Symposium on Radiation Genetics : XIII International Congress of Genetics

NATURE AND CONSEQUENCES OF INDUCED CHROMOSOME DAMAGE IN MAMMALS

A. G. SEARLE

Medical Research Council, Radiobiology Unit, Harwell, Didcot, Berks., U.K.

ABSTRACT

There are marked qualitative and quantitative differences in the patterns of chromosomal damage observed after irradiation of spermatogonia, spermatozoa and oocytes of mice. These differences often result from reduced or zero transmission of particular classes of abberration arising in pre-meiotic germ cells. Probably this is the reason why the level of *X*-chromosomal and autosomal monosomy is not increased after spermatogonial irradiation. Similarly, the reduced transmission of certain *d–se* deficiencies may help to explain their low F_1 frequency after pre-meiotic as compared with later irradiation. Spermatozoal irradiation has revealed no Robertsonian translocations, but has produced some types of reciprocal translocations which apparently are not transmitted to the F_1 after spermatogonial treatment because they prevent maturation of the male pre-meiotic germ cell. Thus they cause sterility in males, but not in females. They include *X*-autosome and *Y*-autosome translocations, those giving a metacentric or sub-metacentric chromosome (with reciprocal product present) and those in which one break-point is in or near the centromeric heterochromatin while the other is more distally placed. This last group (which grades into male sub-fertile conditions) gives a preponderance of chain configurations (often with one separate univalent) in heterozygotes of both sexes at meiosis and a high incidence of somatic marker chromosomes. Nondisjunction associated with the univalent generates tertiary trisomics, which are usually male-sterile also and may show phenotypic abnormalities. Sterile males with complete separation of *X* and *Y* chromosomes have also been reported after mutagenic treatment of meiotic and post-meiotic germ cells. Such separation seems to prevent a primary spermatocyte from forming a secondary one. The usual derivation (in mouse and man) of tertiary trisomics from mothers rather than from fathers may be due to a similar block, together with a general tendency for male heterozygotes for the parental balanced translocation to be sterile or sub-fertile. Mature oocytes tend to resemble spermatoza in the types of aberration produced by irradiation, which include the male-sterile translocation, but more data are needed. Many of the aberrations described contribute to the human cytogenetic load and can be studied in the mouse from this point of view.

THE foundations of all we now know about the induction of structural changes in mammalian chromosomes were laid by HERTWIG, SNELL and others some forty years ago. In 1935, for instance, the term "semi-sterile" was coined by SNELL, to describe those progeny of irradiated animals which consistently produced small litters. SNELL realized that this phenomenon was due to the presence of a reciprocal translocation and made a thorough study of its genetics, while KOLLER and AUERBACH (1941) succeeded in demonstrating its cytological effects

at meiosis. At an early date also it was discovered by HERTWIG (1938) that semi-sterility and full sterility in F_1 males were particularly associated with those offspring resulting from treatment of postmeiotic stages (the so-called *Frühprobanden*) rather than pre-meiotic ones. Dominant lethality induced by *X*-irradiation of these post-meiotic stages was also studied at this period and in 1937 BRENNEKE was able to demonstrate the existence of micronuclei in early embryos sired by irradiated males.

In postwar years, technical advances led to an upsurge of interest in human chromosomal anomalies and a realization of how significant and serious a component of the genetic load these were. The need for related studies in experimental mammals was obvious, especially when it was realized that reciprocal translocations could be induced in spermatogonia and were responsible for most of the overt genetic damage in the next generation (GRIFFEN 1958; LYON, PHILLIPS and SEARLE 1964). Translocations have been much studied in recent years but information is also available on aneuploidy, deficiencies and inversions. The last will not be dealt with here, in view of RODERICK's (1971) review.

Nondisjunction and chromosome loss

Specific locus experiments have provided evidence on some types of chromosomal damage induced in the spermatogonia and oocytes. The original stock (RUSSELL 1951) carries linked genes, namely c^{ch} and *p* on chromosome 7[1] (14 units apart) and *d* and *se* on chromosome *9* (only 0.2 units apart). While no simultaneous c^{ch}–*p* mutations have been reported, two sorts of simultaneous *d*–*se* mutations have been distinguished by RUSSELL and RUSSELL (1960). These comprise (i) presumptive deficiencies covering both loci and transmitting a lethal effect and (ii) non-transmitters of lethal or deleterious effects, behaving exactly as if homozygous for the specific locus alleles. RUSSELL and RUSSELL postulated that these "zero-transmitters" were the result of nondisjunction in both parents, thus receiving two chromosomes *9* from the tester stock carrying *d* and *se* but none from the wild type control or treated parent. This seems the most satisfactory explanation, although other mechanisms of chromsome loss besides nondisjunction may operate in the wild type stock. All four simultaneous *d*–*se* mutations in the Oak Ridge control series were of the zero-transmitting type, but of two found in Harwell controls (CARTER, LYON and PHILLIPS 1958) one was of the zero-transmitting and one of the deficiency type (see SEARLE 1974). The only other definite zero-transmitter in RUSSELL and RUSSELL's *original series* occurred after irradiation of oocytes. Thus there was no evidence for induction of chromosome loss in males and little evidence in females. Data in males came mainly from spermatogonial irradiation, in which autosomal and *X*-chromosome loss presumably act as cell lethals. Autosomal monosomy in metaphase I spermatocytes seems to be very rare after spermatogonial irradiation. Moreover, the low level of extra embryonic lethality after such irradiation mainly arises from unbalanced gametic products of translocation induction (LYON, PHILLIPS and SEARLE 1964; FORD *et al* 1969). The fate of spermatogonial cells lacking a *Y*

