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Meiotic Nondisjunction in the Mouse: Methodology for Genetic Testing and Comparison with Other Methods

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Since trisomies produce adverse effects relatively late in development or even postnatally, they are an important component of the array of genetic damages that might be caused by environmental agents. Whole-chromosome aneuploidy (as opposed to breakage-derived aneuploidy) might come about secondarily from crossover depression, or could follow damage to the meiotic spindle or to kinetochores. For *simplicity, the event — by whichever of the mechanisms — is referred to as meiotic nondisjunction (ND).* A genetic method has been devised which is based on the facts that ND involving the sex chromosomes produces mostly viable mice, and that such exceptional animals can be externally recognized by the use of appropriate markers. The method is compared with the following other ND indicators: univalent and/or chiasma frequencies at M I; number of dyads at M II; extra sex chromosomes in spermatids; karyotypes in cleavage, morula, or blastocyst metaphases; and chromosome constitution of mid-gestation embryos. Some of the cytological endpoints are found to be unreliable. Various biological variables (germ-cell stage, sex, age) are examined with a view toward maximizing the chances for detecting induced nondisjunction. While experimental evidence on this question is equivocal, a consideration of the probable ND mechanisms suggests that the early spermatocyte (in stages including the premeiotic S phase) may be a favorable test object. The numerical sex-chromosome anomaly (NSA) method is useful not only in the study of ND but also in detecting breakage-derived chromosome losses induced in females, where the dominant lethal test is not easily applicable.

It has long been known that exposure of mammalian germ cells to radiation or to certain chemicals can cause chromosome breakage and resulting losses and rearrangements of genetic material. The interest in the induction of events (other than breakage) that lead to aneuploidies involving whole chromsomes is of more recent origin. For simplicity, we shall refer to the end results of all such events as nondisjunction. This paper will focus on the detection of germline nondisjunction in mammals by presenting a method and comparing it with others. Some clues about the biological parameters (such as germcell stage, sex, age) that could maximize the chances for detecting induced nondisjunction are derived from a consideration of the mechanisms that might bring about this endpoint.

With respect to potential human misery caused, the trisomies that result from nondisjunction may be considered intermediate between whole-chromosome losses and transmissible rearrangements. Autosomal monosomies from whatever cause lead to embryonic death, probably so early in a pregnancy as to be nonperceptible, and such losses are obviously not transmitted. At the other end of the scale, such rearrangements as reciprocal translocations and inversions can affect many generations, possibly through associated phenotypic effects (*1, 2*), and certainly by producing unbalanced segregants. At an intermediate level of human concern, pure autosomal trisomies, though not transmitted to future generations, in general produce their adverse effects later in development than do monosomies, and some are even compatible with postnatal survival of abnormal children, as in Down's or Edward's syndrome. The spontaneous frequency of autosomal trisomy in human newborns is about 1 in 600, and, of all trisomies, about 1 in less than 300 (*3*).

The interest in determining whether trisomy can be induced by mutagens has led to the development

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of a number of schemes, most of them cytological, for detecting nondisjunction endpoints in laboratory mammals. Because cytological analysis is time-consuming and requires skilled personnel, and because of a number of other considerations (discussed below), we devised, some time ago, a genetic method based on the fact that nondisjunction that involves the sex chromosomes produces mostly viable mice.

Genetic Method for Detecting Sex-Chromosome Nondisjunction

Background

Simple nondisjunction of the sex chromosome can, depending on the pathway, produce the following types: XO, OY, XXX, XXY, XYY. (Successive nondisjunctional events — e.g., in first and second meiotic divisions, or in a premeiotic and meiotic division — which can produce additional constitutions, are presumably very rare and will not be considered here.) Of these five types, four are presumed to survive to an age at which they can be externally recognized in the mouse by the employment of appropriate genetic markers. Detectability of exceptional types results from the circumstance that, in mammals, the genes on only one X chromosome, at random, are active, so that animals carrying two X's with different genetic markers are mosaics (see Fig. 1).

The viability and fertility of the numerical sex-chromosome anomalies (NSA's) are discussed in some detail elsewhere (*4*) and are therefore only briefly summarized here (Table 1). The OY type in the mouse, and probably also in man, dies shortly after the first cleavage. On the other hand, mouse, unlike human XO females, enjoy normal viability, at least subsequent to day 12 of embryonic life. They are fertile, but their reproductive life is curtailed, probably due to a shortage of oocytes. When an XO female is bred, she produces only about 43% as many XO daughters as expected. This deficiency is due either to preferential inclusion in the egg of the single X, or to early embryonic death of XO's conceived, or to a combination of the two. Surviving mouse XO's are morphologically normal.

