In making these calculations, it has been assumed that the rate of induction of recessive lethals reflects the genome rate for the induction of point mutations in general. This assumption derives support from the fact that recessive lethals represent events as unselected loci and from the fact that a majority of radiation-induced specific-locus mutations studied in the mice behave as recessive lethals in the homozygous condition.

613. There are no data on the induction of recessive lethals in mouse females. Since, under radiation conditions applicable to man, maturing mouse oocytes show a very low level of mutational sensitivity and immature oocytes are virtually insensitive to the induction of specific-locus mutations, it may be presumed that the risk in females will be low, but no quantitative estimates can be given.

(b) *Dominant mutations*

614. There are two sets of data in mice which allow a rough estimate to be made of the risk of inducing mutations with dominant effects. These data refer to (a) dominant visible mutations and (b) skeletal mutations appearing in the F_1 generation after x or gamma irradiation. For chronic gamma irradiation, the mean rate of induction of dominant visible mutations is $1.0\ 10^{-7}$ rad^{-1} per gamete (506). This can be converted into a per-locus rate by dividing it by the number of loci in the mouse (approximately 75) at which dominant visible mutations have occurred. Assuming an equal mutation rate in man, this figure ($1.3\ 10^{-9}$) can then be multiplied by the assumed number of loci (1000) which determine dominant traits in man to get an overall rate for dominant mutations. This procedure was used in the 1972 report. The Committee was, however, aware of the uncertainties and limitations of this approach and have not used it in the present document.

615. As far as skeletal mutations are concerned, there are new data on heritability and rate of induction. The new data of Selby and Selby (528) (paras. 335-339) confirm that abnormalities classified as presumed mutations on the basis of Ehling's criteria (paras. 332-333) are probably mostly heritable. The data of Selby and Selby, when appropriately corrected for dose-fractionation and dose-rate effect, [28] give the same rate of induction as that which could be derived from Ehling's data (namely, a rate of $4\ 10^{-6}$ rad^{-1} per gamete). In both the investigations, the authors recognized that this may be an underestimate of the true rate (paras. 336, 338).

(c) *The relationship between rates of induction of recessive and dominant mutations and the effects of these mutations in the progeny*

616. In its 1972 report, the Committee arrived at estimates of the effects of recessive and dominant mutations in the generation following paternal irradiation (see table 47) in the following way: for recessives, the induction rate (1500 per 10^6 gametes per rad or 36 per 10^6 gametes per rad) was multiplied by 2-5 per cent (this range of figures being based on the average semi-dominant effects of recessive lethals in *Drosophila*) and the resultant figures (30-75 per 10^6 or 1-2 per 10^6) were considered as representing the "expression" of mutations in the F_1 generation. In fact, what these figures imply is that the mean Darwinian fitness of heterozygotes for these mutations will be reduced by 2-5 per cent relative to those not carrying the mutations and that, on the average, these mutant genes will persist in the population for 20 (5% semi-dominance) or 50 (2% semi-dominance) generations subsequent to their induction. In other words, the impact of natural selection on mutant genes is through their heterozygous effects and the duration of persistence of the mutant genes will vary, depending on, among other things, their semi-dominant effect in heterozygotes, i.e., the smaller their semi-dominant effects, the longer will they persist in the population.

617. For dominant visibles, the procedure was simple: the induction rate and the expression in the F_1 were considered the same (2 per 10^6). Based on the limited information then available, mutations causing dominant skeletal defects were considered together with recessive mutations, since it was thought that they might represent heterozygous manifestations of recessive mutations.

618. In the light of new data on mutations causing dominant skeletal defects in the mouse, the finding that

[28] The frequency of $1.4\ 10^{-2}$ is divided by 600 (dose in rad) and further divided by 1.9 (to correct for the enhancement in mutation frequency due to the fractionation régime employed, based on findings in specific-locus work) and by 3 (to get a rate at low dose-rates, again based on specific-locus work) $(1.4\ 10^{-2})/(1.9)\ (3)\ (600) \approx 4\ 10^{-6}$.

four out of four such mutations tested thus far (525) behaved as recessive lethals when made homozygous, other evidence (para. 625), and the premise that dominance and recessivity are attributes of the phenotype and not of genes, the Committee feels that it is now possible to improve the estimates given in 1972 along the following lines: (a) by considering dominant and recessive mutations together to estimate effects in the F_1 and (b) by attempting to express these effects in socially meaningful terms, i.e., in terms of handicaps or disabilities that are expected.

619. An overall estimate of the risk from the induction of mutations causing dominant effects in the progeny (and this includes recessives with semi-dominant effects as well as dominants) can be derived using the data on the rate of induction of skeletal abnormalities in the mouse. The main argument for this rests on the premise that many of the skeletal abnormalities recovered in the mouse experiments are similar to rare dominants and rare irregularly inherited dominant conditions in man, which together constitute a sizeable portion of human genetic diseases. However, to convert the rate of induction of mutations causing skeletal abnormalities in the mouse to an overall rate for all dominant mutations with serious effects in man, one needs information on (a) the proportion of dominant conditions in man whose main effect is in the skeleton and (b) the proportion of skeletal abnormalities studied in the mouse which, at the human level, is likely to cause a serious handicap.

620. Firstly, a perusal of McKusick's latest tabulation of monogenic disorders in man (328) will reveal that out of the 583 "proved" autosomal dominants, 328 are clinically important; in the latter group, 74 (or roughly 20 per cent) involve one or more parts of the skeleton to a varying extent. However, this figure is undoubtedly a reflection of the ease of diagnosis of such abnormalities by phenotypic inspection and/or radiography. The true figure is therefore likely to be lower and, in the opinion of Carter (82) and McKusick (328a), is of the order of 10 per cent.

621. Secondly, not all of the skeletal abnormalities of mutational origin studied in the mouse will impose a serious handicap in humans. Selby (525) suggests that this proportion of abnormalities (leading to serious handicaps) may range from 25 to 75 per cent; in Carter's opinion, a figure of 50 per cent may be accepted as a tentative estimate and this has been confirmed in a detailed discussion of the mutants by Selby (525) and McKusick (328a).

622. It follows that, if the estimated rate of induction of mutations causing skeletal abnormalities in the mouse ($4\ 10^{-6}$ rad^{-1} per gamete under low-LET, low-dose or low dose-rate irradiation; spermatogonia) is multiplied by 10 and then divided by 2, the resultant rate, $20\ 10^{-6}$ rad^{-1} per gamete, is one of induction of mutation having dominant effects on any of the bodily systems in man. In other words, following 1 rad of paternal (spermatogonial) irradiation, 20 out of every million progeny will be expected to carry mutations which cause one or another kind of dominant genetic disease, in the first generation.

623. It is well known in both man and mouse that dominant mutations may show pleiotropism and thus may affect both the skeleton and non-skeletal systems (such as the spleen and stomach in the case of mouse dominant hemimelia). Similar skeletal anomalies may also turn up in different genetic syndromes in both man and mouse. However, in making the calculations referred to above, allowance was made for such pleiotropism.

624. Since in man skeletal abnormalities are frequently observed in association with chromosomal aberrations, such as complete trisomies and partial monosomies and trisomies (208a), it is important to establish to what extent the skeletal conditions described by the Selbys could have been chromosomal rather than genic in origin. Nine mutant lines (mainly involving sterile and semi-sterile individuals) have been tested cytologically and three contained translocation heterozygotes. Two of these three seemed to be associated with the skeletal effects. It should be noted that (a) no unbalanced forms, such as trisomies, were found; (b) the possibility that some rare human apparent dominants are associated with chromosome anomalies cannot be ruled out; and (c) effects of balanced translocations have not been included in any other category in the risk assessments. For these reasons and those given in paragraph 340 the amount of overlap with the chromosomal aberration risk category is likely to be very small, although it cannot be estimated in the absence of detailed refined chromosome analysis.

625. On the other hand, a substantial fraction of these dominant conditions are likely to be lethal in the homozygote and can therefore also be placed in the recessive lethals category. Of 164 dominant mutations in the mouse (apart from those concerned with enzyme polymorphisms and immunological traits) at least 45, or 27 per cent, appear to be lethal in the homozygote. It is quite clear, however, that the dominant manifestation is the important one from the point of view of risk estimation. Homozygotes for human deleterious dominants have rarely been observed, but if so, are lethal.

626. Although the many uncertainties involved in extrapolating from mutations causing dominant skeletal effects in the mouse to the overall number of harmful dominants in man are realized, it is felt that the induction rate of 20 per progeny per rad of paternal irradiation (to spermatogonia), as calculated in paragraph 622, is the best estimate which can be obtained in our present state of knowledge. It is therefore the rate given in table 49.

(d) *Reciprocal translocations*

627. For the induction of reciprocal translocations, more information, including some limited human data, is now available (paras. 131, 184-187). The combined human and marmoset spermatogonial data (at doses of 100 rad and below, acute x-irradiation) indicate a translocation rate of $6.94\ 10^{-4}$ rad^{-1} per cell, on the assumption of linearity (para. 185). A much lower rate could be estimated from the two sets of data now available for the rhesus monkey (paras. 182, 183), but for reasons discussed earlier (paras. 186, 187), it has been decided to base estimates of risk on the human and marmoset data in this report.

628. For estimating the rate at low doses or after chronic exposures, the following lines of evidence from mouse work are pertinent. When a total dose of 300 rad of x rays is administered in 30 or 60 equal fractions, the frequencies of translocations observed in the spermatocytes are reduced by a factor of 4 relative to acute doses (315). At the lowest x-ray dose rate of 0.09 rad/min (300-rad level), the reduction in frequency is by a factor of 2 (512). The efficiency of chronic gamma irradiation at 0.02 rad/min (600-rad level) is lower by a factor of about 10 relative to acute irradiation (517); at a lower dose rate of 0.007 rad/min, over a range of up to 1200 rad, the dose-effect relationship is linear and here too, there is a dose-rate reduction factor of at least 10 (Pomerantzeva *et al.*; refs. 415 and 417). Finally, in the experiments of Searle *et al.* (516) with a gamma-ray dose of 1128 rad delivered over a period of 28 weeks at a rate of 0.004 rad/min, the rate of induction was $1.40\ 10^{-5}$ rad^{-1} per cell, which is lower by a factor of about 20 relative to that after acute irradiation. (In the work of Pomerantzeva *et al.* (417), however, a lowering of the rate from 0.007 to 0.003 rad/min did not lead to any sizeable reduction in the efficiency of gamma irradiation.)

629. If these results are considered applicable to translocation induction in human spermatogonia, the use of reduction factors of, respectively 4, 2 and 10 (to correct the rate of $6.94\ 10^{-4}$ rad^{-1}) for low-dose x rays, low dose-rate x rays, and chronic gamma irradiation, would appear to be reasonable. The corresponding figures then are (rad^{-1} per cell): $1.74\ 10^{-4}$ (low-dose x rays), $3.47\ 10^{-4}$ (low dose-rate x rays), and $0.694\ 10^{-4}$ (chronic gamma irradiation).

630. The induction rate of importance for hazard evaluations is the one that relates to recoverable translocations in the F_1 progeny. Although in its 1972 report the Committee used a reduction factor of 8 to get the above from spermatocyte data (based on the work of Ford *et al.* (170)), it would appear prudent (on the basis of new evidence reported in paragraphs 131 and 132) to assume that the rate of recovery in the F_1 will be one fourth of that in spermatocytes. This means the rates will be (rad^{-1} per gamete): $0.44\ 10^{-4}$ (low-dose x rays), $0.87\ 10^{-4}$ (low dose-rate x rays), and $0.174\ 10^{-4}$ (chronic gamma irradiation).

631. It follows that if males are exposed to low-dose x rays, low dose-rate x rays, or chronic gamma irradiation, the expected number of balanced and unbalanced translocation-carrying zygotes in the F_1 will be, respectively, 44 and 88, 87 and 174, or 17 and 34 per million conceptions per rad of exposure. Assuming further (as the Committee did in its 1972 report) that only about 6 per cent of the unbalanced zygotes will result in children with multiple congenital anomalies, there will be about 5, 10 or 2 such children per million conceptions after one rad of paternal (spermatogonial) exposure. One third of the remaining unbalanced zygotes ($83 \times 1/3 = 28$, $164 \times 1/3 = 55$, $32 \times 1/3 = 11$) would fall into the recognized abortion category, and the remaining two thirds would die so early as to go undetected.

632. It bears reiterating here that the rates of translocation induction used above are based on human and marmoset cytogenetic data with corrections for transmission and expected effects at low doses and dose rates being based on mouse data. Should it turn out that the rate of induction in human spermatogonia is more similar to that in the rhesus monkey, the estimates may need revision, and consequently the quantitative figures arrived at must be considered provisional at present. In the 1972 report, the rate used for human spermatogonia was based on that in the mouse ($\approx 0.3\ 10^{-4}$ rad^{-1} per gamete, acute x irradiation, semi-sterility data), multiplied by 2 to take into account the two-fold difference between man and mouse in effective chromosome arm number and divided by factors of 4 or 9 to get the rates under low dose or chronic exposure conditions, respectively. The change in approach was dictated in part by the lack of validity of the arm number hypothesis (paras. 450-465) for interspecific extrapolations and in part by the availability of new data in man, the marmoset and the mouse on the transmission of translocations to the F_1 progeny.

633. The rate of translocation induction in human females is not known. The data for mouse females discussed earlier (para. 166) show that in maturing oocytes exposed to acute x irradiation, the rate is $0.16\ 10^{-4}$ rad^{-1} per gamete (semi-sterility data). Although there is no direct evidence on the response of immature oocyte stages to the induction of translocations at low doses and dose rates, the data on specific locus mutations and on X-chromosome losses (see paras. 230-231 for the latter) strongly support the view that the rate for translocations is also likely to be low, but no quantitative estimate can be given.

(e) *Other structural aberrations*

634. It seems likely that the risk from the induction of structural aberrations other than reciprocal translocations will be very small. In the mouse, "dominant lethals, due to isochromatid exchange etc., with resultant chromosomal breakage, can be transmitted after oocyte irradiation, though apparently not after spermatogonial irradiation because of germinal selection. It seems likely that most of them act too early to constitute a real hazard. Deficiencies are also induced at comparatively high frequency after oocyte irradiation; however not enough is known yet about their probable overall rate of induction or deleterious effects (especially in the heterozygote) to make any estimate of the genetic damage they may cause" (508).