[1] Nomenclature recommended by COMMITTEE ON STANDARDISED GENETIC NOMENCLATURE FOR MICE (1972).

chromosome is less clear-cut. A clone of presumptive *XO* spermatocytes was found after early embryonic irradiation (SEARLE and PHILLIPS 1971) and sex-reversed (*Sxr*) XO male mice can produce spermatozoa (unlike *XX* males) although these were grossly abnormal (CATTANACH, POLLARD and HAWKES 1971). EVANS, FORD and SEARLE (1969) found no *XO* cells in spermatocytes of an *XO/XYY* male and thought this was due to adverse selection. Such a tendency would help to explain why RUSSELL and MONTGOMERY (1970) found no significantly increased incidence of *XO* offspring after spermatogonial irradiation of mice, in contrast to positive findings after exposure of meiotic and post-meiotic stages (RUSSELL and SAYLORS 1963; RUSSELL and MONTGOMERY 1966).

From these results we can deduce that in the mouse (i) chromosomes differ in the extent to which they undergo spontaneous loss or nondisjunction, (ii) the spontaneous frequency of *d–se* deficiencies is low in males, (iii) any chromosome losses produced in spermatogonia are not normally transmitted to the next generation. It seems doubtful whether complete trisomy (the best evidence for nondisjunction) is induced by spermatogonial irradiation to any marked extent, since an extra chromosome is only very rarely found in mouse spermatocytes at metaphase I or II after gonial irradiation or in controls (EVANS 1973). Much more information is required, however, before we can know to what extent irradiation induces nondisjunction in different mammalian germ cells. The importance of this problem is obvious, in view of the very severe malformations and considerable social load associated with human trisomics.

Deficiencies

The rate of recovery of *d–se* deficiencies from specific locus experiments is lower after X- or γ-irradiation of spermatogonia than of spermatozoa or oocytes, although the effect is less marked after fast neutron irradiation of gonia (see RUSSELL 1971). RUSSELL and RUSSELL (1960) found that *d–se* deficiencies derived from X-irradiated spermatozoa or oocytes often showed markedly reduced transmission, combined with an effect on the size of the heterozygote. From an analysis of litter size and other data the authors concluded that this reduced transmission was probably a gametic rather than a sub-lethal effect. If so, these deficiencies resemble those recessive *t*-locus mutations which show low transmission ratios (see BRADEN 1960, 1972). However, one difference is that transmission ratios are normal in females heterozygous for the *t* alleles but reduced in both male and female carriers of *d–se* deficiencies.

The induction of deficiencies can be inferred at the *c* (albino) locus as well, for a number of lethal albinos "alleles" have been recovered. Since there is evidence that the *c* locus is either the structural or possibly the regulator locus for tyrosinase (FOSTER 1965; HEARING 1973) intragenic mutations should at most lead to complete inactivation of the enzyme, with non-lethal albinism as the consequence. ERICKSON, GLUECKSOHN-WAELSCH and CORI (1968) have found that homozygotes for some of these lethal alleles suffer from hypoglycemia due to a deficiency of glucose-6-phosphatase, an enzyme which metabolically is unrelated to tyrosinase. This suggested that neighbouring loci were involved. The

recent finding by ERICKSON, EICHER and GLUECKSOHN-WAELSCH (1974) that some of these "alleles" involve a deletion of the *Mod-2* locus, 1.1 units from *c*, confirms this belief.

Two of these presumptive *c*-locus deficiencies, one (c^{3H}) induced in foetal female and the other (c^{6H}) in foetal male germ cells, have been studied at Harwell. Neither showed any disturbance of transmission. Nor did a *d–se* deficiency recovered after spermatogonial irradiation and studied by RUSSELL and RUSSELL (1960). RUSSELL (1971) found that *d–se* aberrations tended to be somewhat larger in those groups with a higher total frequency of aberrations (post-gonial stages and oocytes), than in spermatogonia. Perhaps larger deficiencies are also induced in spermatogonia but are not transmitted, or perhaps they are not induced. Either process would help to explain why *d–se* deficiencies are rarer after spermatogonial than after oocyte or post-meiotic irradiation, but RUSSELL and RUSSELL (1960) thought that the difference in frequencies was too great to be due to reduced transmission alone. Work on a range of different deficiencies is needed to solve the many interesting problems connected with them.

Translocations

Turning to reciprocal translocations, we find that the commonest type of multivalent configuration after spermatogonial irradiation is the ring quadrivalent, with chiasmata in all four arms. One recognizable type of chain quadrivalent is particularly rare, namely that involving the *X* chromosome. Only about five percent of the expected number were found in mouse spermatocytes in a series of experiments involving spermatogonial irradiation (SEARLE *et al.* 1971). All reciprocal *X*-autosome translocations known in the mouse cause sterility in the male (see EICHER 1970), as do most recovered in Drosophila also (LIFSCHYZ and LINDSLEY 1972). Moreover, many of the mouse ones lead to failure of spermatogenesis before the onset of meiotic metaphase I (EICHER 1970). If they have an autonomous action on the germ cells carrying them then the resultant cell selection may help to account for the rarity of this type of translocation in metaphase I (RUSSELL and MONTGOMERY 1969). However, there also seems to be a general dearth of *X*-autosome translocations, even after postmeiotic irradiation (LYON and MEREDITH 1966), when germinal selection is unlikely to be operative although zygotic selection may be. RUSSELL and MONTGOMERY (1969) found deleterious effects in females heterozygous for all their translocations involving chromosomes *X* and 7, but these were relatively minor.

