MEIOTIC EXPRESSION OF A RADIATION-INDUCED RECIPROCAL TRANSLOCATION
IN AN F₁ MALE CHINESE HAMSTER (CRICETULUS GRISEUS)

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MEIOTIC EXPRESSION OF A RADIATION-INDUCED RECIPROCAL TRANSLOCATION
IN AN F1 MALE CHINESE HAMSTER (CRICETULUS GRISBUS)

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SUMMARY

Spermatocytes from a sexually mature, breeding male Chinese hamster (Cricetulus griseus), the offspring of a mating between a male irradiated with X-rays at 400 R (80 R per min for five min) and a non-irradiated female, have been examined in propionic orcein squash preparations to obtain information concerning effects of paternal X-irradiation on the occurrence of reciprocal translocations in meiotic cells of the F1 animal. The parental male was bred during the pre-sterile phase immediately after irradiation.

A reciprocal translocation was detected and identified during examination of metaphase I and metaphase II cells. In metaphase I cells, the reciprocal translocation was present at a frequency approaching 100%, as calculated from the frequency of cells containing a quadrivalent association of chromosomes characteristic of such translocations. The quadrivalent association occurred in the RIV (ring-of-four) configuration.

Examination of metaphase II cells revealed alterations in chromatid arm lengths of the two largest chromosomes of the complement, I and II, indicating that these were the chromosomes involved in the exchange. The frequency of chromosomes I-II with arms of unequal length in metaphase II spermatocytes was 49.82%, or 145 of 291 chromosomes examined. Thus, the frequency of spermatocytes carrying the translocation, as calculated both from the frequency of metaphase II chromosomes I-II
with unequal arm lengths \((2 \times 49.8\% = 99.6\%)\) and from the frequency of metaphase I cells containing the RIV quadrivalent \((100.00\%)\), indicated that a reciprocal translocation between chromosomes I and II, induced by paternal X-irradiation, was carried by virtually all of the germ cells of the \(F_1\) male.
CHAPTER I
INTRODUCTION

Chromosome interchange translocations have been found to alter fertility in both plants and animals. Blakeslee and Cartledge (1926) attributed a considerable amount of pollen abortion observed in Datura to chromosomal abnormalities in the secondaries (2n + 1) in which the added chromosome carries a deficiency for one half the chromosome and a duplication for the other half. Brink (1927), in analyzing semi-sterility in Maize, concluded that translocations were the only conceivable aberrations which would yield a single semisterile genotype (AaBb) from two normal types (AAbb and aaBB). This conclusion was based on the observation that, in nature, when aaBB individuals are crossed with AAbb, only the F₁ types (aaBB and AAbb) and the F₁ (AaBb) are fertile to any extent. Thus, Brink concluded that only spores of genotypes aB and Ab are functional, while AB and ab are abortive spores.

Cartledge and Blakeslee (1934) reported two cases of chromosomal mutation and one case of gene mutation leading to pollen abortion in Datura. Chromosomal and gene mutation types were distinguishable on the basis of appearance of the aborted pollen grains. Chromosomal changes produced semisterility in constant proportions, with sterile offspring frequently representing 50% of the total.

Frequency of pollen abortion provides a convenient and precise index to mutation rate. Approximately half the known chromosomal mutations in Datura have been detected by examining pollen. The
pollen abortion mutations were transmitted to offspring through the female plant.

Burnham (1930) discovered a ring-of-four (RIV) translocation configuration in metaphase I in Maize with an exchange involving non-homologous chromosomes. Clarke and Anderson (1935) found a partially sterile strain with 10 separate pairs of chromosomes instead of eight pairs and a ring-of-four. In those cells which did contain a figure of four elements, the translocation group appeared as a chain-of-four (CIV), as described by Burnham (1932) and by Brink and Cooper (1932).

McClintock (1930) demonstrated that the ring-of-four reported by Burnham (1930) arose from a reciprocal translocation. Clarke and Anderson (1935) noted that, in Maize, RIV configurations occur when long segments of chromosomes are involved in the exchange. CIV configurations occur when one short segment is involved; when two short segments are involved, one observes eight pairs at diakinesis, with no four-element configuration.

Burnham (1948) confirmed his previous observations of RIV in semisterile lines of Maize as the most common translocation configuration, although one line examined exhibited a CIV in approximately 2% of the aberrant cells.