(f) *Sex-chromosome losses*

635. The complete data on the x-ray induction of sex-chromosome losses in male mice (para. 229) support the conclusion reached by the Committee in 1972, namely that the risk from this class of genetic damage after spermatogonial irradiation is very low or nil. The new data for female mice (table 32 and para. 231) show that the frequencies of sex-chromosome losses are low and not significantly above the controls both after acute x irradiation with 50 R and after chronic gamma ray exposures (400 R) delivered at a rate of 0.006 R/min. These findings therefore suggest that if the human

oocytes respond like those of the mouse, the risk is probably very low, but no quantitative estimates can be given.

636. The risk from the induction of other chromosomal aberrations cannot be reliably estimated at present. As far as chromosome gains are concerned, there is no clear evidence in the mouse, for their induction after spermatogonial irradiation (450, 506). In oocytes however, Yamamoto *et al.* (627) obtained some presumptive evidence for the x-ray induction of non-disjunction in older mice (para. 237), but these data do not lend themselves to risk estimation because of some statistical problems (para. 238). The work of Uchida and Lee (582) (paras. 239-241) also provides evidence for the induction of non-disjunction in oocytes, but the total number of non-disjunctional events is too scanty to define the form of the dose-effect relationship. The results in humans are conflicting (paras. 33-37) and furthermore, there is a large maternal-age component. In view of these, it seems judicious to refrain from predicting the overall extra risk of trisomy in the offspring of women exposed to irradiation until some more information becomes available.

3. Doubling dose method

637. Turning now to the method of expressing risks in relation to the natural incidence of genetic diseases in man (i.e., the doubling dose method), it should be stressed that this method has certain advantages as well as limitations: these were dealt with in the 1972 report of the Committee. Suffice to point out that its chief advantage lies in the fact that it expresses risks in more comprehensible terms than the direct method, although one of the main assumptions, namely, that there is proportionality between spontaneous and induced rates, still remains an unproved one. To employ the doubling dose method, one needs, in addition to other information, the following kinds of data: (a) the natural incidence of the different classes of genetic disease in man; (b) the relationship between incidence and mutation rate; (c) the doubling dose; (d) the radiation dose to which the population is exposed.

638. The data of Trimble and Doughty (576), with the substantial modifications suggested for regular dominant diseases (para. 11) and those summarized by Nielsen and Sillesen (363) on the incidence of chromosome anomalies (see last column in table 9), provide the requisite information on point (a). Of the total frequency of 0.6 per cent chromosomal anomalies recorded in new-born surveys (table 11), roughly two thirds (0.4 per cent) is constituted by numerical sex-chromosomal and autosomal anomalies plus aneuploid structural abnormalities. This 0.4 per cent can be considered as the rate of incidence of chromosomal diseases, although this figure (which does not include balanced reciprocal translocations) may be an underestimate since there is evidence that heterozygosity for balanced reciprocal translocations in some cases, especially in those involving the sex-chromosomes, can result in a handicap to carriers (143, 144). The evidence of Jacobs *et al.* (250) from new-born surveys that aneuploid structural rearrangements of the chromosomes detectable with non-banding techniques may be a rare cause of congenital malformations needs to be viewed with some caution since such malformations may still be recognized later in life.

639. As far as point (b) is concerned, it is safe to assume that the incidence of autosomal dominants and X-linked diseases is essentially proportional to the mutation rate, i.e., their incidence will increase after radiation exposures. The incidence of recessive diseases is only very indirectly related to mutation rate. The incidence of numerical chromosomal disorders and unbalanced structural anomalies will be directly proportional to mutation rate.

640. For diseases of complex aetiology, such as irregularly inherited dominant diseases, multifactorial diseases and congenital malformations (together consisting about 9 per cent in the British Columbia Survey), as was mentioned earlier (para. 15), we shall assume that the mutational component (i.e., the proportion of these diseases that will respond to radiation exposures in a manner similar to that of simple monogenic dominant diseases) is of the order of 5 per cent (para. 15). In terms of absolute effects expected at equilibrium, this is equivalent to assuming that 0.45 per cent diseases of complex aetiology (i.e., 5 per cent of 9 per cent) are irregularly-inherited dominants, and these will behave like simple dominants in their response to irradiation. The reason for not lumping these two classes is that under the radiation conditions assumed for the estimation of risks (see below) the expected effects in the first generation are different for these two classes.

641. Coming now to the doubling dose, as was discussed earlier, the BEIR report used a range of 20-200 rad; the 1972 UNSCEAR report used the figure of 100 rad for males. The examination of available evidence in the mouse suggests that the use of a 100-rad doubling dose (for both sexes) will not underestimate the risk (495, 510). The ICRP Task Group (381) has also used this figure in its calculations. Further justification for the use of this figure derives from the recent Hiroshima and Nagasaki data (para. 39), which suggest that for normal radiation conditions applicable to the human population (low LET, low dose or low dose rate), the minimal doubling dose for the type of damage resulting in death during the first 17 years of life is at least 138 rad for males and over 1000 rad for females. We shall therefore use 100 rad as the doubling dose in this report.

642. To facilitate comparisons with the estimates given in table 49, we shall assume that the population is exposed at a rate of 1 rad per generation (low-LET, low dose rate). The resultant estimates are given in table 50 (top half). It can be seen that (a) the expected increment in the first generation is estimated to be one fifth of that at equilibrium for simple dominant and X-linked diseases and one tenth of that at equilibrium for diseases of complex aetiology (based on the assumptions used in the BEIR report); (b) for chromosomal diseases, the first generation incidence is nearly the same as at equilibrium; and (c) the total increment expected in the first

generation is 63 new cases of genetic disease per 10^6 (0.06 per cent of current incidence) and 185 cases per 10^6 at equilibrium (0.17 per cent of current incidence).

643. A comparison of the present estimates with those of the BEIR report (bottom half of table 50) will show that there are some discrepancies between the two sets of estimates. The differences in certain numerical estimates derive from the use of (a) different current incidence figures (10.7 per cent in the present report, 6 per cent in the BEIR report); (b) a single doubling dose of 100 rad rather than a range of doubling doses from 20 to 200 rad; (c) a single figure instead of a range of figures to quantify the mutational component in diseases of complex aetiology (5 per cent instead of 5-50 per cent); and (d) a numerical estimate for the effects of radiation on the increment in chromosomal diseases (based on data from new-born surveys) in the present report.

644. It is worth pointing out here that the first-generation effects for simple dominant diseases (20 per 10^6) and for irregularly inherited dominants (5 per 10^6—a total of 25 per 10^6 live-born—is quite similar to the figure of 20 per 10^6 arrived at using the direct method.

4. Summary and conclusions

645. This chapter has been devoted to (a) a comparison of the main conclusions reached by UNSCEAR and BEIR in their 1972 reports; (b) summarizing the major new findings that are pertinent for the evaluation of genetic radiation hazards (details of which were discussed in the different chapters of this annex); and (c) presenting revised estimates of risk on the basis of the information currently available. The estimates arrived at are given in table 49 (direct method) and in table 50 (doubling dose method); these effects are those expected following low-LET irradiation of the germ-cell stages most at risk (spermatogonia in males and oocytes in females) and are for conditions of radiation exposure most relevant, namely, low doses and chronic exposures.

646. The rate of induction of recessive mutations has been estimated to be $0.6\ 10^{-4}$ rad^{-1} per gamete. This figure is derived from the rate of induction of autosomal recessive lethals in male mice following acute x irradiation of spermatogonia ($0.9\ 10^{-4}$ rad^{-1} per gamete), corrected for (a) low dose-rate irradiation conditions (i.e., the use of a reduction factor of 3 which gives $0.9\ 10^{-4} \div 3 = 0.3\ 10^{-4}$) and (b) assuming that the proportion of recessive lethals scored by the pre-natal method represents about one half of all recessive mutations (i.e., multiplying $0.3\ 10^{-4}$ by 2, we obtain $0.6\ 10^{-4}$).

647. The Committee examined the possibility that, at very low dose rates, the mutation-rate reduction factor of 3 (correction (a) above) may not be applicable because of the lack of cell killing in the context of (a) new data on specific locus mutations at low dose rates and (b) new data on the survival of stem-cell spermatogonia at different dose rates and concluded that, at the dose rates studied (one high and two low), there was no correlation between cell killing and the mutation rate. In view of this, the reduction factor of 3 to estimate effects at low dose rates has been retained.

648. Since there are no data on the induction of recessive lethals in female mice, no estimates are possible for this kind of damage in human females. However, the evidence from specific-locus experiments strongly support the view that the risk in females is low.

649. The overall dominant effects from the induction of mutations in man (including recessives with semi-dominant effects and dominants), estimated as $20\ 10^{-6}$ rad^{-1} per gamete, has been arrived at using the expected rate of induction of mutations affecting the skeleton in mice (following spermatogonial irradiation at low dose rates) as the starting point ($4\ 10^{-6}$ rad^{-1} per gamete). This figure was multiplied by 10 to take into account the fact that approximately 10 per cent of dominant mutations recorded in man are associated with skeletal abnormalities and divided by 2 to take into account the possibility that about 50 per cent of the skeletal mutations found in the mouse, if induced in humans, could cause serious handicap.

650. There are no data on the induction of skeletal mutations in female mice, and consequently no attempt has been made to make a quantitative estimate of risk for the induction of dominant mutations in human females. The risk is expected to be low, however, if the results of specific-locus tests in female mice are considered applicable to human females.

651. The rate of induction of reciprocal translocations have been estimated to be $0.44\ 10^{-4}$, $0.87\ 10^{-4}$, and $0.174\ 10^{-4}$ rad^{-1} per gamete for low-dose x rays, low dose-rate x rays, and chronic gamma irradiation, respectively. With these rates of induction, the consequences following 1 rad of paternal exposure are 2-10 congenitally malformed children, 11-55 recognized abortions and 22-109 early embryonic losses per 10^6 conceptions. The rates of induction have been derived using the limited human and marmoset cytogenetic data (spermatogonial irradiation) obtained in experiments involving acute x irradiation and making corrections for expected effects at low doses and dose rates and for transmission to the F_1 progeny using mouse results as a guide. The Committee wishes to stress that the risk from the induction of translocations as given above is subject to uncertainty in view of the limited nature of the primary data and the fact that within primates there is considerable heterogeneity; in fact, the data from Rhesus monkeys suggest that the rates may be much lower than in both the marmoset and the mouse, and should this suggestion be confirmed in subsequent experiments with primates, especially the monkey and/or man, there may be grounds for revision of the estimates.

652. The reason for using human and marmoset data for hazard evaluation (and not the rate of induction in mice with a multiplication factor of 2 to apply to man, as was done in the 1972 report) is that the basic premise for the use of the multiplication factor of 2 (namely, the hypothesis of linear relationship between effective

chromosome arm number and the relative sensitivity to the induction of dicentrics or translocations) now appears invalid.

653. There are no data which will permit an evaluation of the risk from the induction of translocations in human females. Again, on the basis of mouse results with another end-point of genetic damage, namely the loss of X-chromosomes, it would appear that the risks are low, but no quantitative estimates can be given.

654. The risk from the induction of other kinds of structural aberrations is likely to be small by analogy with the mouse, but no estimate can be made at present.

655. For the induction of sex-chromosome losses, the available mouse data suggest that in both males and females (at low doses or after chronic gamma irradiation) the risk is low or negligible. The lack of a significant difference in X-loss frequencies (between controls and the irradiated groups of mice), viewed in conjunction with the fact that in humans, a predominant majority of the XO conceptions are inviable, would suggest that in humans, too, the risk is likely to be quite low.

656. Although in mouse experiments, presumptive evidence for the induction of non-disjunction has been obtained at low doses, in view of some uncertainties (lack of information on dose-effect relationship, statistical problems and complications due to maternal-age effects), it is not possible to make a realistic quantitative estimate for this kind of damage.

657. Estimates of doubling doses arrived at in the mouse for various end-points of genetic damage, as well as the data on the mortality of children born to A-bomb survivors in Hiroshima and Nagasaki, suggest that the doubling dose in man (both sexes) is unlikely to be lower than 100 rad.

658. The doubling dose of 100 rad has been used in conjunction with the frequencies of spontaneous incidence of genetic disease in man to compute, the expected increments. The spontaneous incidence figures used are (per cent): dominant and X-linked diseases, 1; recessive diseases, 0.1; chromosomal diseases, 0.4; diseases with complex aetiology, 9.0. Under the assumption that the population is exposed to low-LET, low dose-rate irradiation at the rate of 1 rad per generation, it was estimated that in the first generation there will be, per million live-born, 20 additional cases suffering from dominant and X-linked diseases, 38 from chromosomal diseases, and 5 cases from diseases of complex aetiology. The total, 63, represents 0.06 per cent of the current incidence (10.5 per cent). At equilibrium (which will be reached after different numbers of generations depending on the kinds of disease), this total increment will be 185 cases per million live-born, or 0.17 per cent of current incidence.

659. The overall increment due to the induction of dominant mutations (simple dominants plus those with irregular penetrance), estimated by the doubling dose method is 25 cases per million (20 + 5; see preceding paragraph) in the first generation and is about the same as that estimated using the direct method (20 per million live-born; see paragraph 649).