The XXY and XYY types are males, which, unless genetically marked or cytologically analyzed, are indistinguishable from XY except by their reproductive performance. The testis of XXY mice lacks all spermatogenic elements. On the other hand, XYY can produce small numbers of spermatozoa, which are, however, abnormal. The XXX type has not yet been described in the mouse, but, on the evidence

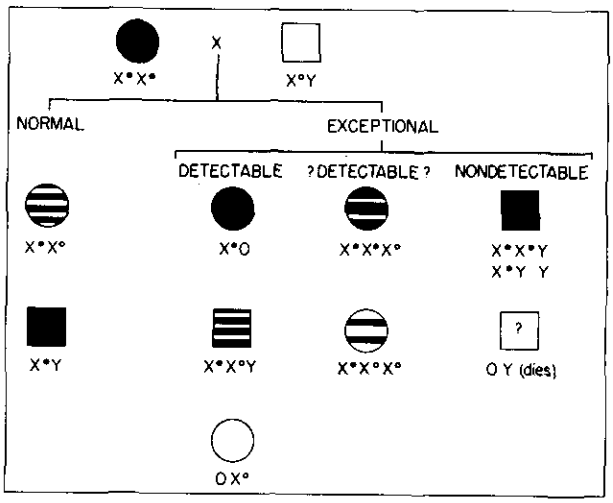

FIGURE 1. Diagrammatic representation of phenotypes in the simplest mating scheme for the detection of numerical sex-chromosome anomalies. Allelic markers on the X chromosome are represented as "black" (X•) and "white" (X°). Females are shown by circles and males by squares. Striping indicates mosaicism, with the relative widths of the stripes representing the approximate proportion of the two cell types in the mosaic.

from other species, may be assumed to survive postnatally, although some human XXX females are small and/or abnormal at birth (*3*).

Any mitotic nondisjunction in the germ line will result in gonads having sectors of cells with NSA's. Available evidence discussed earlier (*4*) indicates that most, if not all, such sectors would be completely or relatively inviable in the testis, although they might survive in the ovary.

Description of the Method

Experimental systems we have developed for the genetic detection of numerical sex-chromosome anomalies (NSA's) utilize X-linked genetic markers that render all of the viable types, except XYY, recognizable on external examination. Any X-linked marker can be used if it produces an external phenotype (e.g., in hair structure or color, ears, eyes) that is clearly different in single-X individuals and X heterozygotes, and if it is compatible with viability. For the simple scheme shown in Figures 1 and 2, matings of $X^{Gs}X^{Gs} \times X^{+}Y$ (or $X^{+}X^{+} \times X^{Gs}Y$) have proved useful. For the more complex scheme (Fig. 3), such combinations as $X^{Gs}X^{+}$ (or $X^{Ta}X^{+}$) $\times$ $X^{le}Y$, or $X^{Gs}X^{Blo} \times X^{le}Y$ may be suggested. The latter will detect XXX exceptions in addition to XXY's; and crossovers, which may complicate the scoring, are relatively rare.

When germ cells are exposed to presumptive mu-

Table 1. Viability and fertility of mice having numerical sex chromosome anomalies.

Sex-chromosome constitution	External sex	Viability	Fertility	Recognizability with markers
OY	—	Dies preimplantation	—	—
XO	♀	Normal after day 12 p.c. <60% mortality before day 12 p.c.	Fertile; some shortage of oocytes.	+
XXY	♂	Probably normal	Sterile; lacks all spermatogenic elements.	+
XYY	♂	Probably normal	Sterile; few and abnormal sperm.	0
XXX	♀	Probably normal	Fertile?	(+)[a]

[a]It is assumed that a mating scheme like that shown in Fig. 3 is used.

tagens during stages when meiotic nondisjunction could theoretically be induced, it is obvious that many other types of genetic damage can also result from the treatment. (Discussion in this paper will be limited to meiotic nondisjunction, and any consideration of pronuclear exposure is therefore omitted.) Some of these, if they involved the sex chromosomes, would yield phenotypes that could mimic those of the NSA's. Figures 2 and 3 show two of these, namely certain translocations [specifically, those T(X;A)'s in which the marker was removed from the influence of X inactivation and became active in all cells], and certain deficiencies (specifically, those deleting the locus opposite the marker). Others are mutations anywhere in the genome that cause sex reversal; deficiencies involving the testis-determining portion of the Y, which would, in effect, also cause sex reversal; and anomalies of fertilization, e.g., polar-body retention. All of these events are presumably quite rare, and their occurrence would be revealed during the follow-up testing (see below) of phenotypically-identified exceptionals.