In studies of a reciprocal translocation in metaphase I pollen of *Sorghum versicolor* (2n = 10), Garber (1948) found an approximately 50:50 ratio of RIV to a configuration which he called "zig-zag." The zig-zag configuration represented an "interchange complex" involving four chromosomes. Those spores in which the metaphase I "interchange complex" was in the form of an open ring were sterile and led to pollen abortion;
those carrying the zig-zag configuration yielded normal spores with a complete chromosome complement. The progeny of RIV plants exhibited the 1:1 ratio of normal to semisterile pollen, with the normal pollen containing only bivalents and semisterile pollen having RIV plus three bivalents. This ratio agrees with reports of Brink (1927) and Burnham (1932), who described an interchange in Maize resulting in chain configurations and low frequency of sterile pollen. An unbalanced translocation results in empty or shriveled pollen grains and nonviable gametes. This leads to pollen abortion because the imbalance prevents the formation of the pollen tube and corresponding tube nuclei.

In animals, the gametes are viable whether the translocation is balanced or not. The unbalanced condition leads to nonviable zygotes. Down's syndrome in humans has been attributed to the presence of an extra chromosome 21. Rosencrans (1971) reported a five-year follow-up study of a patient exhibiting Down's syndrome as a result of a partial duplication of chromosome 21 arising from a reciprocal translocation D(13-15/21), originally detected in the subject's maternal grandmother, mother, and sister. The subject's physical appearance was that of a typical Down's patient, but intellectual capacity was considerably superior to that of patients with trisomy for the entire chromosome. Intellectual defects were similar to those described for chronic brain syndrome, in which scores on some portions of intelligence tests are markedly higher than on other parts. Rosencrans suggested that the extent to which a translocation mongoloid resembles typical Down's patients depends upon the extensiveness of the translocation trisomy for chromosome 21.
Hecht (1972), who also reported a D(13-15/21) translocation, noted that the condition appeared most often to involve chromosome 14, with chromosome 15 less frequently and chromosome 13 only rarely involved. Hecht also cited examples of mental retardation resulting from partial deletions. Cri-du-chat ("cat's cry") syndrome results from a partial deletion of chromosome 5. This type of deletion might, in fact, result from a reciprocal translocation accompanied by a partial loss of one chromosome and a partial duplication of another.

Carr (1971) attributed approximately 27% of spontaneous abortions in humans to reciprocal translocations producing unbalanced (deletion-duplication) gametes and postulated that such unbalanced gametes are more likely when they involve the larger chromosomes. Translocations between homologs (15/15) were found to lead to a spontaneous abortion frequency of 100%; gametes derived from meiocytes carrying such translocations would receive either both chromosomes or neither chromosome, with either type of zygote being nonviable.

Buckton et al. (1971) described a balanced X-14 reciprocal translocation in a phenotypically normal female. Both the two male sibs and the daughter of the woman carried the balanced translocation; the only living female sib was mosaic for the translocation. A majority of her cells contained 46 chromosomes, including only one of the translocation chromosomes. The remainder of her cells contained 45 chromosomes and no translocation figure. The missing region of the chromosome was identified by fluorescent staining as the short arm of the X. The female sib of the patient was phenotypically normal; she exhibited short stature but no other symptoms of Turner's syndrome,
with menarche, menstrual history, and menopause being normal. The ab-
sence of phenotypic manifestations of Turner's syndrome in the female
sib was attributed to the double dose of the short arm of the X chromo-
some in the cells containing 46 chromosomes. The patient described by
Buckton et al. was one of nine children of a mother carrying the
balanced translocation. The patient had one pregnancy resulting in a
balanced translocation daughter, who had two pregnancies, one producing
a normal male and the other terminating in spontaneous abortion. Of the
four male sibs of the patient, two carried the balanced translocation.
One of the balanced translocation males sired a child; the other, upon
testicular biopsy, was found to lack mature spermatozoa. Thus, Buckton
et al. concluded that, while female fertility was not affected by the
balanced X-autosome translocation, the translocation markedly reduced
fertility in males.