VII. SUGGESTIONS FOR FUTURE RESEARCH

660. In this annex, the progress that has been made in mammalian and human genetics, cytogenetics, molecular radiobiology and somatic cell genetics since the publication of the 1972 report has been reviewed, and revised estimates of genetic risks to man from exposure to ionizing radiation have been presented. These estimates reflect our current state of knowledge; as will be evident from the Committee's consecutive reports on this subject, the process of risk assessment is a continuous one: new advances help us to view old problems with a new perspective, to examine the validity of assumptions and extrapolation procedures used, and to identify areas of research which are likely to contribute to a better understanding of the genetic radiosensitivity of human beings. The Committee feels that, in order to increase our precision in risk assessment, more research effort along the following main lines will be useful (the order in which these are listed do not reflect the order of importance):

(a) Additional surveys on the prevalence of hereditary defects and disease in human populations (similar to that carried out in British Columbia), including an ascertainment of chromosomal diseases and diseases of late onset;

(b) Mutation monitoring in man by biochemical techniques;

(c) Continuation of studies on non-disjunction and the effects of radiation thereupon;

(d) Continuation of genetic and cytogenetic studies on Hiroshima and Nagasaki populations;

(e) Continuation of comparative radiation studies in different mammalian species;

(f) Further studies on the relationship between the frequencies of reciprocal translocations scored cytogenetically in spermatocytes and those observed in genetic tests, especially at low doses and after low dose-rate irradiation;

(g) Collection of more cytogenetic data on the induction of reciprocal translocations in primate species using when possible, human testes which happen to have been irradiated for medical reasons;

(h) Continuation of studies on the radiation induction of structural chromosomal aberrations (including translocations) in mammalian oocytes;

(i) Further studies on the role of factors influencing the induction of structural aberrations in somatic as well as germinal cells, such as the amount and distribution of heterochromatin in the chromosomes, the basis for differential sensitivity to radiation-induced breakage observed between chromosomes or chromosome regions;

(j) Further studies on the repair of radiation-induced chromosome breaks;

(k) Continuation of studies aimed at the characterization of the radiation-induced mutational events at biochemically-defined loci in mammals and other organisms such as *Drosophila;*

(l) Continuation of studies on the nature of radiation-induced specific-locus and dominant mutations;

(m) Studies on the induction of autosomal recessive lethals in female mice;

(n) Continuation of studies on the induction and characterization of mutations affecting the skeleton and other apropriate bodily systems in the mouse;

(o) Studies on the induction of mutations in germ cells and somatic cells at very low doses and dose rates and the development of techniques to facilitate such studies;

(p) Continuation of genetic and cytogenetic studies with mice on the effects of incorporated radioisotopes;

(q) Continuation of studies on the factors that modify radiation-induced genetic damage;

(r) Development and exploitation of more mutational assay systems in somatic cells *in vitro* and *in vivo;*

(s) Studies on the nature of radiation-induced damage at the DNA level and its relationship to mutations and chromosome aberrations;

(t) Validation of the assumptions involved in the use of the doubling dose approach for hazard evaluation;

(u) Continuation of studies on the interaction of radiation and chemical mutagens.

TABLE 1. SINGLE GENE DOMINANT DISORDERS BY ICDA CODE

ICDA code[a]	*Number of persons affected*[b]	*ICDA title*[c]
282.0	17	Familial acholuric jaundice
286.3	4	Vascular haemophilia
312.9	1	Moderate idiopathic mental retardation
313.9	1	Severe idiopathic mental retardation
314.9	1	Profound idiopathic mental retardation
330.0	6	Neuropathic muscular atrophy
330.4	4	Myotonia atrophica
330.9	3	Unspecified neuromuscular disorder
331.0	2	Hereditary chorea
350.0	1	Facial paralysis
354.0	2	Polyneuritis and polyradiculitis
358.0	2	Horner's syndrome
377.9	4	Unspecified disease of retina and optic nerve
389.9	3	Impairment of hearing, one or both ears
520.5	3	Hereditary disturbances in tooth structure
733.2	3	Myositis ossificans
743.4	29	Neurofibromatosis
744.2	1	Buphthalmos
744.3	27	Congenital cataract
744.5	8	Aniridia
744.7	3	Congenital blepharoptosis
744.8	24	Anomalies of eye
752.2	2	Hypospadias
753.1	1	Cystic kidney disease
755.0	21	Polydactyly
755.2	2	Reduction deformity of upper limb
755.3	1	Reduction deformity of lower limb
755.5	5	Anomaly of upper limb (including shoulder girdle)
755.8	2	Generalized flexion contracture of limb joints
756.0	16	Anomalies of skull and face bones
756.6	57	Chondrodystrophy
756.6	30	Osteogenesis imperfecta
756.7	1	Generalized anomalies of skeleton
757.0	1	Hereditary oedema of legs
757.2	15	Anomalies of skin
757.9	3	Unspecified anomalies of skin, hair and nails
759.6	12	Tuberous sclerosis
759.8	34	Congenital syndrome, affecting multiple systems
	Total 352	

Source: Based on the British Columbia Survey (576).

[a] ICDA = International Classification of Diseases Adapted, 8th revision (244).

[b] Since ascertainment for many conditions, especially congenital malformations, is not routinely good for all birth cohorts, and since these numerators are not broken down by birth period, valid estimates of rates of individual conditions cannot be calculated from these numbers.

[c] These titles do not describe single disease entities but rather the nature of the groups of conditions to which the ICDA rubrics apply. Detailed diagnostic lists for each ICDA code are given in the ICDA, 8th revision (244).

TABLE 2. SINGLE GENE RECESSIVE DISORDERS BY ICDA CODE

ICDA code	*Number of persons affected*	*ICDA title*
243.0	3	Cretinism of congenital origin
253.1	1	Anterior pituitary hypofunction
253.9	1	Unspecified disease of pituitary gland
270.0	41	Phenylketonuria
270.7[a]	28	Albinism
270.8	11	Congenital disorders of amino acid metabolism
271.0	5	Von Gierke's disease
271.1	8	Unspecified glycogen storage disease
271.2	5	Galactosaemia
272.2	4	Lipid storage disorders
273.0	151	Cystic fibrosis
273.3	1	Hepatolenticular degeneration
273.4	6	Disorders involving metabolism of minerals
273.6	22	Disorders of steroid metabolism
273.8	16	Congenital disorders of metabolism
275.0	7	Agammaglobulinaemia
275.1	5	Hypogammaglobulinaemia
279.0	5	Unspecified metabolic diseases
282.4	10	Mediterranean anaemia
284.0	2	Aplastic anaemia
311.9	1	Mild idiopathic mental retardation
315.9	1	Unspecified idiopathic mental retardation
330.0	3	Neuropathic muscular atrophy
330.1	19	Familial progressive spinal muscular atrophy
330.2	24	Amyotonia congenita
330.3	28	Progressive muscular dystrophy
332.0	5	Hereditary spinal ataxia
333.0	10	Amaurotic family idiocy
333.1	4	Progressive cerebral leukodystrophy
333.9	2	Unspecified hereditary diseases of nervous system
377.9	1	Unspecified diseases of retina and optic nerve
389.0	13	Deafness, both ears
389.9	20	Impairment of hearing, one or both ears
583.0	1	Nephritis, unqualified
742.0	1	Congenital hydrocephalus
743.1	6	Microcephalus
743.8	1	Congenital anomalies of nervous system
744.1	2	Micropthalmos
744.2	16	Buphthalmos
744.3	1	Congenital cataract
747.2	1	Anomaly of aorta
747.9	1	Unspecified anomaly of circulatory system
751.5	1	Atresia of biliary ducts
752.7	5	Pseudohermaphroditism
753.1	19	Cystic kidney disease
756.5	3	Chondrodystrophy
756.7	3	Generalized anomalies of skeleton
757.2	17	Anomalies of skin
759.8	9	Syndromes affecting multiple systems
Total	550	

Source: Based on the British Columbia Survey (576).

Note: See notes to table 1.

[a]Special registry code.

TABLE 3. SINGLE GENE X-LINKED DISORDERS BY ICDA CODE

ICDA code	*Number of persons affected*	*ICDA title*
257.9	1	Unspecified ovarian dysfunction
265.1	2	Rickets, late effect
270.7[a]	4	Albinism
273.4	6	Disorders involving metabolism of minerals
273.8	7	Congenital disorders of metabolism
275.0	11	Agammaglobulinaemia
275.1	20	Hypogammaglobulinaemia
282.2	1	G-6-PD deficiency anaemia
282.9	1	Hereditary haemolytic anaemia
285.0	1	Hypochromic anaemia with iron loading
286.0	40	Haemophilia A
286.1	11	Haemophilia B

TABLE 3 *(continued)*

ICDA code	*Number of persons affected*	*ICDA title*
286.9	1	Coagulation defect
312.9	1	Moderate idiopathic mental retardation
330.3	52	Progressive muscular dystrophy
330.4	1	Myotonia atrophica
377.3	24	Colour blindness
377.9	1	Unspecified disease of retina and optic nerve
742.0	1	Congenital hydrocephalus
752.7	4	Pseudohermaphroditism
756.7	2	Generalized anomalies of skeleton
752.2	3	Anomalies of skin
757.9	1	Unspecified anomaly of skin, hair or nails
759.8	2	Syndromes affecting multiple systems
Total	198	

Source: Based on the British Columbia Survey (576).
Note: See notes to table 1.
[a]Special registry code.

TABLE 4. DISEASES DUE TO SINGLE GENES OF UNCERTAIN DOMINANCE BY ICDA CODE

ICDA code	*Number of persons affected*	*ICDA title*
252.1	1	Hypoparathyroidism
282.4	1	Mediterranean anaemia
282.5	2	Haemoglobinopathies
286.9	3	Coagulation defects
744.6	5	Retinitis pigmentosa
Total	12	

Source: Based on the British Columbia Survey (576).
Note: See notes to table 1.

TABLE 5. AUTOSOMAL CHROMOSOME ANOMALIES BY ICDA CODE

ICDA code	*Number of persons affected*	*ICDA title*
759.3	972	Down's syndrome
759.4	44	Syndromes due to autosomal abnormality except trisomy 21
Total	1 016	

Source: Based on the British Columbia Survey (576).
Note: See notes to table 1.

TABLE 6. SEX CHROMOSOME ANOMALIES BY ICDA CODE

ICDA code	*Number of persons affected*	*ICDA title*
752.0	1	Syndrome due to sex chromosome anomaly with indeterminate sex
752.2	1	Syndrome due to sex chromosome anomaly with hypospadias
756.4	1	Syndrome due to sex chromosome anomaly with anomalies of ribs and sternum
759.5	44	Syndromes due to sex chromosome anomalies
Total	47	

Source: Based on the British Columbia Survey (576).
Note: See notes to table 1.

TABLE 7. CONGENITAL MALFORMATIONS BY ICDA CODE

ICDA code	*Number of persons affected*	*ICDA title*
740.0	123	Anencephalus
741.0	259	Spina bifida with hydrocephalus
741.9	317	Spina bifida without mention of hydrocephalus
742.0	372	Congenital hydrocephalus
743.0	20	Encephalocele
743.1	83	Microcephalus
743.2	85	Anomalies of brain
743.3	2	Anomalies of spinal cord
743.8	6	Anomalies of nervous system
743.9	27	Unspecified anomalies of brain, spinal cord and nervous system
744.0	9	Anopthalmos
744.1	32	Micropthalmos
744.2	18	Buphthalmos
744.3	198	Congenital cataract
744.4	20	Coloboma
744.7	106	Congenital blepharoptosis
744.8	196	Anomalies of eye
744.9	14	Unspecified anomalies of eye
745.0	65	Anomalies of ear causing impairment of hearing
745.1	179	Accessory auricle
745.2	55	Anomalies of ear
745.3	46	Unspecified anomalies of ear
745.4	93	Branchial cleft, cyst or fistula; preauricular sinus
745.5	2	Webbing of neck
745.8	8	Anomalies of face and neck
745.9	3	Unspecified anomalies of face and neck
746.0	22	Common truncus
746.1	72	Transposition of great vessels
746.2	163	Tetralogy of Fallot
746.3	1 014	Ventricular septal defect
746.4	499	Atrial septal defect
746.5	10	Ostium atrioventriculare commune
746.6	219	Anomalies of heart valves
746.7	41	Fibroelastosis cordis
746.8	101	Anomalies of heart
746.9	477	Unspecified anomalies of heart
747.0	347	Patent ductus arteriousus
747.1	91	Coarctation of aorta
747.2	22	Anomalies of aorta
747.3	102	Stenosis or atresia of pulmonary artery
747.4	8	Anomalies of great veins
747.5	13	Absence or hypoplasia of umbilical artery
747.6	12	Anomalies of peripheral vascular system
747.8	8	Anomalies of circulatory system
748.0	23	Choanal atresia
748.1	15	Anomalies of nose
748.2	4	Web of larynx
748.3	38	Anomalies of larynx, trachea and bronchus
748.4	8	Congenital cystic lung
748.5	4	Agenesis of lung
748.6	21	Anomalies of lung
749.0	460	Cleft palate
749.1	291	Cleft lip
749.2	571	Cleft palate with cleft lip
750.0	55	Anomalies of tongue
750.1	746	Pyloric stenosis
750.2	42	Tracheo-oesophageal fistula
750.3	27	Oesophageal atresia and stenosis
750.8	32	Anomalies of upper alimentary tract
750.9	11	Unspecified anomalies of upper alimentary tract
751.0	44	Meckel's diverticulum
751.1	24	Anomalies of intestinal fixation
751.2	73	Hirschsprung's disease
751.3	123	Atresia and stenosis of rectum and anal canal
751.4	107	Anomalies of intestine
751.5	16	Atresia of biliary ducts
751.6	18	Anomalies of gallbladder, bile ducts, and liver
751.7	4	Anomalies of pancreas
751.8	5	Anomalies of digestive system
751.9	2	Unspecified anomaly of digestive system
752.0	11	Indeterminate sex
752.1	612	Undescended testicle
752.2	692	Hypospadias

TABLE 7 *(continued)*

ICDA code	*Number of persons affected*	*ICDA title*
752.3	13	Epispadias
752.5	1	Anomaly of ovary, fallopian tube or uterus
752.6	32	Anomalies of vagina and external female genitalia
752.7	12	Pseudohermaphroditism
752.8	50	Anomalies of genital organs
752.9	5	Unspecified anomalies of genital organs
753.0	66	Renal agenesis
753.1	35	Cystic kidney disease
753.2	123	Obstructive defects of urinary tract
753.3	79	Anomalies of kidney
753.4	46	Anomalies of ureter
753.5	13	Extrophy of urinary bladder
753.6	257	Atresia and stenosis of urethra and bladder neck
753.8	21	Anomalies of bladder and urethra
753.9	10	Unspecified anomalies of urinary system
754.0	2 205	Talipes cavus
755.0	279	Polydactyly
755.1	252	Syndactyly
755.2	182	Reduction deformity of upper limb
755.3	62	Reduction deformity of lower limb
755.5	86	Anomaly of upper limb (including shoulder girdle)
755.6	1 076	Congenital dislocation of hip
755.7	238	Anomaly of lower limb (including pelvic girdle)
755.8	38	Generalized flexion contracture of limb joints
755.9	2	Unspecified anomaly of unspecified limb
756.0	147	Anomalies of skull and face bones
756.1	11	Anomalies of lumbosacral joint
756.2	63	Anomalies of spine
756.3	2	Cervical rib
756.4	125	Anomalies of ribs and sternum
756.5	8	Chondrodystrophy
756.6	3	Osteogenesis imperfecta
756.7	16	Generalized anomalies of skeleton
756.8	151	Anomalies of muscle, tendon and fascia
756.9	57	Anomalies of musculoskeletal system
757.0	4	Hereditary oedema of legs
757.1	123	Pigmented nevus
757.2	40	Anomalies of skin
757.3	4	Anomalies of hair
757.9	8	Anomaly of skin, hair, or nails
758.0	4	Anomalies of spleen
758.1	3	Anomaly of adrenal gland
758.2	43	Anomalies of thyroid gland
758.8	18	Congenital anomalies
758.9	1	Unspecified congenital anomaly
759.0	9	Situs inversus
759.2	14	Forms of monster
759.8	39	Specified syndromes
759.9	14	Unspecified multiple congenital anomalies
Total	15 728	

Source: Based on the British Columbia Survey (576).
Note: See notes to table 1.