Even among the NSA's, however, the monosomy class (XO) could have a variety of causes; and though this class is thus of considerable interest in general mutagenesis testing, only the trisomies can be used as a sure sign of the occurrence of meiotic nondisjunction. As shown in Figure 3, some of the trisomy classes distinguish between nondisjunctional events occurring in the first and second meiotic division, although crossing over between the X centromere and proximal marker, followed by nondisjunction at one of the divisions, can produce the same phenotype as nondisjunction of noncrossover chromosomes at the other division. The detectability of nondisjunctional events in the two sexes is summarized in Table 2.

Spontaneous sex-chromosome trisomies are quite rare in the mouse, so that there is little background "noise" for this genetic endpoint in mutagenesis experiments. The results of many investigations (*4*) are summarized in Table 3. It appears on the basis of available information that spontaneous X^MX^M nondisjunction is rarer than X^PY nondisjunction. Whether the same relationship will hold for induced events has not yet been established.

Protocols

In actual practice, once the treatment has been given and the proper matings set up, the detection of presumed exceptionals requires only minimal effort. New litters are checked as soon as the phenotype determined by the marker can be recognized, and all offspring except the small number of presumed exceptionals are discarded at weaning. If exceptionals appear moribund, a tissue sample for cytogenetic followup (usually a piece of external ear) is obtained as soon as possible. These samples can be studied in short-term tissue culture (*5*). Healthy-looking exceptionals can be subjected to genetic tests first, since the outcome of these may eliminate the need for cytological verification.

All male exceptionals are expected to be sterile, unless mosaic, and initial testing may therefore consist merely of the addition of females from any fertile strain. Presumed XXX should be mated to any wild-type male to see whether they produce three types of sons. In experiments scoring for events additional to nondisjunction, presumed XO probands must also be tested. Ideally they should be crossed with a mate carrying an X-linked marker different from any that were used in the experiment. If the simple scheme (Fig. 2) was employed, mothers of OX^P females should be tested to distinguish a *de novo* loss event from a pre-existing maternal XO condition.