LIFSCHYTZ and LINDSLEY (1972) have suggested that *X*-autosome translocations are male-sterile because they interfere with inactivation of the single *X* chromosome in primary spermatocytes, which they regard as a fundamental control mechanism required for normal spermatogenesis. However, many completely autosomal translocations are male-sterile in the mouse (LYON and MEREDITH 1966) and the immediate cause of this sterility is the same as with *X*-autosome ones, namely spermatogenic failure. It is not clear at present how these translocations would interfere with inactivation of the single *X* chromosome, but, as we shall see, they do have some peculiar properties.

The induction of translocations in mouse spermatogonia leads to two main effects in the next generation. The first is extra embryonic lethality, with consequent reduction in litter size. This occurs because the unbalanced products of translocation heterozygotes, arising at meiosis, are transmitted as efficiently as the balanced translocations themselves, and usually lead to intrauterine death (LYON, PHILLIPS and SEARLE 1964; FORD *et al.* 1969). Second, it leads to the presence of "semi-sterile" individuals, giving litters of half normal size, again because of embryonic lethality. The low incidence of male sterility in the F_1 generation after spermatogonial irradiation contrasts with the high incidence after post meiotic germ cell irradiation. Moreover, the sterility after gonial irradiation does not seem to result from translocation heterozygosity (FORD *et al.* 1969) while that after post-meiotic irradiation certainly does, as we shall see. This suggests that wholly autosomal male-sterile translocations induced in gonia fail to be transmitted, like the *X*-autosomal ones. Evidence for a reduced transmission of translocations was obtained by FORD *et al.* (1969), who found that their incidence in F_1 progeny, and the incidence of embryonic lethality, was only half that expected from the frequency of multivalent configurations in spermatocytes of the irradiated fathers. This suggested that many balanced reciprocal translocations (and about the same proportion of unbalanced ones) were somehow eliminated between metaphase I and the next generation.

In view of this selection against certain translocations during spermatogenesis, it is necessary to study the products of spermatozoal (and perhaps oocyte) irradiation in order to obtain a reasonably complete picture of the spectrum of radiation-induced translocations in mammals. We have recently conducted an experiment (SEARLE *et al.* 1974) in which male mice received gonodal X-ray doses of between 0 and 1200 rad, followed by fertility tests and cytological examination of all sons and many daughters. There were no signs of the heterogeneity and dose-response distortion found after spermatogonial irradiation (see LÉONARD 1971), since the frequency of 0, 1, 2 . . . translocations per F_1 mouse showed a good fit to a Poisson distribution, while the dose-response curve was not humped but had a best-fitting exponent of 1.4, similar to that previously found in Drosophila.

Of 531 F_1 males examined, 57 were judged semi-sterile (on the basis of numbers of dead and total implantations in females mated to them) while 40 were diagnosed as sterile. Thirty-five of these were considered to be translocation carriers, while another had a 41, *XYY* constitution, presumably spontaneous in origin.

Cytologically, these male-sterile translocation have shown a number of interesting differences from those which caused semi-sterility. As Table 1 shows, reciprocal translocations involving the *Y* chromosome, and those forming metacentrics because one of the break-points was in a short arm, were only found in the sterile group. LÉONARD and DEKNUDT (1969) had previously reported a sterile male mouse with a *Y*-autosome translocation. No Robertsonian translocations were found in either group. Nor have any been previously reported in mice after irradiation of various germ cell stages (see SEARLE 1972) although some

TABLE 1

*Types of chromosome anomaly found in F_1 males after spermatozoal irradiation**

Anomaly	Number and where found
41, XYY	1 in sterile male
Insertional translocation	1 in sterile, 1 in semi-sterile male
Reciprocal translocations:	
Y-autosome	4 in sterile males
Involving short arm (giving metacentric)	2 in sterile males
Autosomal—single	25 in sterile and 61 in non-sterile males
Autosomal—double	7 in sterile and 6 in non-sterile males
Autosomal—triple	3 in sterile males
Robertsonian translocations	nil

* From FORD (1973) and SEARLE *et al.* (1974).

have been found in unirradiated stocks. GROPP *et al* (1972) have described their widespread existence in feral Swiss mice, including, of course, the well-known tobacco mice with seven pairs of metacentrics. Nevertheless, it may be premature to conclude that the Robertsonian type of change cannot be induced by irradiation. PARKER (1969) has discussed the recovery of half-translocations from stage-7 oocytes of Drosophila, following radiation-induced chromatid interchange. A Robertsonian arrangement could arise as a half-translocation in which both breaks were very near the centromere. Chromatid interchanges have been reported following irradiation of mouse spermatocytes (WENNSTRÖM 1971) but results obtained so far on oocytes suggest that some interchanges might be of the chromosome type.

About half of those males carrying two translocations, and all of those with three, were sterile (Table 1). In order to test to what extent this latter finding was part of a general phenomenon, we have generated a number of other triply heterozygous translocation stocks by crossing singly homozygous ones to a doubly homozygous stock we had previously manufactured. Most, but not all, of the resultant males have proved to be fertile (in the sense of making females pregnant) although testis weights and sperm counts were below normal (A. G. SEARLE and C. V. BEECHEY, unpublished information, 1973). Therefore many translocations probably have slight effects on spermatogenesis, which may be cumulative, while some have extreme effects.