TABLE 8. OTHER MULTIFACTORIAL DISORDERS BY ICDA CODE

ICDA code	*Number of persons affected*	*ICDA title*
242.0	2	Toxic diffuse goitre
250.0	401	Diabetes mellitus with mention of acidosis or coma
269.0	105	Sprue and steatorrhea
281.0	1	Pernicious anaemia
295.2	2	Schizophrenia, catatonic type
295.8	51	Schizophrenia
295.9	6	Unspecified type of schizophrenia
310.9	542	Borderline idiopathic mental retardation
311.9	327	Mild idiopathic mental retardation
312.9	311	Moderate idiopathic mental retardation
313.9	126	Severe idiopathic mental retardation
314.9	129	Profound idiopathic mental retardation
315.9	321	Unspecified idiopathic mental retardation
345.0	202	Generalized non-convulsive epilepsy
345.1	292	Generalized convulsive epilepsy
345.2	1	Status epilepticus
345.3	19	Partial temporal lobe or psychomotor type epilepsy
345.4	3	Partial motor type epilepsy
345.5	29	Unspecified partial type epilepsy
345.9	842	Unspecified type of epilepsy
370.0	40	Myopia
370.1	2	Hyperopia
370.3	2	Astigmatism
370.9	9	Unspecified refractive errors
373.0	3 517	Strabismus, convergent
373.9	6	Unspecified strabismus
493.0	203	Asthma
722.1	260	Juvenile osteochondrosis of hip
Total	7 751	

Source: Based on the British Columbia Survey (576).
Note: See notes to table 1.

TABLE 9. ESTIMATES OF THE FREQUENCY OF GENETIC DISORDERS IN A BIRTH COHORT

Cases per 100 live-born

Disease category	*Stevenson (566)*	*UNSCEAR (586, 587)*	*British Columbia Survey (576)*		*UNSCEAR present estimate*
			Minimal	*Adjusted*	
Dominant	3.32[a]	0.95	0.06	0.08	1.0[e]
Recessive	0.21	0.21	0.09	0.11	0.1
X-linked	0.04	0.04	0.03	0.04	[f]
Chromosomal	[b]	0.42[c]	0.16	0.20[d]	0.4
Congenital malformations	1.41	2.50	3.58	4.28	9.0
Other multifactorial	1.48	1.50	1.58	4.73	
Unknown	[b]	[b]	(0.60)[g]	(2.70)[g]	
Total[d]	6.46	5.62	5.50	9.44	10.5[h]

[a] Including both trivial and serious anomalies.

[b] No information.

[c] Including Down's syndrome (0.15), other autosomal trisomies (0.05), Klinefelter's syndrome (0.17), Turner's syndrome (0.03) and Cridu-chat syndrome (0.02) but excludes XXX females (0.12) and individuals with translocations (0.50).

[d] All but about 3 per cent are due to Down's syndrome.

[e] Including X-linked disorders.

[f] Included in the dominant category.

[g] Frequency of unknown diseases is not included in the total.

[h] For the evaluation of radiation hazards the figures used are slightly different; see table 50 and paragraphs 12 and 19.

TABLE 10. ESTIMATES OF MUTATION RATES FOR HUMAN GENES

Trait	*Population examined*	*Mutation rate*	*Number of mutants per 10^6 gametes*
	A. AUTOSOMAL MUTATIONS		
Achondroplasia	Denmark	$1\ 10^{-5}$	10
	Northern Ireland, UK	$1.3\ 10^{-5}$	13
	Münster, Germany, Fed. Rep. of	$6\text{-}9\ 10^{-6}$	6-9
Aniridia	Denmark	$2.9\text{-}5\ 10^{-6}$	2.9-5
	Michigan, USA	$2.6\ 10^{-6}$	2.6
Dystrophia myotonica	Northern Ireland, UK	$8\ 10^{-6}$	8
	Switzerland	$1.1\ 10^{-5}$	11
Retinoblastoma	England, UK; Michigan, USA Switzerland; Germany, Fed. Rep. of	$6\text{-}7\ 10^{-6}$	6-7
	Hungary	$6\ 10^{-6}$	6
	Netherlands	$1.23\ 10^{-5}$	12.3
	Japan	$8\ 10^{-6}$	8
	France	$5\ 10^{-6}$	5
Acrocephalosyndactyly (Apert's syndrome)	England, UK	$3\ 10^{-6}$	3
	Münster, Germany, Fed. Rep. of	$4\ 10^{-6}$	4
Osteogenesis imperfecta	Sweden	$0.7\text{-}1.3\ 10^{-5}$	7-13
	Münster, Germany, Fed. Rep. of	$1.0\ 10^{-5}$	10
Tuberous sclerosis (epiloia)	Oxford, England, UK	$1.05\ 10^{-5}$	10.5
	China	$6\ 10^{-6}$	6
Neurofibromatosis	Michigan, USA	$1\ 10^{-4}$	100
	Moscow, USSR	$4.4\text{-}4.9\ 10^{-5}$	44-49
Polyposis intestini	Michigan, USA	$1.3\ 10^{-5}$	13
Marfan's syndrome	Northern Ireland, UK	$4.2\text{-}5.8\ 10^{-6}$	4.2-5.8
Polycystic disease of the kidneys	Denmark	$6.5\text{-}12\ 10^{-5}$	65-120
Diaphyseal aclasis (multiple exostoses)	Münster, Germany, Fed. Rep. of	$6.3\text{-}9.1\ 10^{-6}$	6.3-9.1
von Hippel-Lindau syndrome	Germany, Fed. Rep. of	$1.8\ 10^{-7}$	0.18
	B. SEX-LINKED RECESSIVE MUTATIONS		
Haemophilia	Denmark	$3.2\ 10^{-5}$	32
	Switzerland	$2.2\ 10^{-5}$	22
	Münster, Germany, Fed. Rep. of	$2.3\ 10^{-5}$	23
Haemophilia A	Hamburg, Germany, Fed. Rep. of	$5.7\ 10^{-5}$	57
	Finland	$3.2\ 10^{-5}$	32
Haemophilia B	Hamburg, Germany, Fed. Rep. of	$3\ 10^{-6}$	3
	Finland	$2\ 10^{-6}$	2
Duchenne-type muscular dystrophy	Utah, USA	$9.5\ 10^{-5}$	95
	Northumberland and Durham, UK	$4.3\ 10^{-5}$	43
	Südbaden, Germany, Fed. Rep. of	$4.8\ 10^{-5}$	48
	Northern Ireland, UK	$6.0\ 10^{-5}$	60
	Leeds, England, UK	$4.7\ 10^{-5}$	47
	Wisconsin, USA	$9.2\ 10^{-5}$	92
	Bern, Switzerland	$7.3\ 10^{-5}$	73
	Fukuoko, Japan	$6.5\ 10^{-5}$	65
	North-east England, UK	$10.5\ 10^{-5}$	105
	Warsaw, Poland	$4.6\ 10^{-5}$	46
Incontinenta pigmenti (Bloch-Sulzberger)	Münster, Germany, Fed. Rep. of	$0.6\text{-}2.0\ 10^{-5}$	6-20
Oculofaciodigital syndrome (OFD)	Münster, Germany, Fed. Rep. of	$5\ 10^{-6}$	5

Source: Reference 599.

TABLE 11. INCIDENCE OF CHROMOSOMAL ABNORMALITIES IN NEW-BORN INFANTS

Survey centre	Number of infants			Sex-chromosome anomalies						Autosomal abnormalities														Total	Frequency (%)
				Males			Females			Numerical (trisomics)				Structural (euploid)				Structural (aneuploid)							
														Robertsonian		Reciprocal T and insertional	Inversions	Robertsonian	Reciprocal T	Inversions	Deletions	Supernumerary			
	Male	Female	Total	47, XYY	47, XXY	Others[a]	45, X	47, XXX	Others[a]	+D	+E	+G	Others[a]	D/D	D/G										
Hamilton, Canada	493	437	930[e]	2	1	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	5	0.54
London, Canada	1 066	1 015	2 081	4	1	–	–	–	–	–	–	2	–	1	–	–	–	1	–	–	1	–	–	10	0.48
Winnipeg, Canada	7 176	6 763	13 939	4	6	2	–	5	2	1	3	14	–	12	1	11	1	–	1	–	–	–	2	65	0.47
Arhus, Denmark	5 751	5 387	11 148	3	6	9	1	7	3	1	1	16	–	15	2	15	1	–	6[b]	–	1	6[c]	–	93	0.83
Edinburgh, UK	7 849	3 831	11 680	10	9	5	–	5	2	–	2	17	1	6	4	10	2	1	–	1	–	1	2	78	0.67
Boston, USA	9 048	–	9 048	3	6	8	–	–	–	–	–	7	–	5	2	5	2	–	–		2	4	–	44	0.49
New Haven, USA	2 176	2 177	4 353	3	4	–	1	3	–	1	1	3	–	2	1	3	–	–	–	–	–	–	–	22	0.51
Moscow, USSR	1 303	1 197	2 500[d]	–	1	6	–	–	–	–	–	4	2	–	1	3	1	–	–	–	1	–	–	19	0.76
Total	34 872	20 807	55 679	29	34	30	2	20	7	3	7	63	3	41	11	48	7	2	7	1	5	11	4	336	0.60
				122 (0.22%)						76 (0.14%)				107 (0.19%)				30 (0.05%)							

Sources: References 38, 212, 250, 289, 363.

[a] Most of these are mosaics.

[b] Unbalanced Y-autosome translocations.

[c] Supernumerary small metacentric chromosomes including two mosaics.

[d] These 2500 infants are a random sample of 10 237 infants born during the study period (1969-1972) and included 15 twin-pairs, 29 new-born infants with various congenital malformations, 5 stillborn and 13 delivered prematurely (38).

[e] Including 1 twin-pair and 1 triplet.

TABLE 12. CHROMOSOMAL ANOMALIES IN SPONTANEOUS ABORTIONS[a]

Karyotype	Canada Carr	United Kingdom Creasy et al.	France Boué	Germany, Fed. Rep. of Degenhardt	United Kingdom Dhadial	United States Arakaki	United States Szulman	United States Thiede	USSR Kuliev	Japan Kaji et al.	Denmark Therkelsson
Monosomy X	12	68	140	8	31	18	5	5	1	12	39
Autosomal	–	–	1 (G)	–	–	1 (A)	–	–	–	1	–
Trisomy A	1	11	12	2	–	–	–	2	1	2	1
B	1	2	6	–	–	–	–	–	–	1	2
C	2	17	86	2	6	1	1	–	3	7	11
D	6	21	109	3	11	1	–	2	2	11	16
E	9	60	172	7	12	14	2	7	2	20	27
F	1	3	7	–	–	2	–	–	1	–	–
G	6	29	87	6	10	–	3	4	3	9	6
Double trisomy	1	–	16	2	2	–	–	–	–	1	2
Triploidy XXX	6	18	57	1	5	3	–	1	1	3	9
XXY	2	18	92	–	8	3	4	–	1	5	5
XYY	1	1	7	–	–	1	–	–	–	–	–
Not karyotyped	–	–	27	–	–	–	–	–	2	–	–
Mosaics 46/49	–	–	2	1	–	–	–	–	–	2	–
Hypertriploids	–	2[b]	–	–	–	–	–	–	–	–	–
Tetraploidy XXXX	–	7	33	–	3	–	–	2	–	2	6
XXYY	–	5	20	–	1	1	–	1	–	1	4
Not karyotyped	2	–	–	–	–	–	–	–	1	–	–
Other anomalies	–	25[c]	43	3	5	17	–	–	2	5[d]	10
Total	50	287	919	35	94	63	15	24	20	82	138

Sources: References 45, 403.

[a]Results from 11 series in 9 different countries.

[b]One had an extra C-group chromosome (70, XXY, +C), while the other was tetrasomic for an E-group chromosome (70, XXY, +E).

[c]Including 10 structural anomalies, the remainder being mixoploids or other anomalies.

[d]Including 3 structural anomalies and 2 mosaics.

TABLE 13. COMPARISON OF THE FREQUENCIES OF INCIDENCE OF DIFFERENT KINDS OF CHROMOSOMAL ANOMALIES IN SPONTANEOUS ABORTIONS

Kind of anomaly	Boué and Boué (45) Number	Boué and Boué (45) Frequency (%)	Other authors Number	Other authors Frequency (%)
Monosomy-X	140	15.2	199	24.6
Monosomy (autosomal)	1	0.1	2	0.2
Trisomy	479	52.1	393	48.6
Double trisomy	16	1.7	8	1.0
Triploidy	185	20.1	101	12.5
Tetraploidy	55	6.0	36	4.5
Others	43	4.7	69	8.5
Total	919	100	808	100

Source: Compiled from the data in table 12.