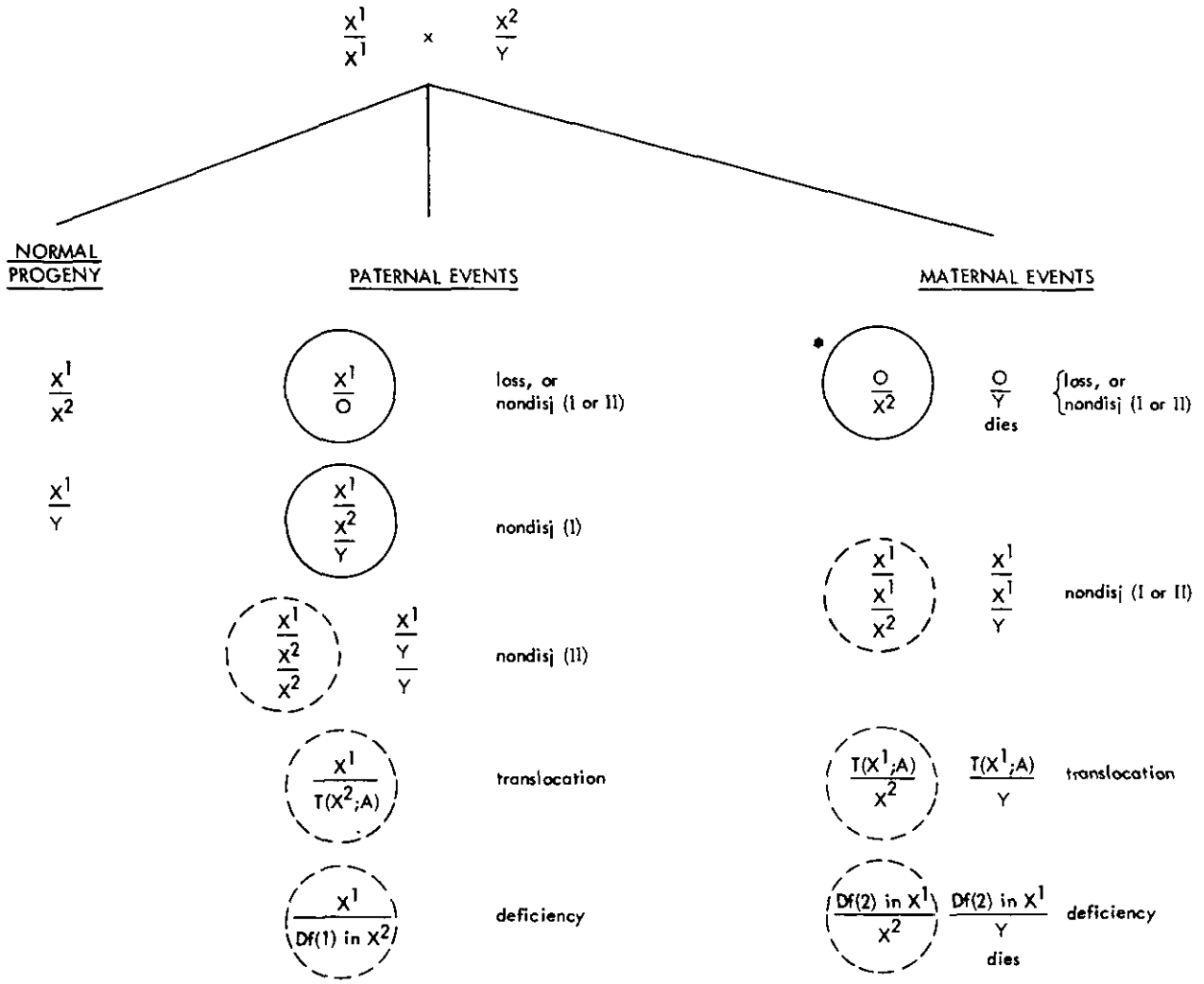

FIGURE 2. Simple mating scheme for detecting induced or spontaneous abnormal events involving sex chromosomes. This scheme is most suitable for experiments involving treatment of male germcells. With precautions, it can also be used to measure induction of X^M losses, but not of maternal nondisjunction. The superscripts 1 and 2 represent distinguishable markers on the X chromosomes: most conveniently, they are a pair of alleles of which either one may be wild type. Exceptional genotypes that are phenotypically recognizable are encircled with an unbroken line. A broken circle indicates those that are only questionably recognizable, or recognizable under certain genetic circumstances. Uncircled genotypes are not phenotypically distinguishable from normal segregants. "I" and "II" indicate the first and second meiotic divisions. Df(1) represents deficiency of the locus at which marker 1 is located, and correspondingly for Df(2). [Note: where 1 and 2 are alleles, this is, of course, the same locus.] — (Discovery of an animal of the type marked * requires further testing to rule out a pre-existing XO condition of the mother.) Reproduced, by permission, from Russell (*4*).