More information on the characteristics of those translocations with extreme effects is given by the 15 sterile males in which single translocation configurations could be seen in metaphase I. In two of these, hardly any metaphase spermatocytes were present. Analysis of the other 13 (Table 2) showed that the proportion of chain configurations was much higher than in spermatocytes of semi-sterile males resulting from spermatozoal irradiation or in affected spermatocytes of semi-sterile males resulting from spermatozoal irradiation or in affected spermatocytes from spermatogonial irradiation. The percentage of chains with univalents was also increased. This agrees with the original observation of LYON and MEREDITH (1966) that the five male-sterile translocations they recovered

TABLE 2

Analysis of single quadrivalent configurations induced by X-rays in male mice

Exposed germ-cells	Observed derivatives	Number examined	Percent chains	Percent of chains showing III + I	Source of data
Spermatogonia	Spermatocytes	935	42.9	16.0	EVANS *et al.* (1970) SEARLE *et al.* (1972 a, b)
Spermatozoa	Semi-sterile F_1 ♂♂	61*	40.9	7.6	FORD *et al.*, (unpublished)
Spermatozoa	Sterile F_1 ♂♂	13*	90.6	27.1†	FORD *et al.*, (unpublished)

* 50 or 100 cells from each (2 steriles with few MI cells excluded).
† Omitting one with very small element (classification uncertain).

from spermatozoal irradiation had a much higher frequency of chain configurations and of univalents than were found in semi-sterile males. The same marked tendency showed in the results of LÉONARD and DEKNUDT (1968), analyzed by SEARLE (1971). It is interesting to note that CHANDLEY *et al.* (1972) have reported azoospermia in a man heterozygous for a reciprocal translocation giving chain configurations (including III and I) in all 22 primary spermatocytes examined. However, another azoospermic male had ring quadrivalents.

In our data, there was a marked tendency for a positive correlation between severity of effect and percentage of chains. Thus the percentage rose from only 12 in the single sterile male with a normal sperm count to 85 in five with a reduced sperm count and 99 in nine with a sperm count of nil. *Y*-autosome translocations and metacentrics were only found in this last group, which was noteworthy also in the frequent presence of somatic marker chromosomes, usually both long and short.

Somatic marker chromosomes, and *Y*-autosome translocations, were also found in the most severely affected group of sterile males, namely those in which spermatogenesis had apparently ceased before meiotic metaphase I. CATTANACH, POLLARD and ISAACSON (1968) and CACHEIRO (1971) had previously reported translocations giving this type of sterility after mutagenic treatment, while LYON and MEREDITH (1966) found very few metaphase spermatocytes in some of their male-sterile translocations. CACHEIRO also reported that 10 out of 16 sterile males in which diakinesis was not reached had abnormally long and/or abnormally short chromosomes, a much higher proportion than would be expected with semi-sterile translocations. CATTANACH, POLLARD and ISAACSON found the same tendency in their material.

Let us see what conclusions can be drawn from these various findings about the nature of male-sterile translocations in the mouse. Their very high frequency of chains, implying failure of association, suggests that one or both break-points lie near the ends of chromosomes (FORD 1972). The sterility of metacentrics, the frequent occurrence of univalents (III + I association) and of long and short somatic markers all suggest that the proximity of one break-point to a centromere, and the other to the more central or distal part of a chromosome, is particularly likely to generate male sterility by spermatogenic failure (Figure 1). If both

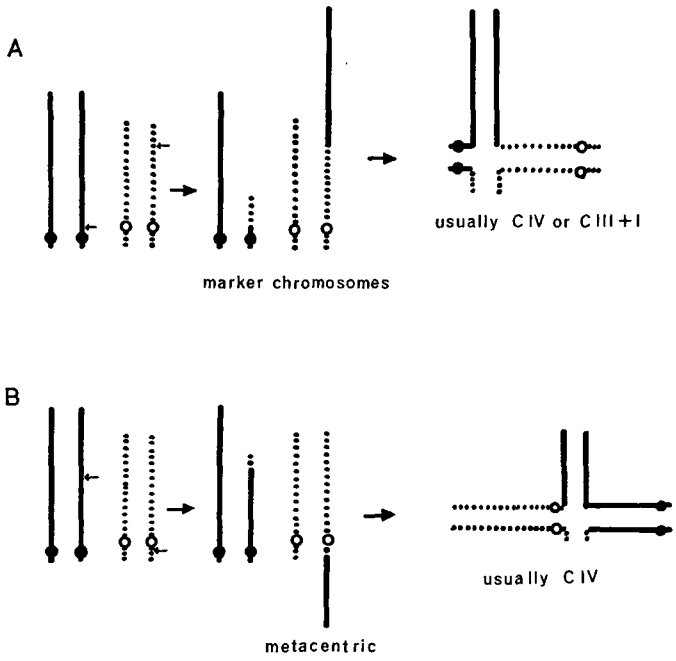

FIGURE 1.—Break-point positions (small arrows) and resultant mitotic and meiotic configurations giving male-sterile reciprocal translocations. A: both break-points in long arm. B: one break-point in short arm.

break-points were proximal or both were distal, failures of association would tend to occur in opposite arms, giving a high frequency of bivalents rather than chains. There is no evidence that this type, which would not give somatic marker chromosomes, is associated with male sterility, for the frequency of bivalents is very similar in translocation heterozygotes with few or no spermatozoa and in those with normal sperm numbers (see SEARLE 1971).

In order to obtain more stocks of male-sterile translocations which could be investigated further, semi-sterile daughters of irradiated males were tested to see if they produced sterile sons. This method, used previously by LYON and MEREDITH (1966), proved successful, yielding twelve such stocks, nine of which are being kept for further study (SEARLE and BEECHEY 1973) by banding methods, linkage tests and crosses to other translocations.

