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, $(1.48 \pm 0.13)\ 10^{-4}$ rad^{-1} compared with $(0.94 \pm 0.07)\ 10^{-4}$ rad^{-1} in guinea-pigs and $(0.93 \pm 0.09)\ 10^{-4}$ rad^{-1} in the golden hamsters; definite plus possible translocations: rabbit, $(1.70 \pm 0.14)\ 10^{-4}$ rad^{-1} compared with $(1.10 \pm 0.10)\ 10^{-4}$ 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, $(0.93 \pm 0.07)\ 10^{-4}$ 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 nave 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

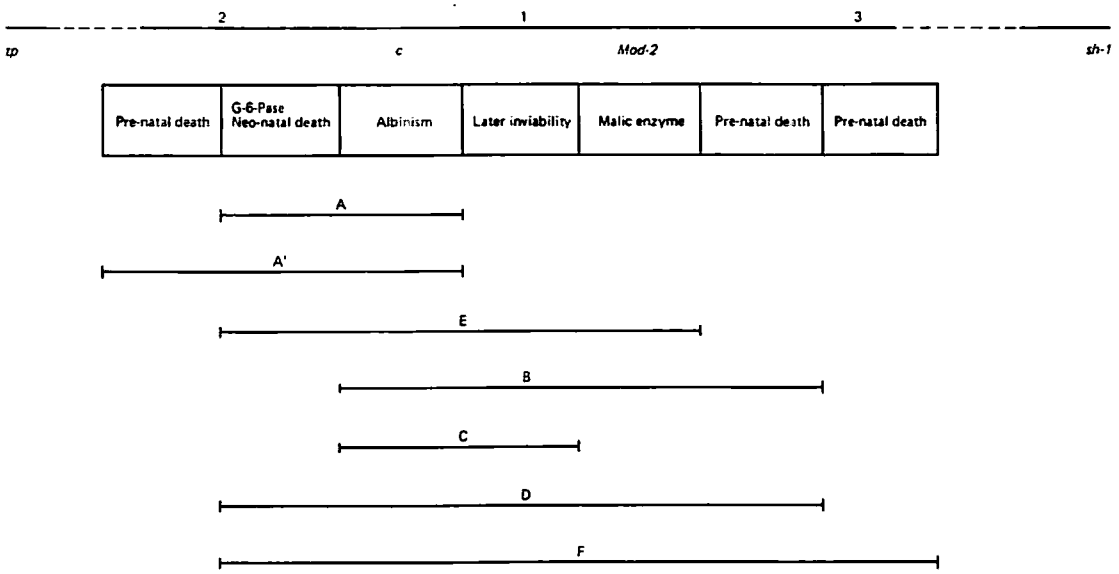
311. The 1972 report considered in some detail the work of L. B. Russell (453), who analysed, by means of complementation tests, the mouse chromosome 9 region between and surrounding *d*, *se* and *sv* (recombination frequency *d-se*, 0.16 per cent; *se-sv*, 2 per cent). This analysis revealed the existence 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:

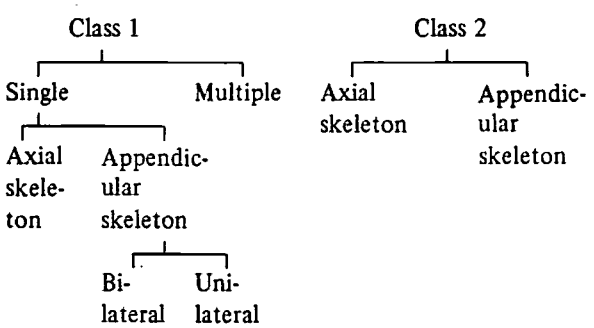
(a) For the evaluation of genetic radiation hazards to a population, it is desirable to obtain more information on the frequency of induced dominant mutations;

(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.