In a comparative analysis of reciprocal and Robertsonian trans-
locations in man, Jacobs et al. (1970) noted that Robertsonian
translocations, or whole-arm translocations resulting from centric
fusion, are easily detected in somatic cells since the chromosome number
is reduced to 45. Due to the nature of their origin, the number of
types of Robertsonian translocations is obviously limited. By contrast,
reciprocal translocations can occur through breaks at many different
points along the chromosome, so that many different types of exchange
are possible; thus, each reciprocal translocation can be considered
unique. Jacobs et al. detected and distinguished these types of
translocation by cytogenetic examination of both phenotypically abnormal
subjects and their relatives. Generally, the origin of an unbalanced
reciprocal translocation can be traced to one of the parents of the affected individual, with the parent carrying a balanced translocation. Familial groups carrying Robertsonian translocations are detected both by identifying individuals with phenotypic manifestations characteristic of described syndromes (e.g., G group Down's syndrome and certain cases of D trisomy), or by random search (most D group trisomic individuals).

Although their characterizations were based on studies of leucocytes and cells in skin culture, with no studies of meiotic cells, Jacobs et al. defined balanced reciprocal translocations in terms of the presence of two abnormal chromosomes, with the difference in length between one abnormal chromosome and its normal homolog being equal to the difference in length between the other abnormal chromosome and its normal homolog, irrespective of the phenotype of the carrier. Within the restrictions of their limited data, Jacobs et al. described a segregation ratio of 1:1 balanced translocation individuals to normal offspring in reciprocal translocation families, with no carriers of unbalanced translocations reported. The absence of unbalanced translocation carriers might be attributed to loss of such individuals through spontaneous abortion or stillbirth of unbalanced embryos, as described by Buckton et al. (1971) and Carr (1971). The 1:1 ratio of balanced translocations to normal individuals was also observed in families carrying Robertsonian translocations (Jacobs et al., 1970).

Subjects identified by random search as carriers of either reciprocal or Robertsonian translocations did not deviate from the expected 1:1 ratio of normal to balanced translocation individuals, while families detected by the identification of an abnormal member were
found to include living unbalanced translocation individuals, with a
greater percentage of unbalanced translocation offspring being produced
if the mother was the carrier than if the father carried the balanced
translocation. Children carrying unbalanced translocations represented
8% of the offspring of families carrying balanced translocations if the
male was the carrier and 15% if the female was the carrier. In families
carrying Robertsonian translocations detected as Down's syndrome individ-
uals, 10% of the offspring of female heterozygotes and 2% of the off-
spring of male heterozygotes exhibited unbalanced translocations.
These observations tended to suggest either that oogenesis produces
unbalanced gametes more frequently than spermatogenesis or that males
have a more efficient mechanism for selection against unbalanced
gametes than do females.

Fluorescent and Giemsa chromosome banding techniques have
considerably improved the ability to determine the exact locations of
reciprocal translocations and the exact portions of the chromosomes
involved. These techniques employ staining with fluorescent quinacrine
dyes or modified Giemsa staining, to produce differential banding
patterns. Francke (1972) suggested that differential banding produced
by staining with quinacrine mustard (QM) depends both upon the relative
amount of DNA present for binding of the dye in each location and on
the pattern of chromosomal proteins binding to DNA in that location,
which would determine the relative accessibility of the stain to DNA in
that region.
Chromosome banding techniques are especially useful in organisms such as the mouse, which has 20 pairs of telocentric chromosomes with a gradient in size differential from large to small, with no distinctive groupings. The fluorescent staining technique produces bands which permit specific identification of each chromosome. Thus, as noted by Miller and Miller (1972), reciprocally translocated chromosomes can be matched with their normal homologs and, with experience, one can determine which chromosomes participated in the reciprocal translocation, even when normal homologs are not present (e.g., in cells with homozygous reciprocal translocations). Miller et al. (1971), using quinacrine fluorescence staining, completed identification of the chromosomes of the mouse karyotype and tentatively assigned them to seven linkage groups.