This series of male-sterile translocation stocks has provided further evidence in support of the proximal–distal break-point idea. Eighty percent showed somatic marker chromosomes and a sub-metacentric chromosome was formed in one. Giemsa banding studies, using the trypsin method of SEABRIGHT (1972), have revealed that in at least two of the translocations the constitutive heterochromatin lying around the centromere is directly affected. In one of our mouse translocations, practically all of chromosome *16*, with most of its centromeric heterochromatin, has been translocated on to another chromosome. DEV *et al.* (1974) have recently found essentially the same phenomenon in T (**10, 13**)*199H*, one of

LYON and MEREDITH's male-sterile translocations. In addition, there is a deficiency of C-band material in the submetacentric chromosome found in one of our male-sterile stocks, suggesting that some heterochromatin has been translocated with the short arm.

The functions of centromeric heterochromatin have been recently discussed by YUNIS and YASMINEH (1972) and by HSU (1974). It is composed of satellite DNA consisting of repetitious sequences (JONES 1970; PARDUE and GALL, 1970) which is located round centromeres and secondary constrictions possibly to confer some strength to these areas, and whose function is to protect vital genes and to maintain proper spatial relationships among the chromosomes for efficient operation of the cell cycle. Thus it provides points of attachment to the nuclear membranes and sites for the recognition and association of homologous chromosomes (see also POLANI 1972). The observations of WOOLLAM, FORD and MILLEN (1966) suggested that synaptonemal complexes and therefore bivalents were attached at both ends to the nuclear membrane in primary spermatocytes, centromeric ends being clustered around the sex vesicle. It is, perhaps, not surprising that disturbances to spermatocyte development should occur when homologous C-bands are attached to synaptonemal complexes of very different lengths, as would be the case when one breakpoint is proximal and one distal. The usual attachments of both ends to the nuclear membrane might become impossible.

Tertiary trisomy

Another type of chromosome anomaly associated with male sterility was found in the male-sterile stocks from semi-sterile females. This was teritary trisomy, in the form of an additional small univalent as well as the normal complement of twenty bivalents. This would not be expected in immediate offspring of males given post-meiotic irradiation, though it might occur after pre-meiotic treatment (see GRIFFEN 1967). In our males it must have occurred by nondisjunction in the mother, presumably associated with the frequent formation of a III + I translocation arrangement (see CATTANACH, POLLARD and ISAACSON 1968).

Such tertiary (or partial) trisomic males were found by LYON and MEREDITH (1966) in their sterile group derived from semi-sterile females and also by CATTANACH (1967) in the well-known *T6Ca* marker translocation (also from spermatozoal irradiation), during his tests for distributive pairing. They tend to show abnormal morphology and behavior as well as spermatogenic failure and are, of course, of great interest as animal models of human trisomy and for the study of effects of gene duplication. Thus, CHANDLEY *et al.* (1972) reported that the azoospermic man with chain configurations, mentioned earlier, had a phenotypically abnormal brother with tertiary trisomy. Because the extra element is likely to be small if it is to remain as a separate univalent (i.e., part of a III + I configuration) at the meiosis at which nondisjunction occurs, the translocation concerned is likely to be of the male-sterile type, in mice anyway. It is interesting to note that although *T6Ca* does not fall into this category, heterozygous males are frequently sterile or sub-fertile and the sperm count is low, so that many eggs are unfertilized (CARTER, LYON and PHILLIPS 1955; BARANOV and DYBAN 1968).

Moreover, E. P. EVANS (personal communication) found that 73% of first metaphases showed that CIII + I configuration (see EICHER and GREEN 1972), so a high frequency of non-disjunction is expected, and is indeed found in female heterozygotes.

Tertiary trisomic males are usually sterile but similar females may be fertile or sub-fertile (LYON and MEREDITH 1966; CATTANACH 1967; EICHER 1973). Very recently, DE BOER (1973) has described a tertiary trisomic stock in which both sexes are fertile but have abnormal skulls. This stock was derived from the Harwell translocation *T*(**1**, **13**)*70H* in which males are seldom sterile. It is interesting to note that spermatogenesis tends to be less severely affected in sterile male tertiary trisomics than in the corresponding balanced translocation heterozygote.

In male-sterile stocks, of course, tertiary trisomics are necessarily derived from female translocation heterozygotes. However, EICHER (1973) has reported that even in *T6Ca* all the trisomics she has recovered were derived from maternal heterozygotes rather than paternal ones. The same phenomenon is seen in the foetal data on *T6Ca* by BARANOV and DYBAN (1970). Exactly the same puzzling phenomenon has been found in man (HAMERTON 1971) and investigations on the mouse may well reveal its cause. Possibly it is related to the male sterility with azoospermia found in both mice and men when *X* and *Y* chromosomes fail to associate at meiosis (CATTANACH, POLLARD and ISAACSON 1968; CACHEIRO 1971; CHANDLEY and EDMOND 1971; BEECHEY 1973). In males with a high frequency of *X*–*Y* separation at metaphase I, BEECHEY found no signs of the expected aneuploidy in spermatocytes at metaphase II. Probably, therefore, the *X*–*Y* association is necessary for continued maturation of the spermatocyte concerned. Similarly, primary spermatocytes with *autosomal* univalents (either for whole chromosomes or translocated products) may fail to complete their reduction division. Evidence in support of this idea comes from the observations of E. P. EVANS and B. J. WEST (unpublished information, 1973) who found that the frequency of nondisjunctional arrangements at metaphase II in spermatocytes of *T6Ca* heterozygotes was much less than expected from the frequency of III + I configurations at metaphase I.