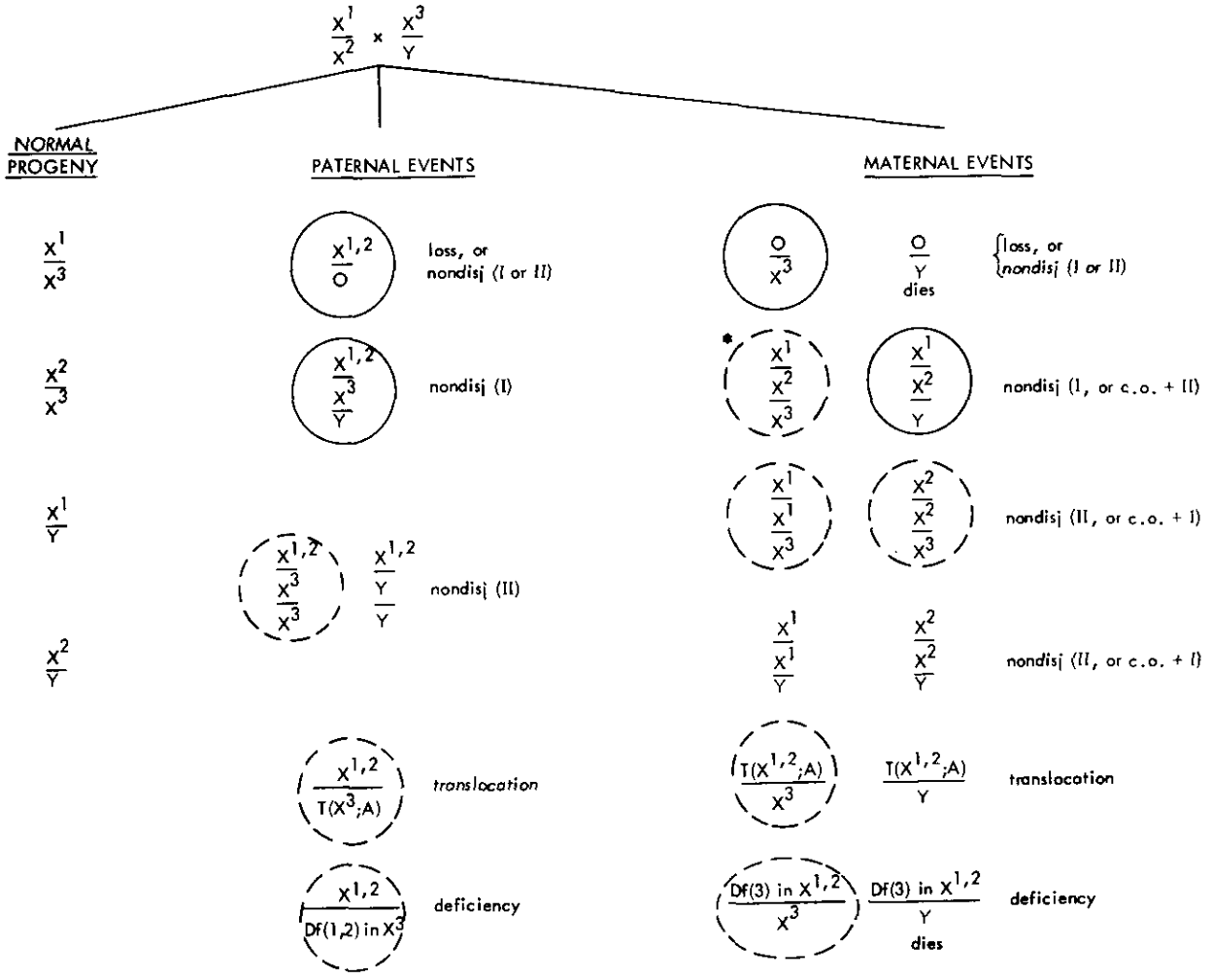

FIGURE 3. More complex mating scheme for detecting induced or spontaneous abnormal events involving sex chromosomes. This is the method of choice for experiments involving treatment of female germ cells but is unnecessarily complex for experiments involving male germ cells. The superscripts 1, 2, and 3 represent distinguishable markers on the X chromosomes: most conveniently, 1 and 2 represent a pair of alleles of which one is wild type. (Where this is not the case, recombination 1 and 2 must also be considered. This has not been done in the figure.) For 1, 2, read: 1 or 2. "I" and "II" indicate the meiotic division at which nondisjunction has occurred, and "c.o." signifies crossing over between X centromere and marker. Broken and unbroken circles and other descriptive symbols as in Fig. 2. The genotype marked by the asterisk (*) will be clearly recognizable where neither 1 nor 2 are wild type, and three appropriate markers are chosen. *Reproduced, by permission, from Russell (4).*

Table 2. Detectability of nondisjunctional events by the numerical sex chromosome anomaly method.[a]

Event	No. of sex chromosomes going to gamete: Zero	Two
Nondisjunction I		
In ♂	1.0	1.0
In ♀	0.5	1.0
Nondisjunction II		
In ♂	1.0	<0.5
In ♀	0.5	<0.5

[a]It is assumed that the mating scheme of Fig. 3 is used.

Table 3. Approximate frequencies of spontaneous occurrence of numerical sex chromosome anomalies[a]

Event	Approximate frequency	
Nondisjunction of X^MX^M	0	(upper 95% conf. limit, 0.04%)
Nondisjunction of X^PY	0.01%	
Noninclusion of X^M	0.05%	
Noninclusion of X^P or Y	0.2 to 0.3	(very variable; can be higher)

[a]Frequencies are most reasonable estimates derived from numerous separate determinations (*4*) and are very approximate.

Events That Can Produce Nondisjunction in Mammals

Mechanisms can be postulated that produce monosomy and trisomy as complementary conditions. Monosomy alone can also have additional causes, such as breakage (breakage-fusion-bridge cycle), lagging, etc.; and trisomy alone might theoretically be caused from extra divisions of specific chromosomes. The discussion in this paper is however restricted only to those mechanisms that produce the complementary types, an event which, for simplicity, we refer to as nondisjunction (ND). A consideration of what these mechanisms might be can provide some clues to the biological parameters that could be used to maximize the chances for detecting induced nondisjunction. While the mammalian material has not yet provided information on mechanisms, some conclusions may be drawn from other species.

Extensive and ingenious studies in Drosophila (*6*) have shown that nondisjunction can be increased secondarily by influences (rearrangements, recombination-defective mutants, heat) whose primary effect is to decrease crossing over. However, the production of homologous univalents does not necessarily lead to nondisjunction, since distributive pairing (which follows exchange pairing) can bring about regular segregation. Distributive pairing probably occurs in man as well as Drosophila (*7*), but no valid attempt has yet been made to find it in the mouse. Nondisjunction does occur when the distributive pool contains univalent heterologs, and the univalents then pair primarily according to size. It may be assumed that agents that decrease crossing over will secondarily cause nondisjunction.

There is also increasing evidence from Drosophila that certain agents can cause damage to the meiotic spindle (*8, 9*) and thus interfere with proper segregation of chromosomes. It is also not inconceivable that there might be agents that affect the kinetochore of chromosomes. Obviously, just which stages are sensitive to induction of nondisjunction by a certain agent would depend on the mechanism by which ND was produced.