Francke (1972) employed QM staining to determine precisely which chromosomes were involved in several reciprocal translocations in man. She was able to determine not only which chromosomes were involved, but also the points of breakage and rejoining and the occurrence of deletions. Shaw (1972) argued that certain of the translocations reported by Francke (1972) were not reciprocal, but represented attachment of fragments to ends of unaffected chromosomes, followed by "healing" of the shortened, broken chromosomes at their free ends. This conclusion contradicts the "telomere theory," in which chromosome ends were postulated to be inviolate. Banding techniques have permitted such identification and localization of fragments, as well as providing specific criteria for identification of members of groups (A-G) of human...
Among effects of paternal X-irradiation on mammalian reproduction are alterations in the ability of the irradiated animal to produce viable gametes and changes in the ability of the progeny to produce viable offspring (Mandl, 1964). After exposure of male mice to X-rays in the range of 400-1000 R, Russell (1954) observed the occurrence of an initial "pre-sterile" period in which fertility is not significantly decreased. Since both late spermatids and mature spermatozoa are highly resistant to lethal effects of X-rays (Oakberg and Clark, 1964), fertility is not altered immediately by X-irradiation. The pre-sterile phase is followed by a "sterile" period, whose duration increases with dosage. Fertility is restored during a subsequent "post-sterile" period; the resurgence of fertility during the post-sterile phase is attributed to the repopulation of the testis by proliferation from radiation-resistant spermatogonia which survive dosages to which the more sensitive majority of gonial cells succumb during the sterile phase (Russell, 1954).

Both dominant sterility and semisterility have been reported in offspring of X-irradiated male mice mated during the pre-sterile phase (Russell, 1954). Evidence from cytogenetic studies indicates that reciprocal translocations play a significant role in both dominant sterile (Leonard and Deknudt, 1968) and semisterile conditions (Snell, 1935 and 1946; Russell, 1954).

Snell (1935) reported that the size of litters was reduced in approximately 25% of the F1 offspring of X-irradiated male mice;
he designated these F1 animals semisteriles. Ratios of genetic crosses of semisterile mice to normal animals were consistent with the genetic behavior of reciprocal translocations, and translocations have been demonstrated cytologically in semisterile mice (Koller and Auerbach, 1941).

In studies of meiotic cells of the mouse and other species, the occurrence of reciprocal translocations is detected as a reduction of the number of units found in metaphase I. Fredga and Rayner (1967) reported the occurrence of a reciprocal translocation in man characterized by the appearance of 22 units instead of the normal 23 bivalents in the majority of undamaged meiotic cells at metaphase I. In some of the cells, the quadrivalent was easily discernible as a chain-of-four chromosomes. The translocation was found to occur between chromosomes 15 and 18; thus, at metaphase II, the cells contained one chromosome 15, one chromosome 18, and two new chromosomes which were designated \( \text{Tr}_1 \) and \( \text{Tr}_2 \). Other workers have confirmed the correlation between reciprocal translocations and the loss of a bivalent at metaphase I (Reddi, 1965; Leonard and Deknudt, 1968 and 1969; Lyon et al., 1970).

The most common chromosomal configurations in reciprocal translocations are the ring-of-four (R1V) and the chain-of-four (CV), with the R1V configuration occurring more frequently (Koller and Auerbach, 1941; Lyon et al., 1970). Simple reciprocal translocations in the mouse are represented by a configuration resembling a cross, with the length of the arms dependent on the positions of exchange points (Griffen, 1958). Griffen and Bunker (1967) attributed the R1V configuration to a repulsion at diakinesis which develops between
homologous pairs of chromatids; the repulsion results in outward rotation of the arms of the cross-figure and, eventually, in the formation of a circle with the four components oriented end-to-end. The non-centromeric ends remain associated via terminalizing chiasmata.

The CIV configuration results when chiasmata are not formed in one of the distal arms of the cross-figure or when those which are formed complete terminalization so that the association is not maintained. The RIV and CIV translocation figures observed at meiotic metaphase I in the mouse conform to the pattern described above (Griffen, 1958; Griffen and Bunker, 1967).

Numerous investigators have analyzed single and reciprocal X-ray-induced translocations in chromosomes of the mouse (e.g., Ford and Evans, 1964; Ashwood-Smith et al., 1965; Leonard and Deknudt, 1966 and 1970; Griffen and Bunker, 1967; Ford and Clegg, 1969; Evans et al., 1970). However, information concerning X-ray-induced translocations in other mammalian species is somewhat more limited. In the present study, spermatocytes from the sexually mature, breeding offspring of an X-irradiated male Chinese hamster (*Cricetulus griseus*) have been examined to obtain information concerning effects of paternal X-irradiation on the frequency of reciprocal translocations in meiotic cells of male progeny in this species.