TABLE 14. RELATIVE FREQUENCY OF DIFFERENT TYPES OF AUTOSOMAL TRISOMIES IN SPONTANEOUS ABORTIONS

Extra autosome involved	Group of 392 trisomies[a]		Group of 479 trisomies[b]	
	Number	Frequency (%)	Number	Frequency (%)
A	20	5.1	12	2.5
B	6	1.5	6	1.3
C	50	12.8	86	17.9
D	73	18.6	109	22.8
E	160	40.8	172	35.9
F	7	1.8	7	1.5
G	76	19.4	87	18.2
Total	392	100	479	100

[a] The series of table 12, that of Boué excepted.
[b] References 45, 253.

TABLE 15. MATERNAL AGE IN RELATION TO DIFFERENT TYPES OF CHROMOSOMAL ANOMALIES

Karyotype	Number of cases observed	Mean maternal age (years)
Monosomy X	134	27.57 ± 0.88
Trisomy	448	31.25 ± 0.60
Triploidy	167	27.38 ± 0.79
Tetraploidy	53	26.79 ± 1.40
Translocation	26	26.96 ± 2.32
Normal	509	27.48 ± 0.45
Autosomal trisomy		
47,A+	13	29.62 ± 2.18
47,B+	7	33.43 ± 7.13
47,C+	72	30.93 ± 1.67
47,D+	92	32.49 ± 1.33
47,E+	157	29.58 ± 0.88
47,F+	8	30.13 ± 5.32
47,G+	78	33.17 ± 1.40
48	14	35.00 ± 6.09

Source: Reference 45.

TABLE 16. X-RAY-INDUCED DOMINANT LETHALS IN MALE MICE AT DIFFERENT POST-IRRADIATION INTERVALS

Mating ratio, 1 male to 3 females per week; 30 irradiated and 10 control males

Irradiation level 200 R

Group	Week of mating	Number of females paired	Number of females pregnant	Proportion of total (%)	Corpora lutea	Implants	Live embryos	Average number of live embryos per pregnant female	Pre-implantation loss (%)	Post-implantation loss (%)	Induced dominant lethality (%)	
											A[a]	B[b]
Control		31	28	90	259	239	221	7.9	7.7	7.5		
Irradiated	1	15	15	100	134	111	89	5.9	17.2	19.8	25	13
	2	16	15	94	118	103	78	5.2	12.7	24.3	34	18
	3	14	12	86	110	83	55	4.6	24.5	33.7	42	28
	4	14	12	86	102	71	57	4.8	30.4	19.7	39	13
	5	15	14	93	121	92	69	4.9	24.0	25.0	38	19
	6	13	8	62	70	47	36	4.5	32.9	23.4	43	17
	7	14	1	7	8	2	2	2.0	75.0	–	–	–
	8	13	11	85	126	93	84	7.6	26.2	9.7	4	2
	9	5	5	100	54	39	34	6.8	27.8	12.8	14	6

Source: Reference 133.
[a] Index A for dominant lethality is based on the number of live embryos per pregnant female.
[b] Index B for dominant lethality is based on the number of live embryos per total implantation.

TABLE 17. FREQUENCY OF DOMINANT LETHALITY IN LITTERS CONCEIVED IN THE FIRST AND THIRD WEEKS AFTER X IRRADIATION OF FEMALE MICE

Dose (rad)	Number of females	Corpora lutea	Total implants	Live embryos	Corpora lutea per female	Implants per female	Pre-implantation[a] loss (%)	Live embryos / Total implants	Induced post-implantation[b] loss (%)
0	22	222	201	194	10.1	9.14	9.5	0.965	–
First week									
100	18	199	162	151	11.1	9.00	18.6	0.932	3.4
200	16	154	125	110	9.6	7.81	18.8	0.880	8.8
300	15	164	138	121	10.9	9.20	15.9	0.877	9.1
400	14	165	141	116	11.8	10.07	14.5	0.823	14.7
Third week									
100	15	151	121	116	10.1	8.07	19.9	0.959	0.7
200	19	201	154	122	10.6	8.11	23.4	0.792	17.9
300	18	181	94	67	10.1	5.22	48.1	0.713	26.1
400	18	187	107	67	10.4	5.94	42.8	0.626	35.1

Source: Reference 513.

[a] Calculated as–Number of implants/Number of corpora lutea) × 100

[b] Calculated as $\left(1 - \frac{\text{Number of embryos/Number of implants in the irradiation series}}{\text{Number of embryos/Number of implants in the controls}}\right) \times 100$

TABLE 18. DOMINANT LETHALITY AFTER FISSION-NEUTRON IRRADIATION OF FEMALES

First-week mating

Dose (rad)	Pregnant females	Corpora lutea per female	Implants per female	Live embryos per female	Live embryos / Corpora lutea	Live embryos / Total implants	Induced lethality: Post-implantation	Induced lethality: Total
0	16	10.0	9.6	8.6	0.856	0.890	–	–
50	15	12.7	11.4	10.2	0.805	0.895	−0.6	6.0
100	18	11.3	9.7	8.4	0.749	0.869	2.4	12.5
150	16	9.6	8.7	6.0	0.627	0.691	22.4	26.8
200	16	9.9	7.8	5.0	0.503	0.645	27.5	41.2

Source: Reference 513.

TABLE 19. FERTILITY AND DOMINANT LETHALITY IN FEMALE GUINEA-PIGS AND GOLDEN HAMSTERS TREATED WITH X RAYS

Dose (rad)	Total number of females	Number not pregnant	Corpora lutea	Implants	Live embryos	Moles	Dead	Abnormal	Corpora lutea per female	Implants per female	Live embryos per female
					GUINEA-PIG						
					Mating at first oestrus						
0	20	0	72	62	60	2	0	0	3.6	3.1	3.0
100	25	0	101	85	82	3	0	0	4.0	3.4	3.3
200	21	0	93	69	61	7	1	0	4.4	3.3	2.9
400	26	5	94	60	49	9	2	0	4.8	2.9	2.3
					Second oestrus						
0	20	0	74	68	66	1	0	1	3.7	3.4	3.3
100	21	1	69	65	65	0	0	0	3.5	3.3	3.3
200	20	0	78	69	65	1	3	0	3.9	3.5	3.3
400	20	0	73	55	52	3	0	0	3.7	2.8	2.6
					Three months						
0	23	2	79	70	65	4	1	0	3.8	3.3	3.1
100	21	0	74	66	65	1	0	0	3.5	3.1	3.1
200	24	1	92	79	72	6	1	1	4.0	3.4	3.1
400	21	0	77	68	58	9	1	0	3.7	3.2	2.8
					GOLDEN HAMSTER						
					Mating at first oestrus						
0	11	1	178	165	138	19	3	5	12.7	11.8	9.8
100	23	2	270	245	196	38	6	5	12.9	11.7	9.3
200	21	2	245	192	147	36	3	6	12.9	10.1	7.7
400	23	4	278	180	112	65	1	2	14.6	9.5	5.9

Dose (rad)	Total number of females	Number not pregnant	Corpora lutea	Implants	Live embryos	Moles	Dead	Abnormal	Corpora lutea per female	Implants per female	Live embryos per female
				GOLDEN HAMSTER *(continued)*							
				Second oestrus							
0	13	2	143	133	104	21	6	2	13.0	12.0	9.5
100	20	2	226	204	161	32	5	6	12.6	11.3	8.9
200	20	2	231	190	135	40	12	3	12.8	10.6	7.5
400	20	3	280	211	142	47	11	11	16.5	12.4	8.4
				One month							
0	20	1	230	207	171	34	1	1	12.1	9.5	8.5
100	25	5	252	223	175	40	5	3	12.6	11.2	8.8
200	22	2	254	214	178	27	4	5	12.7	10.7	8.9
400	31	12	275	219	175	33	2	9	14.5	11.5	9.2
				Three months							
0	12	2	126	111	91	14	3	3	12.6	11.0	9.1
400	13	10	34	28	20	5	2	1	11.3	8.5	6.7

Source: Reference 121.

TABLE 20. FREQUENCY OF DOMINANT LETHALS IN FEMALE GUINEA-PIGS AND GOLDEN HAMSTERS TREATED WITH X RAYS

Dose (rad)	Implants / Corpora lutea	Live embryos / Implants	Live embryos / Corpora lutea	Fraction of dominant lethals (%)		
				Pre-implantation	Post-implantation	Total
			GUINEA-PIG			
			Mating at first oestrus			
0	0.861 ± 0.041	0.986 ± 0.022	0.833 ± 0.044			
100	0.842 ± 0.036	0.965 ± 0.020	0.812 ± 0.039	2.2	0.4	2.6 ± 6.9
200	0.742 ± 0.045	0.884 ± 0.039	0.656 ± 0.049	13.2	8.6	21.3 ± 7.2
400	0.638 ± 0.050	0.817 ± 0.050	0.521 ± 0.052	25.8	15.6	37.5 ± 7.1
			Second oestrus			
0	0.919 ± 0.032	0.971 ± 0.021	0.892 ± 0.036			
100	0.942 ± 0.028	1.00 ± 0.030	0.942 ± 0.028			
200	0.885 ± 0.036	0.942 ± 0.028	0.833 ± 0.042	3.7	2.9	6.6 ± 6.0
400	0.753 ± 0.050	0.946 ± 0.031	0.712 ± 0.053	18.0	2.6	20.1 ± 6.7
			Three months			
0	0.886 ± 0.036	0.929 ± 0.031	0.823 ± 0.043			
100	0.892 ± 0.036	0.985 ± 0.015	0.878 ± 0.038			
200	0.859 ± 0.036	0.911 ± 0.032	0.783 ± 0.043	1.8	3.1	4.9 ± 7.2
400	0.883 ± 0.037	0.853 ± 0.043	0.753 ± 0.049	0.3	8.1	8.5 ± 7.6
			GOLDEN HAMSTER			
			Mating at first oestrus			
0	0.927 ± 0.020	0.836 ± 0.029	0.775 ± 0.031			
100	0.907 ± 0.018	0.800 ± 0.026	0.726 ± 0.027	2.1	4.4	6.4 ± 5.1
200	0.784 ± 0.048	0.766 ± 0.031	0.600 ± 0.031	15.5	8.5	22.6 ± 5.1
400	0.648 ± 0.033	0.622 ± 0.036	0.403 ± 0.029	30.1	25.6	48.1 ± 4.3
			Second oestrus			
0	0.930 ± 0.021	0.782 ± 0.036	0.727 ± 0.037			
100	0.903 ± 0.020	0.789 ± 0.028	0.712 ± 0.030	2.9		2.1 ± 6.5
200	0.823 ± 0.047	0.711 ± 0.055	0.584 ± 0.059	11.6	9.1	19.6 ± 9.1
400	0.754 ± 0.033	0.673 ± 0.047	0.507 ± 0.039	18.2	14.9	30.3 ± 6.5
			One month			
0	0.900 ± 0.031	0.855 ± 0.025	0.770 ± 0.051			
100	0.885 ± 0.020	0.785 ± 0.041	0.694 ± 0.038	1.7	8.2	9.8 ± 7.8
200	0.843 ± 0.039	0.832 ± 0.026	0.701 ± 0.042	6.4	2.7	8.9 ± 8.1
400	0.796 ± 0.041	0.799 ± 0.027	0.636 ± 0.043	11.6	6.5	17.3 ± 7.8
			Three months			
0	0.881 ± 0.029	0.820 ± 0.036	0.722 ± 0.040			
400	0.824 ± 0.065	0.714 ± 0.085	0.588 ± 0.022	6.5	13.0	18.6

Source: Reference 121.

TABLE 21. DOSE-EFFECT DATA FOR THE INDUCTION OF RECIPROCAL TRANSLOCATIONS IN MOUSE SPERMATOGONIA

Single exposures at a high exposure rate

Exposure (R)	*Number of animals*	*Number of cells scored*	*Time interval[a] (days)*	*Proportion of abnormal cells (%)*	*Proportion of translocations (%)*
0	6	600		0.0	0.0
50	6	600	27	0.7 ± 0.3	0.7 ± 0.3
100	6	600	28	1.5 ± 0.5	1.5 ± 0.5
150	6	600	32	2.5 ± 0.6	2.5 ± 0.6
200	6	600	35	4.0 ± 0.8	4.3 ± 0.8
300	6	600	48	7.8 ± 1.1	7.8 ± 1.1
400	8	800	54	12.1 ± 1.2	13.5 ± 1.3
500	8	800	62	14.8 ± 1.4	17.0 ± 1.5
600	8	800	68	16.0 ± 1.4	19.5 ± 1.6
700	8	800	91	11.6 ± 1.2	13.5 ± 1.3
800	9	900	121	8.7 ± 1.0	10.2 ± 1.1
1200	6	600	182	3.8 ± 0.8	14.5 ± 0.9

Source: Reference 419.

[a]Between irradiation and sampling.

TABLE 22. YIELD OF RECIPROCAL TRANSLOCATIONS IN SPERMATOCYTES FOLLOWING X IRRADIATION OF MOUSE SPERMATOGONIA

Repeated 400-R fractions separated by 8-week intervals

Total exposure (R)	*Number of cells scored*	*Yield of translocations (%)*
0	1 000	0
400	2 150	13.8 ± 0.8
800	1 450	19.7 ± 1.2
1 200	900	31.1 ± 1.9
1 600	600	47.8 ± 2.8
2 000	600	60.2 ± 3.2
2 400	600	64.5 ± 3.3
2 800	600	80.7 ± 3.7

Source: Reference 420.

TABLE 23. FREQUENCY OF CYTOLOGICALLY SCORED AND GENETICALLY RECOVERED RECIPROCAL TRANSLOCATIONS FOLLOWING X IRRADIATION OF MOUSE SPERMATOGONIA

Exposure (R)	*Frequency of translocations scored[a] (%)*	*Number of F_1 males tested*	*Minimum frequency of translocation heterozygotes[b] (%)*	*Ratio recovered/ scored*
0	0.0	2 633	0.0	–
150	2.5 (2.5)	993	0.6	0.25
300	7.8 (5.7)	1 018	0.88	0.12
600	17.2 (16.2)	1 075	1.90	0.11
1 200	4.5 (3.8)	1 038	0.50	0.13

Source: Reference 56.

[a]Values in parentheses are proportions of cells with a single translocation.

[b]Cytologically confirmed semisteriles.