Biological Parameters That May Maximize Induced Meiotic ND

Stage and Sex

To the extent that nondisjunction results secondarily from reduced meiotic crossing over, one would expect the period during or prior to the crossing-over process, i.e., during (or prior to) premeiotic DNA synthesis (premeiotic S) (*10*), to be vulnerable to environmental agents that induce nondisjunctional endpoints. Where spindle damage is the primary cause of ND, vulnerability would occur after the meiotic prophase. Kinetochore damage could presumably be induced at any stage.

In mouse germcell production, the interval between the last mitotic division and the end of meiosis is very different in the two sexes. In the male, the entire period occurs during adult life, with the treatment-to-ejaculation interval determining at what stage the exposure was received (*11*). (Oakberg gives intervals to release of sperm from testis. An additional ca. 7 days are required for sperm to reach the ejaculate.)

Sperm exposed during preleptotene (and thus perhaps in premeiotic S) is ejaculated 32-34 days later. In the female (*12, 13*) the first part of the interval occurs during prenatal life. Premeiotic S occurs soon after the midpoint of the 19- to 20-day intrauterine period. Diplotene is reached neonatally, after which the oocyte goes quickly into dictyate and remains thus (through various follicle stages) until about 9 hr before ovulation, when dynamic processes are resumed with the appearance of diakinesis (*14*). The oocyte is in M II at the time of sperm penetration.

What evidence we have on exposure during premeiotic S is scanty. Irradiation of 11- or 13-day female embryos yielded no hyperploid M II's (*15*), and no cases of X^MX^MY in the progeny (*16*), but the number of observations was quite small in each case. In the male mouse, irradiation at "preleptotene" (probably close to leptotene) yielded a significant increase in hypo and hyperploid M II's (*17, 18*). Early primary spermatocytes were the stage most sensitive to the production of extra sex chromosomes in Microtus (*19*). With the NSA system of the mouse, X^PY nondisjunction was observed when spermatocytes were irradiated in the interval that includes preleptotene, though the experiment was not large enough to establish that this period was preferentially sensitive (*4*).

Irradiation of stages that are clearly postrecombinational have yielded equivocal results for cytological nondisjunctional indicators. In the male, pachytene has been found slightly sensitive in M II analysis (*17*); and zygotene, pachytene, and diplotene in M I analysis (*20*). In the female, irradiation of dictyate oocytes in old mice has been claimed to increase nondisjunctional indicators (*21, 22*). In young adult females, irradiation during dictyate, or in the interval between dictyate and diakinesis, failed to cause increases in ND indicators (*23*) or caused increases that were restricted to one group (*24*), or nonsignificant (*22, 25*), or on the borderline of significance (*26*). Certain problems exist with the end-

points studied in these various investigations, and these are discussed further below. For example, it is possible that the aneuploidies seen in M II of mice could be secondary effects of radiation-induced structural rearrangements, rather than resulting from nondisjunction in the sense in which the term is used here.

For irradiation, the results from cytological ND-indicators in mammals are thus not very instructive with respect to revealing sensitive stages. The NSA system yields clear stage-sensitivity results for XO's, but, as discussed, the bulk of these are due to loss, rather than ND, events. In Drosophila, the frequency of nondisjunction induction by x-rays appears not to be stage-dependent (*6*).

Among experiments with other environmental mutagens, only one stage comparison is available for an ND indicator, hyperploidy in M II (*27*). Both the period between late oogonia and pachytene (which entails exposure of embryos) and the "preovulatory stage" (probably diakinesis-metaphase I) were found to be more sensitive than was dictyate to amethopterin, but there was considerable cell selection after the former treatment. Most other experiments with chemical mutagens in females have concentrated on the "preovulatory stage" (*28-33*), which was some time ago shown to be differentially sensitive to dominant-lethal induction (*14*). Structural anomalies and chromosome losses complicate the picture in each case. Chemical mutagenesis experiments in males (*34-37*) have, for the most part, used chronic exposures and/or imperfect ND indicators (univalent frequencies at M I), and are therefore not useful for revealing sensitive stages.

It appears likely that at least some chemical mutagens may be able to induce nondisjunction by one or more of the mechanisms discussed above. For one of the pathways, kinetochore damage, there may be little differential stage sensitivity. The pathway involving spindle damage is probably best tested by exposing during the "preovulatory period" of the oocyte, but the circumstance that this stage is differentially sensitive to chromosome-breakage-related events may complicate the interpretation of results. The pathway involving reduction in meiotic crossing over requires exposure during or before the premeiotic S phase. In the absence of actual experimental evidence on the subject of maximum stage sensitivity, it may be suggested that the early spermatocyte of mammals (in stages including S phase) could be a favorable test object for the induction of meiotic nondisjunction. The oocyte in equivalent stages is not as easily accessible, since treatment at the appropriate stage could entail complications such as embryotoxicity or teratogenicity.