Female mice

Let us now consider the nature of the transmissible chromosome damage associated with the irradiation of female mammals. As RUSSELL and MONTGOMERY (1966) have shown, there is quite a high rate of induction of maternal *X* chromosome loss after irradiation of mouse mature oocytes (namely 3% after 100R), as well as of the *d–se* deficiencies already mentioned. In their pioneer studies on the extent to which translocations could be induced in irradiated female mice, RUSSELL and WICKHAM (1957) tested sons for fertility after a 400R exposure to the mothers. Only 1 in 319 showed typical inherited semi-sterility but another 2% were completely sterile and others produced very few litters. Some of the sterile males may have carried translocations so the actual translocation frequency may have been higher than 1/319 (0.3%). However, GILLIAVOD and LEONARD (1973) failed to find translocations in 107 sons of female mice given 50R and 78 sons of females given 200R.

In our own studies (SEARLE 1970; A. G. SEARLE and C. V. BEECHEY, 1974) female mice have been given 300 rad X-rays and both sons and daughters have been tested for fertility. None out of 385 sons gave any indication of translocation heterozygosity. However, 9 out of 294 daughters were diagnosed as semi-sterile on the basis of low litter size. Follow-up studies have revealed the presence or probable presence of translocations in four of these, although in only three could the diagnosis be made with complete confidence. One of these gave five sterile sons, either with an autosomal translocation or with tertiary trisomy. Banding studies have shown that the two break-points were proximal and distal, as with male-sterile translocations derived from spermatozoal irradiation.

Cytological examination of oocytes at diakinesis-metaphase I taken from female mice exposed to radiation one or more days earlier has revealed the presence of ring and chain quadrivalents (GILLIAVOD and LÉONARD 1973; A. G. SEARLE and C. V. BEECHEY, 1974). Some appear to be of essentially the same types as those observed in spermatocytes after spermatogonial irradiation, which might suggest that the interchange was between pairs of chromatids, rather than single chromatids. This would contrast with WENNSTRÖM's (1971) conclusions on examining metaphase I spermatocytes one day after irradiation, but further observations are needed to see if a real difference exists.

In conclusion, it can be seen that our knowledge of the different patterns of chromosomal damage which can result from irradiation of mammalian germ-cells has increased greatly in recent years. Germinal selection and cell lethality obviously play an important part in the end result, leading to the elimination of many aneuploids, some deficiencies and the whole group of male-sterile translocations from the germ line after spermatogonial irradiation. Other types of chromosomal male sterility, also reported from man (see FERGUSON-SMITH 1972), have been found in mice after mutagenic treatment of meiotic and post-meiotic germ cells; for instance, *X*-autosome and *Y*-autosome translocations as well as failures of X–Y association. The rarity of chromosomal anomalies causing sterility in females contrasts with their relative abundance in males, and we are beginning to glimpse the possible reasons for this.

I am grateful to C. V. BEECHEY, P. DE BOER, EVA M. EICHER, E. P. EVANS, C. E. FORD and O. J. MILLER for letting me refer to unpublished information.

LITERATURE CITED

BARANOV, V. S. and A. P. DYBAN, 1968 Analysis of spermatogenic and embryogenic abnormalities in mice heterozygous for the chromosome translocation T6. Genetika (USSR) **4**: 70–83. ——, 1970 Correlation between abnormal embryonic development and the type of chromosome abnormalities arising during meiosis in mice that are heterozygous with regard to T6 translocations. Ontogenez **1**: 248–261.

BEECHEY, C. V., 1973 X–Y chromosome dissociation and sterility in the mouse. Cytogenet. Cell Genet. **12**: 60–67.

BRADEN, A. W. H., 1960 Genetic influences on the morphology and function of gametes. J. Cellular Comp. Physiol. Suppl. **56**: 17–29. ——, 1972 T-locus in mice; segregation distortion and sterility in the male. pp. 289–305. In: *The Genetics of the spermatozoon*. Edited by R. A. BEATTY and S. GLUECKSOHN-WAELSCH. Edinburg University.

BRENNEKE, H., 1937 Strahlenschädigung an Mäuse-und Rattensperma, beobachtet an der Frühentwicklung der Eier. Strahlentherapie **60**: 214–238.

CACHEIRO, N. L. A., 1971 Cytological studies of sterility in sons of mice treated with mutagens. Genetics **68**: s9.

CARTER, T. C., M. F. LYON and R. J. S. PHILLIPS, 1955 Gene-tagged chromosome translocations in eleven stocks of mice. J. Genet. **53**: 154–166. ——, 1958 Genetic hazard of ionizing radiations. Nature **182**: 409.

CATTANACH, B. M., 1967 A test of distributive between two specific non-homologous chromosomes of the mouse. Cytogentics **6**: 67.

CATTANACH, B. M., C. E. POLLARD and J. H. ISAACSON, 1968 Ethyl methanesulfonate-induced chromosome breakage in the mouse. Mutation Res. **6**: 297–307.

CATTANACH, B. M., C. E. POLLARD and S. G. HAWKES, 1971 Sex-reversed mice: XX and XO males. Cytogenetics **10**: 318–337.

CHANDLEY, A. and P. EDMOND, 1971 Meiotic studies on a subfertile patient with a ring Y chromosome. Cytogenetics **10**: 295–304.

CHANDLEY, A. C., S. CHRISTIE, J. FLETCHER, A. FRANCKIEWICZ and P. A. JACOBS, 1972 Translocation heterozygosity and associated subfertility in man. Cytogenetics **11**: 516–533.