TABLE 24. FREQUENCY OF CHROMATID ABERRATIONS IN MI OOCYTES AT VARIOUS TIMES AFTER AN ACUTE X-RAY EXPOSURE OF 200 R

Interval (days)	*Number of cells scored*	*Frequency of deletions (%)*	*Frequency of interchanges (%)*
1	100	1.0 ± 1.0	1.0 ± 1.0
3	100	4.0 ± 2.0	1.0 ± 1.0
5	125	4.8 ± 2.0	0.8 ± 0.8
7	100	6.0 ± 2.5	6.0 ± 2.5
14	125	10.4 ± 2.9	12.8 ± 3.2
21	100	6.0 ± 2.5	6.0 ± 2.5

Source: Reference 58.

TABLE 25. FREQUENCY OF CHROMATID ABERRATIONS IN MI OOCYTES 14 DAYS AFTER VARIOUS X-RAY EXPOSURES

Exposure (R)	*Number of cells scored*	*Frequency of deletions (%)*	*Frequency of interchanges (%)*
0	200		
50	150	1.3 ± 0.9	1.3 ± 0.9
100	150	4.0 ± 1.6	4.7 ± 1.8
200	125	10.4 ± 2.9	12.8 ± 3.2
300	150	16.0 ± 3.3	23.3 ± 3.9
400	100	37.0 ± 6.1	56.0 ± 7.5

Source: Reference 58.

TABLE 26. YIELD OF TRANSLOCATIONS IN SPERMATOCYTES FOLLOWING SPERMATOGONIAL IRRADIATION IN DIFFERENT MAMMALIAN SPECIES

(a) Data of Lyon and Cox (309).

	Rabbit				*Golden hamster*				*Guinea-pig*			
			Yield of translocations (%)				*Yield of translocations (%)*				*Yield of translocations (%)*	
Dose or exposure (rad or R)	*Animals*	*Cells*	*Definite and possible*	*Definite*	*Animals*	*Cells*	*Definite and possible*	*Definite*	*Animals*	*Cells*	*Definite and possible*	*Definite*
0	3	1 117	0.2	0.1	5	1 013	0.2	0.1	5	1 406	0.3	0.1
100	3	1 285	1.8	1.6	5	1 408	1.6	1.2	4	1 273	1.1	0.7
200	3	1 008	3.1	2.6	6	1 633	2.3	1.7	5	1 504	2.3	1.7
300	4	1 923	4.9	4.2	5	1 518	2.2	2.0	5	1 873	3.6	3.0
400	3	967	1.0	0.8	5	1 245	1.8	1.4	4	1 127	1.3	1.2
500	3	1 248	1.1	0.6	5	1 307	2.1	1.6	4	968	0.5	0.3
600	3	1 055	0.5	0.3	5	1 051	1.6	1.0	5	1 268	0.8	0.5

(b) Data of Brewen and Preston (54).

Dose or exposure (rad or R)	*Yield of translocations (%)*	
	Guinea-pig	*Chinese hamster*
0	0	0
50	1.4 ± 0.6	1.0 ± 0.6
100	2.7 ± 0.9	2.0 ± 0.8
200	6.1 ± 1.1	4.3 ± 1.2
300	8.0 ± 2.0	–

TABLE 27. TRANSLOCATION YIELDS AFTER VARIOUS X-RAY DOSES TO MONKEY SPERMATOGONIA

Monkey No.	Dose (rad)	Interval between irradiation and examination (months)	Number of cells analysed	Translocation yield	
				Definite and possible	Definite
		English monkeys (ref. 314)[a]			
1L	0 + S	8	124	0.8	0.8
R	300 + S	8	186	2.7	2.7
2L	S + 200	11	517	1.0	0.8
R	200 + S	9	82	4.9	4.9
3L	100	9	391	4.1	1.8
R	100	6.5	253	2.0	1.2
4L	100	9	248	0.4	0.4
R	100	6.5	146	1.4	1.4
		Dutch monkeys (ref. 314)			
1	300	8	291	0.0	0.0
2	300	8	1	0.0	0.0
3	200	8	36	0.0	0.0
4	200	8	495	1.6	1.2
5	100	8	777	0.3	0.3
Control	0		30	0.0	0.0
		Combined results			
	100		1 815	1.4	0.8
	200		1 130	1.5	1.2
	300		478	1.0	1.0
		Dutch monkeys (ref. 70)			
1-3	0		100 + 100 + 300		0.0
4	100	8	1 700		0.06
5	100	8	350		1.1
6	200	8	850		2.0
7	200	8	550		1.1
8	300	8	400		0.0
9	300	8	175		1.1
		Combined results			
	100		2 050		0.2
	200		1 400		1.6
	300		575		0.3

[a]English monkeys No. 1 and No. 2 were unilaterally irradiated; all others were bilaterally irradiated. L = left testis; R = right testis; S = shielded.

TABLE 28. YIELD OF RECIPROCAL TRANSLOCATIONS IN SPERMATOCYTES FOLLOWING SPERMATOGONIAL IRRADIATION IN MARMOSET AND MAN COMPARED WITH MOUSE DATA

Dose (rad)	Man			Marmoset			Mouse		
	Number of individuals	Cells	Yield (%)	Number of animals	Cells	Yield (%)	Number of animals	Cells	Yield (%)
0	2	200	0	2	600	0	6	600	0
25	–			2	600	2.8 ± 0.7	–		
50	–			2	600	3.3 ± 0.8	6	600	0.7 ± 0.3
78	2	371	4.0 ± 1.0	–			–		
100	–			2	600	7.8 ± 1.1	6	600	1.5 ± 0.5
200	2	300	7.0 ± 1.3	1	200	7.5 ± 1.9	6	600	4.3 ± 0.8
300	–			2	600	7.0 ± 1.1	6	600	7.8 ± 1.1
600	2	180	6.1 ± 1.8	–			8	800	19.5 ± 1.6

Source: References 57, 419.

TABLE 29. YIELD OF TRANSLOCATIONS IN SPERMATOCYTES FOLLOWING SPERMATOGONIAL IRRADIATION WITH SINGLE OR FRACTIONATED EXPOSURES IN THE GOLDEN HAMSTER AND GUINEA-PIG

			Golden hamster				Guinea-pig			
					Yield (%)				Yield (%)	
Total dose (rad)	Radiation procedure	Interval between fractions	Number of animals	Number of cells scored	Definite and possible	Definite	Number of animals	Number of cells scored	Definite and possible	Definite
0[a]			5	1 013	0.2	0.1	5	1 406	0.3	0.1
0[b]			5	1 118	0.2	0.1	4	778	0.1	0.0
0[c]			5	906	0.1	0.0	3	604	0.2	0.0
0[d]			–				4	765	0.3	0.0
200	single		6	1 633	2.3	1.7	5	1 504	2.3	1.7
400	single		5	1 245	1.8	1.4	4	1 127	1.3	1.2
400	2 X 200	24 h	6	1 607	4.5	3.9	4	881	4.8	4.1
400	2 X 200	8 weeks	5	1 350	3.5	3.1	4	1 039	3.2	2.5
400	8 X 50	1 week	7	1 800	2.7	1.9	–			
300	single		–				5	1 873	3.6	3.0
600	single		5	1 051	1.6	1.0	5	1 268	0.8	0.5
600	2 X 300	24 h	–				4	763	7.6	7.0
600	2 X 300	8 weeks	–				4	1 042	3.1	2.0
600	12 X 50	1 week	6	1 446	3.1	2.5	3	1 193	5.0	4.1

Source: Reference 308.

[a]Control for single-dose experiments.

[b]Control for 2 X 200 rad (24 h), 2 X 200 rad (8 week) experiments in golden hamster and for 2 X 200 rad (24 h) and 2 X 300 rad (24 h) experiments in guinea-pig.

[c]Control for 12 X 50 rad (1 week), 8 X 50 rad (1 week) experiments in golden hamster for 12 X 50 rad (1 week) experiments in guinea-pig.

[d]Control for 2 X 300 rad (8 week) and 2 X 200 rad (8 week) experiments in guinea-pig.

TABLE 30. EMBRYONIC MORTALITY IN PROGENY OF MALES WITH OR WITHOUT DETECTABLE TYPES OF CHROMOSOME ABERRATION

Category of male	Number of pregnant females	Total implants	Live embryos	Dead implants	Embryonic mortality (%)	Implants per female	Live embryos per female
With 1 RT[a]	120	806	369	437	54.2	6.72 ± 0.20	3.08 ± 0.15
With 2 RT	8	58	18	40	69.0	7.25	2.25
With insertion	2	14	7	7	50.0	7.00	3.50
Normal	849	6 743	6 161	582	8.63	7.94	7.26

Source: Reference 520.

[a]RT = Reciprocal translocation.

TABLE 31. EMBRYONIC MORTALITY IN PROGENY OF MALES WITH RECIPROCAL TRANSLOCATIONS (RT)

Male Number	Number of RT	Total number of females	Number not pregnant	Corpora lutea	Implants	Live embryos	Dead embryos	Moles	Embryonic mortality (%)	Implants per female	Live embryos per female
					Golden hamster						
1	2	5	0	71	45	11	1	33	75.6	9.0	2.2
2	1	9	0	125	99	39	3	57	60.6	11.0	4.3
3	1	8	0	104	76	36	3	37	52.6	9.5	4.5
4	1	9	0	107	78	31	4	43	60.3	8.7	3.4
6	1	8	0	108	93	47	8	38	49.5	11.6	5.9
8	1	10	0	112	106	69	4	33	34.9	10.6	6.9
12	1	3	0	34	33	14	0	19	57.6	11.0	4.7
13	1	9	0	105	85	40	2	43	52.9	9.4	4.4
Total (1RT)[a]		56	0	695	570	276	24	270			
								Average	52.6	10.3	4.87
Control[b]		43		542	478	393	12	53	13.6	11.1	9.14

TABLE 31 *(continued)*

Male Number	*Number of RT*	*Total number of females*	*Number not pregnant*	*Corpora lutea*	*Implants*	*Live embryos*	*Dead embryos*	*Moles*	*Embryonic mortality (%)*	*Implants per female*	*Live embryos per female*
					Guinea-pig						
2	1	10	5	22	14	7	0	7	50.0	2.8	1.4
4	1	10	1	42	31	25	0	6	19.4	3.4	2.8
6	1	8	1	34	30	15	0	15	50.0	4.3	2.1
9	1	12	1	52	37	30	1	6	18.9	3.4	2.7
11	1	6	3	13	8	5	0	3	37.5	2.7	1.7
15	1	4	0	21	13	9	1	3	30.8	3.3	2.3
17	1	7	4	9	8	1	0	7	87.5	2.7	0.3
Total (1RT)[a]		57	15	193	141	92	2	47			
								Average	42.0	3.2	1.9
Control[b]		40		146	130	126	0	4	3.1	3.3	3.2

Source: Reference 123.

[a]1RT = one reciprocal translocation.

[b]Control data taken from Cox and Lyon (121).

TABLE 32. FREQUENCY OF INDUCED X-CHROMOSOME ANOMALIES FROM IRRADIATION OF FEMALE GERM-CELL STAGES IN MICE

Cell stage sampled	*Interval, irradiation to mating (days)*	*Exposure R*	*Exposure rate (R/min)*	*Number of females*	*Frequency of OX^P*		*Reference*
					Total (%)	*Induced[a] (10^{-5} R^{-1})*	
Preleptotene through diplotene	> 42	221	*ca.* 80	1 221	0.08-0.33[b]	0.13-1.24[b]	452, 456
Control		0		391	0		
Dictyate	1-42[c]	400	66-78	15 006	0.61-0.77	1.38-1.78	468, 489, 490
Dictyate	1-42	50	66-78	12 256	0.02-0.08	0[d]	
Dictyate	> 42[c]	50	66-78	10 172	0.09	0[d]	
Dictyate	1-42	400	0.6	10 900	0.36-0.40	0.76-0.88	
Control		0		11 528	0.06		
Dictyate	0-7[e]	400	0.006	1 906	0.26	0.52	468, 484, 485
Dictyate	8-42[f]	400	0.006	1 779	0-0.06	0[d]	
Dictyate	> 42[g]	400	0.006	6 644	0.08	0[d]	
Control		0		12 163	0.04-0.05		
Dictyate	1-49	400	65-70	5 170	0.77-0.87	1.80-2.04	76, 468
Dictyate	1-49	8 X 50[h]	65-70	6 099	0.46-0.51	1.02-1.14	
Control		0		5 154	0.04		

Source: Reference 450.

[a]Calculated as treated minus overall spontaneous control frequency. Data for the contemporary control are shown grouped with the treated sets.

[b]The lower number (or the single number, where only one is given) represents only genetically and/or cytologically verified cases; the higher number represents cases observed in original phenotypic scoring. All are derived from XX mothers only.

[c]Considerable differences in frequency are observed within this interval. Readers wishing to make more precise quantitative comparisons between these results and those from lower dose rates are referred to the full data.

[d]Frequency of treated group not significantly higher than contemporary control.

[e]Matings made during this interval utilized oocytes that received almost the entire 400-R dose during the last six weeks of their development.

[f]Most matings made during this interval utilized oocytes that received only a small part of the dose during the last six weeks of their development.

[g]Matings made during this interval utilized oocytes irradiated prior to their last six weeks of development.

[h]With 75-minute intervals between the fractions.

TABLE 33. SPECIFIC-LOCUS MUTATION FREQUENCIES RESULTING FROM X IRRADIATION OF MALE MICE AT VARIOUS AGES

X rays, 300 R

Age at irradiation (days)	*Number of offspring screened*	*Number of mutations*	*Frequency per locus (10^{-5} R^{-1})*	*95% confidence limits*	
				Lower	*Upper*
New-born	55 456	16[a]	13.7	7.1[b]	22.6[b]
2	8 127	2	11.7	2.1	39.2
4	8 784	3	16.3	4.4	43.9
6	7 559	4	25.2	8.6	60.5
8	8 463	7	39.4	18.5	77.5
10	8 580	3	16.6	4.5	45.0
14	8 397	9	51.0	25.3	95.1
21	8 920	5[c]	26.7	7.3[b]	72.1[b]
28	10 009	6	28.5	12.4	61.0
35	9 056	4	21.0	7.2	50.5
Adult[d]	65 548	40	29.1	21.0	39.0

Source: References 526, 527.