Age

Because of the finding that the frequency of spontaneous trisomy 21 (Down's syndrome) and of other trisomies in man increases with maternal age, it has been suggested that old mouse females might provide sensitive experimental material for the study of nondisjunction. The findings of several investigators indicate that certain spontaneous phenomena that may be related to nondisjunction do become more frequent with advancing maternal age. Thus, there is a decrease in chiasma frequency at diakinesis or metaphase I (*38-41*), and an increase in univalents (*38-40*). An increase with age in hyperploid metaphase II configurations has also been reported (*42*), but this may not continue past middle age (*43*), and since it may be absent altogether, some authors (*40*) have suggested that univalents at meiosis I are, for the most part, artifacts. The age effect on the spontaneous incidence of hyperdiploid F_1 embryos is only on the borderline of significance or otherwise doubtful (*23, 44, 45*). On balance, advancing maternal age does seem to have some influence on spontaneous nondisjunction, but, at least for radiation, appears to produce no differential increase in induced nondisjunction (*21, 23, 46*). Some investigators (*47*) have, in fact, not been able to show any effect of irradiation on trisomy induction in oocytes, even in very old females.

Comparison with Other Test Systems

In chemical mutagenesis studies of induced chromosomal anomalies, *in vivo* mammalian exposure has some obvious advantages over *in vitro* screening and over tests that use nonmammalian species: metabolic alterations of the chemical occur naturally; effects on dynamic systems of reproductive cells can be studied; and chromosomal structure and behavior may be assumed to be relatively similar to those of man. Whether these features outweigh the advantages of other test objects will depend on the specific aims of an investigation. The discussion here has thus been restricted to two types of comparisons: (a) with other measures of germ-line chromosomal damage in mammals; and (b) with other methods for the detection of meiotic nondisjunction in mammals.

Other Measures of Germ-Line Chromosomal Damage in Mammals

Standard tests for this type of damage are dominant lethals and heritable translocations. The second of these detects a type of genetic damage — chromo-

some interchange — that has great significance to human risk (see Introduction), and, for this and other reasons, can not be adequately substituted for by other procedures. The dominant-lethal test is a useful prescreen for detecting chromosomal damage in the male. However, when females are to be exposed, a dominant-lethal effect can be superficially mimicked by physiological damage to the mother. Interestingly, even the absence of damage can be mimicked if the agent induces superovulation, as we found to be the case for radiation (*14*). Time-consuming procedures are required to prove that neither is the case. For these reasons, the NSA test is highly useful for detecting chromosome-loss events in females by scoring for XO's. In the case of some chemicals, hycanthone, for example (*48*), XO induction was the only clear heritable effect that could be detected in the mouse.

Other Methods for Detecting Meiotic Nondisjunction in Mammals

In addition to the NSA system, the following endpoints have been used as "nondisjunction indicators" in mammals: univalent and/or chiasma frequencies at diakinesis or first meiotic metaphase, M I; number of dyads at second meiotic metaphase, M II; extra sex chromosomes in spermatids; karyotypes of cleavage, morula, or blastocyst metaphases; and chromosome constitution of midgestation embryos.

Diakinesis and metaphase I have been studied by a number of investigators, both with respect to chiasma frequency and the incidence of univalents (*20, 24, 34-41*). A number of difficulties with this endpoint should be pointed out. The probable existence of distributive pairing (6) casts doubt on the conclusion that univalents necessarily indicate nondisjunction. Polani and Jagiello (*40*), who compared M I and M II, thought that many univalents were artifacts and that their artifactual nature might be age-related. It has also been suggested (*20*) that radiation-associated increases in apparent univalent frequency might result from radiation-induced "fragility" that causes precocious univalent separation under the hypotonic treatment used in slide preparation. Certainly, lack of association of X and Y at M I is not predictive of nondisjunction. Strains vary in frequency of unassociated sex chromosomes (probably due to differences in the degree of precocity with which the sex chromosomes disjoin), with frequencies of 7-8% (*41*) not being unusual; yet the spontaneous XY or O condition at M II is very rare [none in 1460 cells (*49*), none in 400 cells (*18*)].

Another ND indicator involves dyad counting in metaphase II, and this is being used with increasing frequency (*15, 17, 18, 21, 25-27, 29, 32, 40, 42, 43, 50*). One problem with M II cytology is that the identity of individual chromosomes cannot be adequately established. For example, hyperploidy resulting from mis-segregation of a translocation quadrivalent could probably not be distinguished from pure trisomy. Yet if one were, in fact, detecting evidence of translocations, the predictions for human risk would be quite different, as discussed in the Introduction. There is the further possibility (*40*) that some of the M II figures scored could belong to polar bodies rather than to secondary oocytes. If so, the pertinent total will be something between one-half and all the cells scored. It should be noted that a great discrepancy between the frequency of hyperploids diagnosed in M II and that in the pronucleus has been reported (*31*). Since it is unlikely that selection can have occurred during the few intervening hours, one possible conclusion is that some of the M II findings are artifacts. This conclusion receives further support from the fact that projections of dominant-lethal frequency made on the basis of M II results greatly exceed the actual frequencies determined experimentally.