COMMITTEE ON STANDARDIZED GENETIC NOMENCLATURE FOR MICE, 1972 Standard karyotype of the mouse, *Mus musculus*. J. Hered. **63**: 69–72.

DE BOER, P., 1973 Fertile tertiary trisomy in the mouse. Cytogenet. Cell Genet. **12**: 435–442.

DEV, V. G., D. A. MILLER, J. CHAREN and O. J. MILLER, 1974 Translocation of centromeric heterochromatin in the T(10;13)199H stock of *Mus musculus* and localization of chromosome break points. Cytogenet. Cell Genet. **13**: 256–267.

EICHER, E. M., 1970 X-autosome translocations in the mouse: total inactivation versus partial inactivation of the X-chromosome. Advan. Genet. **15**: 170–259. ——, 1973 Translocation trisomic mice: production by female but not male translocation carriers. Science **180**: 81.

EICHER, E. M. and M. C. GREEN, 1972 The T6 translocation in the mouse: its use in trisomy mapping, centromere localization and cytological identification of linkage group III. Genetics **71**: 621–632.

ERICKSON, R. P., S. GLUECKSOHN-WAELSCH and C. F. CORI, 1968 Glucose-6-phosphatase deficiency caused by radiation-induced alleles at the albino locus in the mouse. Proc. Natl. Acad. Sci. U.S. **59**: 437–444.

ERICKSON, R. P., E. M. EICHER and S. GLUECKSOHN-WAELSCH, 1974 Demonstration in mouse of X-ray-induced deletions for a known enzyme structural locus. Nature **248**: 416–417.

EVANS, E. P., C. E. FORD and A. G. SEARLE, 1969 A 39,X/41,XYY mosaic mouse. Cytogenetics **8**: 87–96.

EVANS, E. P., C. E. FORD, A. G. SEARLE and B. J. WEST, 1970 Studies on the induction of translocations in mouse spermatogonia. III. Effects of X-irradiation. Mutation Res. **9**: 501–506.

FERGUSON-SMITH, M. A., 1972 Human chromosomes in meiosis. Proc. IV Intern. Congr. Human Genet., pp. 195–211. Excerpta Medica. Amsterdam.

FORD, C. E., 1972 Remark in discussion of D. L. LINDSLEY and E. LIFSCHYTZ. (*loc. cit.*)

FORD, C. E., 1973 Personal communication. Mouse News Letter **49**: 42–43.

FORD, C. E., A. G. SEARLE, E. P. EVANS and B. J. WEST, 1969 Differential transmission of translocations induced in spermatogonia of mice by irradiation. Cytogenetics **8**: 447–470.

FOSTER, M., 1965 Mammalian pigment genetics. Advan. Genet. **13**: 311–339.

GILLIAVOD, N. and A. LÉONARD, 1973 Sensitivity of the mouse oocyte to the induction of translocations by ionizing radiation. Can. J. Genet. Cytol. **15**: 363–366.

GRIFFEN, A. B., 1958 Occurrence of chromosomal aberrations in pre-spermatocytic cells of irradiated male mice. Proc. Natl. Acad. Sci. U.S. **44**: 691–694. ——, 1967 A case of tertiary trisomy in the mouse, and its implications for the cytological classification of trisomics in other mammals. Can. J. Genet. Cytol. **9**: 503–510.

GROPP, A., H. WINKLING, L. ZECH and H. MULLER, 1972 Robertsonian chromosomal variation and identification of metacentric chromosomes in feral mice. Chromosoma **39**: 265–288.

HAMERTON, J. L., 1971 *Human Cytogenetics*. Academic Press, New York.

HEARING, V. J., 1973 Tryosinase activity in subcellular fractions of black and albino mice. Nature New Biol. **245**: 81–83.

HERTWIG, P., 1938 Unterschiede in der Entwicklungsfähigkeit von F_1-Mäusen nach Röntgenbestrahlung von Spermatogonien, fertigen und unfertigen Spermatozoen. Biol. Zbl. **58**: 273–301.

HSU, T. C., 1974 Longitudinal differentiation of chromosomes and the possible function of constitutive heterochromatin. Genetics **78**: (In press.)

JONES, K. W., 1970 Chromosomal and nuclear location of mouse satellite DNA in individual cells. Nature **225**: 912–915.

KOLLER, P. C. and C. AUERBACH, 1941 Chromosome breakage and sterility in the mouse. Nature **148**: 501–502.

LÉONARD, A., 1971 Radiation-induced translocations in spermatogonia of mice. Mutation Res. **11**: 71–88.

LÉONARD, A. and G. DEKNUDT, 1968 The sensitivity of various germ-cell stages of the male mouse to radiation induced translocations. Can. J. Genet. Cytol. **10**: 495–507. ——, 1969 Cytological study of a translocation between the Y-chromosome and an autosome in the mouse. Experientia **25**: 876–877.

LIFSCHYTZ, E. and D. L. LINDSLEY, 1972 The role of X-chromosome inactivation during spermatogenesis. Proc. Natl. Acad. Sci. U.S. **69**: 181–186.

LINDSLEY, D. L. and E. LIFSCHYTZ, 1972 The genetic control of spermatogenesis in Drosophila. pp. 203–222. In: *The Genetics of the spermatozoon*. Edited by R. A. BEATTY and S. GLUECKSOHN-WAELSCH. Edinburgh University.