[a] Including three clusters of two mutations each.

[b] Including correction for clusters using Russell's approach (480).

[c] Including two clusters of two mutations each.

[d] Data of W. L. Russell (474).

TABLE 34. ARBITRARY GROUPING OF SPECIFIC-LOCUS MUTATION RATE DATA FOR YOUNG MALE MICE TO ILLUSTRATE THE LIKELY TIME FOR TRANSITION IN MUTATIONAL RESPONSE

Age at the time of irradiation (days)	*Mutation frequency per locus (10^{-5} R^{-1})*	*95% confidence limits*	
		Lower	*Upper*
New-born	13.7	7.1[b]	22.6[b]
2-6	17.5	8.7	32.6
8-35	30.6	20.4[b]	42.3[b]
Adult[a]	29.1	21.0	39.0

Source: Reference 527.

[a] Data of W. L. Russell (474).

[b] Including correction for clusters using Russell's approach (480).

TABLE 35. A COMPARISON OF THE FREQUENCIES OF SPECIFIC-LOCUS MUTATION AFTER IRRADIATION OF MOUSE SPERMATOGONIA GIVEN IN ONE DOSE OR IN TWO EQUAL FRACTIONS SEPARATED BY VARIOUS TIME INTERVALS

X rays, 1000 R

Interval between fractions	*Total offspring*	*Number of mutations*	*Mutation frequency per locus (10^{-5})*	*95% confidence limits*	
				Lower	*Upper*
None (single exposure[a]	44 649	29	8.5	6.21	13.33
2 h[a]	148 791	12	11.52	5.95	20.12
24 h[a]	11 164	39	49.91	35.45	68.22
4 days[b]	7 168	11	21.92	10.94	39.22
7 days[b]	8 271	11	19.00	10.94	39.22
> 15 weeks[a, c]	4 904	10	29.13	13.98	53.57

[a] Data of W. L. Russell (472).

[b] Data of Cattanach and Moseley (92).

[c] 600 R + 400 R.

TABLE 36. YIELD OF SPECIFIC-LOCUS MUTATIONS OBTAINED AFTER IRRADIATION OF MOUSE FEMALES WITH ACUTE AND FRACTIONATED DOSES OF X RAYS

Group	Dose	Total progeny	Number of mutants	Mutations per locus (10^{-7} rad^{-1})	95% confidence limits		Total progeny	Number of mutants	Mutations per locus (10^{-7} rad^{-1})	95% confidence limits	
			Up to 20 days[a]		Lower	Upper		Up to 7 weeks[a]		Lower	Upper
A + B[b]	I X 200	21 578	7	2.32	0.93	4.78	34 813	9	1.85	0.85	3.51
C + D[c]	20 X 10	20 398	1	0.35	0.0088	1.95	39 887	1	0.18	0.0045	1.00

Source: Reference 312.

Note: The mutation rates given above are not corrected for controls.

[a] Duration from end of radiation treatment to conception.

[b] In group A, mating was soon after irradiation; in group B, it was one week later.

[c] In group C, 4 fractions each of 10 rad were administered at 2-h intervals in one day; radiation was given over a 5-day period (4 X 10 rad X 5); mating: 3 days after the last dose. In group D, 20 fractions each of 10 rad were administered over 5 days in a week (separated by 24-h intervals) for 4 weeks; mating: soon after completion of the last treatment.

TABLE 37. DISTRIBUTION OF OFFSPRING AND MUTATIONS AT SEVEN SPECIFIC LOCI IN CONCEPTIONS OCCURRING DURING SUCCESSIVE WEEKS AFTER IRRADIATION OF FEMALE MICE WITH VARIOUS EXPOSURES AND EXPOSURE RATES

Week	X rays, 200 R at 90 R/min		X rays, 50 R at 90 R/min		Gamma rays, 400 R at 0.8 R/min	
	Off-spring	Muta-tions	Off-spring	Muta-tions	Off-spring	Muta-tions
1	28 547	13	84 614	6	49 039	14
2	3 604	2	8 016	0	7 928	3
3	454	1	2 904	0	737	1
4	1 138	0	9 587	1	844	4
5	8 395	12	45 179	3	7 342	6
6	3 327	5	16 304	3	4 990	2
7	328	0	13 868	0	170	0
8	17	0	9 092	0	2	0
9	5	0	11 096	0	4	0
10	1	0	7 433	0	–	
11 on	–		50 570	0	–	

Source: Reference 483a.

TABLE 38. NUMBER OF MUTATIONS AT SEVEN SPECIFIC LOCI IN VARIOUS LOW-LEVEL RADIATION AND FRACTIONATION EXPERIMENTS ON FEMALE MICE

Exposure or dose given (R or rad)	Exposure rate or dose rate (R/min or rad/min)	Weighted mean effective exposure or dose[a] (R or rad)	Number of offspring	Number of mutations	Mutation frequency per locus (10^{-6})	Reference
0	51.		204 639	3 or 8	2.1 or 5.6	483a
215	51.6[b]	172	14 671	1	9.7	312
258	0.009	207	7 692	1	18.6	471
215	51.6[c]	212	21 204	0	0.0	312
400	0.009	283	13 742	2	20.8	471[d]
400	0.009	284	14 402	1	9.9	471
615	0.05	615	10 177	1	14.0	84a

[a] Dose delivered to oocytes in the 6 weeks prior to evaluation.

[b] Dose delivered over 4 weeks in 20 fractions of 10.7 rad.

[c] Dose delivered over 5 days in 20 fractions of 10.7 rad.

[d] Animals irradiated were old, previously bred females.

TABLE 39. INDUCTION OF DOMINANT LETHALS BY ^{239}Pu IN THE GERM CELLS OF MALE MICE

Experimental series	*Group*	*Week after mating*	*Total number of implants* N	*Dead implants (early deaths)* n_{ED}	*Frequency*[a] *(%)*	*Dead implants (late deaths)* n_{LD}	*Frequency*[b] *(%)*	*Total dead implants* n_T	*Frequency*[c] *(%)*
E4	Control	1-13	2 502	221	8.8	45	2.0	266	10.6
	0.05 μCi	1-13	2 962	298	10.1	71	2.7	369	12.5
E5	Control	1-18	3 971	331	8.3	40	1.1	371	9.3
	0.05 μCi	1-18	3 663	368	10.0	113	3.4	481	13.1
E3	Control	1-24	5 492	384	7.0	55	1.1	439	8.0
	0.1 μCi	1-24	5 182	564	10.9	161	3.5	725	14.0
E4	Control	1-13	2 502	221	8.8	45	2.0	266	10.6
	0.25 μCi	1-13	2 436	248	10.2	42	1.9	290	11.9
E5	Control	1-18	3 971	331	8.3	40	1.1	371	9.3
	0.25 μCi	1-18	3 773	424	11.2	112	3.3	536	14.2
E3	Control	1-24	5 492	384	7.0	55	1.1	439	8.0
	0.5 μCi	1-23	2 467	287	11.6	44	2.0	331	13.4

Source: Reference 304.

[a] $100\, n_{ED}/N$.

[b] $100\, n_{LD}/(N-n_{ED})$.

[c] $100\, n_T/N$.

TABLE 40. CHROMOSOME ABERRATIONS IN METAPHASE-I SPERMATOCYTES OF MICE AT DIFFERENT TIMES AFTER INJECTION OF ^{239}Pu OR AFTER CHRONIC GAMMA-RAY EXPOSURES

Series	*Weeks of exposure*	*Estimated testis dose*[a] *(rad)*	*Testis mass (mg)* *Right*	*Left*	*Number of spermatocytes scored*[b]	*Number*[d] *with* *Fragments*	*Quadrivalents*[c]	*Reciprocal translocations* *Rings*	*Chains*	*Totals*[d]	*Reference*
I: ^{239}Pu	0	0 0	125 ± 2		300	4 (1.3)	1 (0.3)	0	0	0	33
	6	14 (10)[e]	91 ± 6		400	8 (2.0)	4 (1.0)	3	1	4 (1.0)	
	12	30 (25)[e]	88 ± 2		400	9 (2.2)	16 (4.0)	15[f]	4	19 (4.8)	
	18	44	73 ± 8		400	12 (3.0)	9 (2.3)	2	3	5 (1.3)	
II: ^{239}Pu	0	0	119.2 ± 3.7	–	600	2 (0.3)	2 (0.3)	0	2	2 (0.3)	516
	21	13 ± 1	111.4 ± 6.4	103.4 ± 6.4	700	11 (2.6)[j]	5 (0.7)	4	1	5 (0.7)	
	28	18 ± 2	102.7 ± 5.3	96.3 ± 8.0	800	12 (1.5)	8 (1.0)	6	2	8 (1.0)	
	34	18 ± 1	109.0 ± 5.8	111.0 ± 3.5	800	12 (1.9)[k]	4 (0.5)[g]	1	3[g]	4 (0.5)	
	Mean[h]	17 ± 1; 15.5[e]	107.7 ± 3.1	103.0 ± 3.4	–	(1.9)[j]	–	–	–	– (0.74)	
^{60}Co	0	0	118.9 ± 6.1	–	300	2 (1.0)[j]	–	–	–	0	
	28	1128; 1055[e]	42.2 ± 1.3	–	1 000	39 (4.1)[j]	16 (1.6)[h]	10[i]	6	17 (1.7)	
	Mean controls (^{239}Pu and ^{60}Co of series II)	–	–	–	–	–	–	–	–	0.22	

[a] Estimated from left testis.

[b] Scored in the right testis.

[c] Resulting from reciprocal translocations arising in spermatogonia (see next columns) or chromatid interchanges.

[d] Percentage in parentheses.

[e] Estimated spermatogonial dose, not taking into account the inhomogenous distribution of ^{239}Pu in the testis in the plutonium series.

[f] Three cells each had 2 rings of 4.

[g] Including a chain of 3 + univalent and 2 chains of 4.

[h] Including 1 ring of 6.

[i] Including 1 ring of 6, 9 rings of 4 and 6 chains of 4.

[j] Fragments per cell.

[k] One cell recorded as having both single and double fragments.

TABLE 41. RECOVERY OF FERTILITY OF X-IRRADIATED MALE MICE FOLLOWING SINGLE OR FRACTIONATED (24-H INTERVAL) EXPOSURES

Age (days)	*Number of replicates*	*Total number of females tested*	*Overall median day of return to fertility*
	(a) Various ages at 300 R single		
5	1	10	42
10	1	10	51
Adult	1	10	51
	(b) Various ages at 500 R single		
5	1	10	44
10	1	11	53
Adult	1	10	56
	(c) Various ages at 1000 R single		
3	1	10	79
5	1	10	84
10	1	10	74
Adult	2	20	102[a]
	(d) Adults at various exposures		
Exposure (R)			
300	2	20	52
400	2	20	53
100 + 300	1	7	53
300 + 100	1	10	51
500	5	47	56
600	5	50	67
100 + 500	2	20	62
500 + 100	3	29	62
300 + 300	1	10	57
700	2	20	67
800	4	40	71
100 + 700	2	20	81
700 + 100	2	20	73
300 + 500	1	10	82
500 + 300	2	20	81
900	2	20	88
1000	2	20	102
700 + 300	1	10	111
100 + 900	1	10	135
900 + 100	1	10	108
500 + 500	2	20	102

Sources: References 90, 94.

[a] Same data as for the adult at 1000 R in part (d)

TABLE 42. COMPARISON OF RADIATION-INDUCED MUTATION RATES WITH FREQUENCY OF LABELLED SPERMATOGONIAL STEM CELLS IN THE MOUSE 207 AND 414 HOURS AFTER X IRRADIATION

Exposure (R)	*Mutations per locus[a] (10^{-5})*	*Frequency of labelled cells (%)*				*Cell survival compared with control (%)*
		Labelling 6 × 12.5 μCi of ^{3}H-TdR 24 h before x irradiation		*Labelling 3 × 12.5 μCi of ^{3}H-TdR 24 h before x irradiation*		
		Time after irradiation		*Time after irradiation*		
		207 h	*414 h*	*207 h*	*414 h*	
0	0.75	7.8	0.7	29.2	22.8	
100		15.6	1.6	29.0	33.8[b]	
300	8.72			24.3	33.3[b]	26.8
500		16.3	1.7	27.5	39.9[b]	11.2
600	13.29			26.3	36.5[b]	9.3
1000	9.28	2.4	0.2	10.2[c]	15.3[b]	3.2
500 + 500[d]	49.91	39.1	3.1	47.2[b]	35.5[b]	2.3

Sources: References 367a, 657.

[a] From W. L. Russell (471).

[b] Significantly above control.

[c] Significantly below control.

[d] Fractions given 24 h apart.

TABLE 43. COMPARISON OF THE YIELDS OF DICENTRICS (IN LYMPHOCYTES) AND RECIPROCAL TRANSLOCATIONS (IN SPERMATOCYTES, AFTER SPERMATOGONIAL X IRRADIATION) IN MAN, MARMOSET, RABBIT AND THE RHESUS MONKEY

Dose (rad)	Dicentric yield (%)	Translocation yield (%)		Dicentric yield / Translocation yield
		Definite and possible	Definite	
		Man[a]		
0	–	–	0	–
78	8.0	–	4.0 ± 1.0	2
200	35.2	–	7.0 ± 1.3	5
600	250.0	–	6.1 ± 1.8	42
		Marmoset[a]		
0	–	–	0	–
25	3.0	–	2.8 ± 0.7	1
50	6.7	–	3.3 ± 0.8	2
100	16.3	–	7.8 ± 1.1	2
200	44.6	–	7.5 ± 1.9	6
300	84.9	–	7.0 ± 1.1	12
		Rabbit		
100	8.0[b] 6.3[c]	1.84 ± 0.38[d]	1.56 ± 0.34[d]	4-5
153	13.0	2.5 (estimated)	1.8 (estimated)	5-7
200	20.5	3.08 ± 0.54	2.59 ± 0.50	~7-8
203	23.0			
300	33.6	4.94 ± 0.85	4.20 ± 0.77	~7-12
305	50.0			
		Rhesus monkey[e]		
0	– (600)[f]	–	0- (500)[f]	–
100	4.9 (600)	–	0.2 (1 950)	25
200	13.9 (600)	–	1.6 (1 400)	8
300	26.6 (600)	–	0.3 (515)	89

[a] Data given in the paper of Brewen and Preston (57).
[b] Estimated from the data of Scott and Bigger (502); x irradiation at 100 rad/min.
[c] Data of Bajerska and Liniecki ((20) and personal communication); x irradiation at 18 rad/min.
[d] Data of Lyon and Cox (309), x irradiation at 88 rad/min.
[e] Data of van Buul (70).
[f] Number of cells in parentheses.