The scoring of extra sex chromosomes in the spermatids of Microtus (*19*) is a relatively new method that has not yet had wide application. Since the observations involve specially stained bodies, rather than karyotypes, the procedure is quick, but could detect anomalies additional to whole-chromosome aneuploidy.

A fourth ND indicator involves the scoring of cells in mid-gestation embryos (*17, 18, 22, 23, 45*). This has certain disadvantages, one practical one being that several cells per conceptus must be scored instead of just one, as in M-II analysis. When chromosomal mosaics are found, it is often difficult to decide whether they were purely mitotic (post-conception) events, or meiotic hyperploidies plus subsequent mitotic loss. Conversely, non-mosaic hyperploidies need not necessarily be due to meiotic events: since the embryo derives from only a very few descendants of the blastomeres, early mitotic nondisjunction does not always result in a mosaic (*51*). Another disadvantage of the procedure is that, the later the embryos are scored, presumably the smaller is the chance of discovering autosomal trisomies. The chance of finding monosomies is probably nil, even in 9-day embryos.

Of the cytological methods available, scoring in early cleavage divisions through blastocyst (*28, 30, 31, 33, 44, 52*) appears to be most likely to avoid some of the difficulties enumerated. The main problem with this procedure is its laboriousness, but this is partly offset by the fact that trisomy can be detected in any one of 20 chromosomes, instead of just the sex

chromosomes as in the NSA method.

The chief disadvantage of the NSA method in the study of nondisjunction is that the diagnostic (trisomy) type will, at best, be relatively rare. However, with the spontaneous frequencies as low as they are, even a small number of trisomic probands are indicative of a positive effect. As for XO's, they are living proof of monosomy (from whatever mechanism) — unlike hypoploid M II cells, which are likely to be artifacts. There is no evidence of selection against sex-chromosome hyperploids in meiosis or against sex-chromosome trisomics in embryonic life, so that it may be tentatively assumed that all nondisjunctional events are recovered. The genotype of sex-chromosome trisomics gives clues as to the mechanism of their origin in a way that cytological analysis cannot accomplish. For example, recovery of X^MX^PY proves that nondisjunction could not have been a mitotic event in the embryo (which would have produced X^MX^MY). Detection of nondisjunction induction by means of NSA might be of limited value if sex chromosomes were less likely to be affected than the average autosome. However, the limited data available on this point indicate that, if anything, the opposite is the case (*4*, *17*).

Applications of the Genetic Method for Detecting Nondisjunction

The NSA system has been useful in deriving relative spontaneous frequencies of various events (see Table 3). It has been extensively used with radiation (*4*), where it yielded a pattern of differential stage sensitivities for the induction of chromosome loss (from causes other than ND). The NSA system has also seen some limited use with chemical mutagens such as TEM, MMS, IMS, and hycanthone (*4*), benzo[a]pyrene (W. L. Russell, personal communication) and methylmercuric hydroxide (W. M. Generoso, personal communication). No clearly substantiated induction of trisomy has been found. However, the monosomic (XO) category has been useful in diagnosing induced chromosome losses with certainty when females were exposed and when dominant-lethal tests would have given equivocal results.

Genetic methods for detecting autosomal trisomies have been designed. Some of these involve the use of chromosomally normal animals (*53*). One may either mate treated m^h/m^h by untreated m^a/m^a, where m^h and m^a are hypomorph and amorph respectively, such that $m^h/m^h/m^a$ is distinguishable from m^h/m^a; or one may mate treated $a+/b+$ by ab/ab, where a and b are very closely linked markers. In the second scheme, any + + progeny would be potentially trisomic for the marked chromosome and could be distinguished from a recombinant by a chromosome count. By "manufacturing" trisomics from matings involving Robertsonian translocations in the mouse (*54, 55*), it has now been shown for many chromosomes that the trisomic condition is lethal between midgestation and the day of birth, depending on the chromosome involved. Therefore, any marker genes used would have to be expressed and readily detectable during this interval, and, as of now, not enough such markers are available to tag more than a very few chromosomes.

Other methods which detect nondisjunction by complementation, i.e., without resulting chromosomal unbalance, involve the use of reciprocal or Robertsonian translocations or of isochromosomes (which are not yet available in the mouse) (*56*). All of these systems employ marker genes in the detection of the nondisjunctional event. However, since spontaneous frequencies are relatively high, at least in the case of Robertsonian translocations, it may be difficult to detect small mutagen-induced effects.

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