LYON, M. F. and R. MEREDITH, 1966 Autosomal translocations causing male sterility and viable aneuploidy in the mouse. Cytogenetics **5**: 335–354.

LYON, M. F., R. J. S. PHILLIPS and A. G. SEARLE, 1964 The overall rates of dominant and recessive lethal and visible mutation induced by spermatogonial X-irradiation of mice. Genet. Res. **8**: 447–470.

PARDUE, M. L. and J. G. GALL, 1970 Chromosomal localization of mouse satellite DNA. Science **168**: 1356–1358.

PARKER, D. R., 1969 Heterologous interchange at meiosis in Drosophila. II. Some disjunctional consequences of interchange. Mutation Res. **7**: 393–407.

POLANI, P. E., 1972 Centromere localization in meiosis and the position of chiasmata in the male and female mouse. Chromosoma **36**: 343–374.

RODERICK, T. H., 1971 Producing and detecting paracentric chromosomal inversions in mice. Mutation Res. **11**: 59–69.

RUSSELL, L. B., 1971 Definition of functional units in a small chromosomal segment of the mouse and its use in interpreting the nature of radiation-induced mutations. Mutation Res. **11**: 107–123.

RUSSELL, L. B. and C. S. MONTGOMERY, 1966 Radiation-induced sex-chromosome abnormalities in female germ-cell stages of the mouse. Genetics **54**: 358. ——, 1969 Comparative studies on X-autosome translocations in the mouse. I. Origin, viability, fertility and weight of

five T(X;1)'s. Genetics **63**: 103–120. ——, 1970 Sex-chromosome loss induced in mouse spermatogonia by single and fractionated doses of X-rays. Abstr. IV Intern. Congr. Radiation Res. p. 186.

RUSSELL, L. B. and W. L. RUSSELL, 1960 Genetic analysis of induced deletions and of spontaneous nondisjunction involving chromosome 2 of the mouse. J. Cellular Comp. Physiol. **56,** (Suppl.: 169–188.

RUSSELL, L. B., and C. L. SAYLORS, 1963 The relative sensitivity of various germ-cell stages of the mouse to radiation-induced nondisjunction, chromosome loss and deficiencies. pp. 313–342. In: *Repair from Genetic Radiation Damage*. Edited by F. H. SOBELS. Pergamon, Oxford.

RUSSELL, L. B. and L. WICKHAM, 1957 The incidence of disturbed fertility among male mice conceived at various intervals after irradiation of the mother. Genetics **42**: 392.

RUSSELL, W. L., 1951 X-ray-induced mutations in mice. Cold Spring Harbour Symp. Quant. Biol. **16**: 327–336.

SEABRIGHT, M., 1972 The use of proteolytic enzymes for the mapping of structural rearrangements in the chromosomes of man. Chromosoma **36**: 204–210.

SEARLE, A. G., 1964 Genetic effects of spermatogonial x-irradiation on productivity of F_1 female mice. Mutation Res. **1**: 99–108. ——, 1970 Attempts to induce translocation in female mice. p. 194. Abstr. IV Intern. Congr. Radiation Res. ——, 1971 Symposium on Mammalian Radiation Genetics, Summary and synthesis. Mutation Res. **11**: 133–147. ——, 1972 Chromosome damage and risk assessment. pp. 58–66. Proc. IV Intern. Congr. Human Genetics. Excerpta Medica, Amsterdam. ——, 1974 Mutation induction in mice. Advances in Radiation Biology. **4**: 131–207.

SEARLE, A. G. and C. V. BEECHEY, 1973 Personal communication. Mouse News Letter **49**: 28–29. ——, 1974 Cytogenetic effects of x-rays and fission neutrons in female mice. Mutation Res. **24**: 171–186.

SEARLE, A. G. and R. J. S. PHILLIPS, 1971 The mutagenic effectiveness of fast neutrons in male and female mice. Mutation Res. **11**: 97–105.

SEARLE, A. G., C. V. BEECHEY, E. P. EVANS, C. E. FORD and D. G. PAPWORTH, 1971 Studies on the induction of translocations in mouse spermatogonia. IV. Effects of acute gamma-irradiation. Mutation Res. **12**: 411–416.

SEARLE, A. G., C. V. BEECHEY, M. J. CORP. and D. G. PAPWORTH, 1972a A dose-rate effect on translocation induction by X-irradiation of mouse spermatogonia. Mutation Res. **15**: 89–91.

SEARLE, A. G., C. V. BEECHEY, E. P. EVANS and C. E. FORD, 1972b Studies on the induction of translocations in mouse spermatogonia. V. Effects of short-term fractionation. Mutation Res. **15**: 169–174.

SEARLE, A. G., C. E. FORD, E. P. EVANS, C. V. BEECHEY, M. D. BURTENSHAW and H. M. CLEGG, 1974 The induction of translocations in mouse spermatozoa. I. Kinetics of dose-response with acute X-irradiation. Mutation Res. **22**: 157–174.

SNELL, G. D., 1935 The induction by X-rays of hereditary changes in mice. Genetics **20**: 545–567.

WENNSTRÖM, J. 1971 Effect of ionizing radiation on the chromosomes in meiotic and mitotic cells. Commentationes Biologicae **45**: 60.

WOOLLAM, D. H. M., E. H. R. FORD and J. W. MILLEN, 1966 The attachment of pachytene chromosomes to the nuclear membrane in mammalian spermatocytes. Exptl. Cell Res. **42**: 657–661.

YUNIS, J. J. and W. G. YASMINEH, 1972 Model for mammalian constitutive heterochromatin. Advan. Cell Molec. Biol. **2**: 1–46.