TABLE 44. COMPARISON OF INTRA-LOCUS FORWARD MUTATION RATES PER HAPLOID GENOME IN DIFFERENT ORGANISMS[a]

Low-LET radiation

Test system	Haploid DNA content (pg)	Loci and cell stages where mutations were studied	Mutation rate per locus (rad^{-1})	References: DNA content	References: Mutation rate
Hordeum vulgare (barley)	6.7 (10.0, 11.5)[b]	44 eceriferum loci	$3.0\ 10^{-9}$	440, 441	395, 442
		59 eceriferum loci	$2.2\ 10^{-9}$		443
		26 erectoides loci	$1.4\ 10^{-8}$		395
		86 chloroplast loci	$1.3\text{-}1.7\ 10^{-8}$		444, 445
		130 chloroplast loci	$0.85\text{-}1.2\ 10^{-8}$		443, 444
		1000 chloroplast loci (estimated)	$1.1\text{-}1.5\ 10^{-9}$		443
		1 locus (DDT resistant)	$1.7\ 10^{-8}$		444, 446
Zea mays (maize)	5.5	1 locus (bronze)	$<2.3\ 10^{-8}$	440, 441	447
Mus musculus (mouse)	3.0 (2.26)[b]	Spermatogonia			
		7 loci	$2.2\ 10^{-7}$[c]	448	195, 419
		7 loci minimum	$8.1\ 10^{-8}$[d]		see text
		6 loci	$7.8\ 10^{-8}$		449
		Maturing oocytes			
		7 loci, 50 R	$1.78\ 10^{-7}$[e]		195, 419
		200 R	$3.95\ 10^{-7}$		195, 419
		200 R	$1.78\ 10^{-7}$		211
		400 R	$5.50\ 10^{-7}$		195, 419
		Immature oocystes			
		7 loci, 50 R	≈0		195, 419
Arabidopsis thaliana (cruciferous plant)	0.27	4 thiamine loci	$1.1\text{-}1.5\ 10$	440	450

TABLE 44 *(continued)*

Test system	*Haploid DNA content (pg)*	*Loci and cell stages where mutations were studied*	*Mutation rate per locus (rad^{-1})*	*References*	
				DNA content	*Mutation rate*
Drosophila melanogaster (fruit fly)	0.14	Spermatogonia			
	0.18	8 chromosome III loci	$1.28\ 10^{-8}$	433, 448 451	405
	(0.17, 0.22)[b]	10 oogonia			
		10 X-chromosome loci	$0.5\text{-}1.54\ 10^{-8}$		452
		Post-meiotic sperm			
		8 chromosome III loci	$4.7\ 10^{-8}$		405
			$3.0\ 10^{-8}$		453, 454
		4 X-chromosome loci	$2.9\ 10^{-8}$		455
		5 loci	$1.4\ 10^{-8}$		456
		5 loci	$1.2\ 10^{-8}$		
Neurospora crassa (mold)	0.038	2 adenine-3 loci	$3.0\ 10^{-9}$	457	458
	(0.042)[b]	*(ad-3A, 3B)*	$5\text{-}6\ 10^{-9}$		459
		1 aza-3 locus	$2.0\ 10^{-8}$		410
		All 3 loci considered	$8.7\ 10^{-9}$		410, 458
		All 3 loci considered	$1.0\ 10^{-8}$		410, 459
Saccharomyces cerevisiae (yeast)	0.008	6 early adenine loci	$6.0\ 10^{-9}$	425, 460	416
	0.014, 0.015	1 can-1 locus	$1.6\ 10^{-9}$		358
	(0.024)[b]	1 can-1 locus	$1.0\ 10^{-10}$ (maximum)		411
Schizosaccaromyces pombe (yeast)	0.012, 0.015(1C)	5 early adenine loci	$0.8\text{-}1.1\ 10^{-9}$		414
	0.023, 0.029(2C)	5 early adenine loci	$0.4\text{-}0.8\ 10^{-9}$ [f]	461, 462	415

Source: Reference 562.

[a] The intra-locus rates given here must be viewed in conjunction with the differences between systems in their resolving power to detect intragenic change.

[b] DNA values given by Abrahamson *et al.* (4).

[c] This rate is not intragenic.

[d] For the mouse, except for the rate $8.1\ 10^{-8}$, the rates given may not all represent intra-locus rates.

[e] The rates obtained in the radiation experiments were corrected using the spontaneous rate of $1.4\ 10^{-6}$ per locus (589).

[f] The two ranges of mutation rate estimates were obtained by taking the rates given by the respective authors and dividing by 2 to allow for the fact that cells with 2C DNA amounts were treated.

TABLE 45. INTRA-LOCUS MUTATION RATE ESTIMATES FOR THE INDUCTION OF SPECIFIC LOCUS MUTATIONS IN POST-MEIOTIC MALE GERM CELLS OF *DROSOPHILA MELANOGASTER*, CYTOLOGICAL POSITION OF THE LOCI AND ESTIMATES FOR THE SIZE OF THE BANDS IN THE SALIVARY CHROMOSOME

Locus[a]	*Mutation rate per locus (R^{-1})*	*Salivary band position*[b]	*Estimated relative size of the bands from 1 = small to 5 = large*
g	$5.0\ 10^{-8}$	12B6-7±	1
f	(between that of	15F1-2	4
rb	*g* and *w*)	4C6-8	2
y		1B1	2[c]
w	$2.5\ 10^{-8}$	3C2 or 3C1.5	1[c]
ct[d]	(between that of	7B3 or 4	1
sn	*w* and *pn*)	7D1-2	4
v		10A1	5
cm		6E6	1
pn	$9.0\ 10^{-9}$	2D6-2E1	?
m	(between that of *pn* and *car*)	1OE1 or 2	3 or 4
car	$3.3\ 10^{-9}$ ($2.0\ 10^{-8}$)[d]	18D1-2	4
ras	$1.6\ 10^{-9}$ ($6.8\ 10^{-8}$)[d]	9E4-7	1
ac	$\approx 1.6\ 10^{-9}$	1B2	2[c]
sc		1B3-4	5

Sources: References 275, 593.

[a] The loci are ranked in descending order of mutation rate.

[b] Salivary band positions are from Lefevre (275), who in a personal communication makes the following amendment: *rb*, *cm* and *car* are not exactly where they are supposed to be.

[c] According to Lefevre (274), there appears to be more than a single complementation group associated with at least some of the bands in 1B. In addition, Lefevre (personal communication) points out that *y* and *ac* are the sole occupants of 1B1-2 and that cytogenetic analysis in collaboration with Garcia-Bellido suggests that *sc* is a duplicated gene which occupies 1B3-4. The possibility that *w* is associated with a very faint band between 3C1 and 3C2 has been discussed by Sorsa, Green and Beerman (546).

[d] In consideration of the arguments of Muller (351) that at *ct*, *car* and *ras* most of the mutations unaccompanied by chromosome aberrations are male lethal (and may therefore be considered as true gene mutations giving a lethal effect), the intra-locus rates given in parentheses include these mutations as well; for the *ct* locus the rate is $3.3\ 10^{-8}$.

TABLE 46. FACTORS WHICH POSSIBLY DISTURB THE EXACT DETERMINATION OF MUTATION FREQUENCIES IN CULTURED MAMMALIAN CELLS

Phenomena involved	*Factors possibly disturbing the exact determination of mutation frequencies*	*Factors as such relevant for selection of HG-PRT-deficient mutants in*		
		Human diploid skin (fibroblastic cells)	*V79 Chinese hamster (epithelioid cells)*	*L5178Y mouse lymphoma (cells in suspension)*
A. Recovery of mutants				
Growth	Occurrence of spontaneous mutants in course of experiment	+	+	+
	Differential growth rate of wild-type cells and fully expressed mutants	–	–	–
	Differential growth rate of wild-type cells and newly induced mutants	+	+	+
Cloning efficiency	Differential cloning efficiency of wild-type cells and fully expressed mutants	–	–	–
	Differential cloning efficiency of wild-type cells and newly induced mutants	+	+	+
	Absolute cloning efficiency of wild-type cells	+	–	+
Metabolic co-operation	Humoral metabolic co-operation	–	–	–
	Intraclone metabolic co-operation	+	+	+
	Interclone metabolic co-operation	+	±	–
B. Determination of expression time				
Rate of decrease of wild-type gene products	Dilution of normal gene products by cell division	+	+	+
	Rate of turnover of normal gene products	+	+	+
Treatment of cells	Dose-dependent reduction in growth rate	+	+	+
	Delay in mutation induction			

Source: Reference 538.

TABLE 47. RISK OF INDUCTION OF VARIOUS KINDS OF GENETIC DAMAGE IN MAN PER 1 RAD AT LOW DOSES OR AFTER CHRONIC EXPOSURES

Estimated in 1972

End-point	*Expected rate of induction per million*		*Expression in F_1 per million conceptions after spermatogonial irradiation*
	Spermatogonia	*Oocytes*	
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[k]
6. Other chromosome anomalies	Very low	–	Very low
Total genetic damage	1 521[g] (57)[h]		
Total genetic damage[i]	300		6-15[j]

Source: Reference 589.

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 end-point 1 above (see paragraph 594 of Annex E of reference 589).

[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 of Annex E of reference 589).

[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.

[k] After oocyte irradiation.

TABLE 48. ESTIMATED EFFECT OF 5 RAD PER GENERATION ON A POPULATION OF ONE MILLION, INCLUDING CONDITIONS FOR WHICH THERE IS SOME EVIDENCE OF A GENETIC COMPONENT

Disease classification	*Current incidence*	*Effect of 5 rad per generation*	
		First generation	*Equilibrium*
Dominant diseases	10 000	50-500	250-2 500
Chromosomal and recessive diseases	10 000	Relatively slight	Very slow increase
Congenital anomalies	15 000		
Anomalies expressed later	10 000	5-500	50-5 000
Constitutional and degenerative diseases	15 000		
Total	60 000	60-1 000	300-7 500

Source: Reference 34.

TABLE 49. RISK OF INDUCTION OF VARIOUS KINDS OF GENETIC DAMAGE IN MAN PER 1 RAD AT LOW DOSE RATES OR LOW DOSES OF LOW-LET IRRADIATION

End-point	*Expected rate of induction per million gametes resulting from irradiation of*		*Expression in first generation per million births*
	Spermatogonia	*Oocytes*	
1. Autosomal mutations[a]	60	–	20[d]
2. Dominant visibles[b]	Very low	–	
3. Skeletal mutations[c]	4	–	
4. Balanced reciprocal translocations[e]	17-87	Low	Low[f]
5. Unbalanced products of end-point 4 above	34-174	–	2-10[g]
6. X-chromosome loss[h]	Very low	Low	Low
7. Other chromosome anomalies	–	–	–

Note: Dashes indicate that inadequate or no information is available.

[a]Presumed to include small deficiencies. Based on rate of induction of mutations in mice that are lethal in the homozygous condition, which is doubled to give the overall rate (para. 611).

[b]Based on those scored in the course of specific-locus experiments in mice.

[c]Detected in mice by dominant effects.

[d]Overall rate of dominant effects, based on skeletal mutations and presumably including dominant visibles and heterozygous effects of autosomal mutations.

[e]Derived from human and marmoset cytogenetic data under the assumption that the frequency of translocations in the F_1 progeny is one fourth of that observed in spermatocytes.

[f]Effects such as those given for end-point 5 in the next footnote will become manifest in generations following the first.

[g]Expressed as congenital malformations. In addition, there would be 11-55 recognized abortions and 22-109 early embryonic losses.

[h]Detected in mice by X-chromosomal markers.

TABLE 50. ESTIMATED EFFECT OF 1 RAD PER GENERATION OF LOW-DOSE, LOW DOSE-RATE, LOW-LET IRRADIATION ON A POPULATION OF ONE MILLION LIVE-BORN INDIVIDUALS

Assumed doubling dose, 100 rad

Disease classification[a]	*Current incidence*[b]	*Effect of 1 rad per generation*	
		First generation[c]	*Equilibrium*
Autosomal dominant and X-linked diseases	10 000[d]	20	100
Recessive diseases	1 100	Relatively slight	Very slow increase
Chromosomal diseases	4 000[e]	38[f]	40
Congenital anomalies Anomalies expressed later Constitutional and degenerative diseases	90 000[g]	5[h]	45[h]
Total	105 200	63	185
Percentage of current incidence		0.06	0.17
Recalculated BEIR assessments			
Autosomal dominant and X-linked diseases	10 000	20	100
Recessive and chromosomal diseases	10 000	Relatively slight	Very slow increase
Congenital anomalies Anomalies expressed later Constitutional and degenerative diseases	40 000	2-20[i]	20-200[i]
Total	60 000	25-40[j]	125-300[j]
Percentage of current incidence	100	0.04-0.07	0.21-0.50

[a] Follows that given in the BEIR Report (34).

[b] Based on the results of the British Columbia Survey with certain modifications; see table 9.

[c] The first generation incidence is assumed to be about one fifth of the equilibrium incidence for autosomal dominant and X-linked diseases; for those included under the heading "congenital anomalies etc." it is one tenth of the equilibrium incidence. For rationale see reference (34).

[d] See table 9.

[e] Based on the pooled values cited in Nielsen and Sillesen (363); includes mosaics but excludes balanced translocations.

[f] The first generation incidence is assumed to include all the numerical anomalies and three fifths of the unbalanced translocations (the remaining two fifths being derived from a balanced translocation in one parent).

[g] Includes an unknown proportion of numerical (other than Down's syndrome) and structural chromosomal anomalies.

[h] Based on the assumption of a 5 per cent mutational component.

[i] The range reflects the assumption of 5 and 50 per cent mutational components; see text for explanation.

[j] Rounded-off figures.

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