The Chinese hamster is a particularly favorable mammalian species for cytogenetic analysis since the normal karyotype includes only eleven chromosome pairs, all but three small pairs of which are cytologically distinguishable without the use of banding techniques (Yerganian, 1958 and 1959). Criteria commonly employed for identification of individual
chromosome pairs as they appear in c-metaphases include arm ratio, or ratio of the length of the longer arm of the chromosome to shorter arm length, and centromere index, or ratio of the shorter arm length to the length of the entire chromosome (Yerganian, 1963). Based on similarities in arm ratio and centromere index, Yerganian (1959) divided the autosomes of C. griseus into four groups: (1) pairs 1 and 2; (2) pairs 4 and 5; (3) pairs 9, 10, and 11; and (4) pairs 6, 7, and 8. All members of groups (1), (2), and (3) are metacentric; members of group (4) have subterminal centromeres. The metacentric X is slightly larger than the autosomes of group (2), and the Y chromosome, which has a submedian centromere, is somewhat smaller than group (2) autosomes.
CHAPTER II

MATERIALS AND METHODS

Source of Material

Meiotic cells were obtained from testes of a single male Chinese hamster (Cricetulus griseus Milne-Edw.). This hamster was among the offspring of a male which had been irradiated with X-rays at a dose rate of 80 r per minute for a total whole-body dose of 400 r. The parental male was bred during the pre-sterile phase immediately after irradiation. The F1 male used as a source of material in the present study was a mature, breeding animal at the time of sacrifice.

Preparation of Seminiferous Tubules for Subsequent Squashing

The squashing technique was a modification of that of Brooks and Lengemann (1967). Pretreatment with colchicine was omitted from the procedure since (1) mitotic metaphases were not of interest in the examination of testicular material and (2) the lungs of the same animal were to be cultured and examined in a study by another investigator.

A further modification of the procedure cited above consisted of rinsing the seminiferous tubules in a 45% acetic acid solution prior to placement on slides for squashing. Squashed tubules in acetic acid solution are much more readily frozen than are tubules in alcoholic solution. The tubules were allowed to remain in acetic acid solution for one hour.
Preparation of Squash Slides for Staining

Tubules were removed from the acetic acid solution and were placed individually on clean slides. Care was taken to transfer as little liquid as possible from beaker to slide. To insure the chemical cleanliness of the slides, they were soaked in a 1:1 mixture of saturated aqueous potassium dichromate and concentrated sulfuric acid for a minimum of twenty-four hours prior to use.

A large coverslip (22 x 40 mm) was placed carefully over the tubule, and pressure sufficient to effect squashing was applied with a pencil eraser. The squash preparation was then placed on a block of dry ice for 15-30 min (Conger and Fairchild, 1953). At the end of this interval, with the slide still in place on the block of dry ice, the coverslip was flipped off with a razor blade.

The slide was removed from the dry ice block, was numbered with an etching tool, and was allowed to air-dry. At this stage, squash preparations could be stored for several weeks.

Staining Procedures

A saturated solution of orcein in 50% propionic acid was prepared daily by heating to boiling a sufficient amount of powdered orcein to effect saturation in several milliliters of propionic acid. The solution was allowed to cool to room temperature and was placed in an ice-cold water bath for several minutes. The cooled solution was then filtered and was used immediately. Cooling permits maximal precipitation of stain prior to filtration, reducing the amount of
precipitated orcein on the prepared slide.

Air-dried slides were stained by placing one or two drops of the filtered orcein solution over the tubule and covering with a large coverslip. Slides were blotted with a paper towel to remove excess stain. Gentle pressure was applied to the towel above the coverslip to ensure a uniform and minimal volume of stain remaining under the coverslip. Slides were then sealed temporarily with paraffin. After preliminary microscopic examination, selected slides were made permanent according to the following procedure:

1. The slides were placed again on dry ice until the stained material was frozen, 15-30 min.
2. Coverslips were removed with a razor blade, as described above. (Freezing facilitates coverslip removal since it renders the paraffin seal brittle.)
3. In preparation for the introduction of permount to the squash preparations, slides were then passed through a series of rinses for time intervals specified below.
   a. Twenty minutes in 95% ethanol (to remove excess stain);
   b. One hour in a 1:1 mixture of ethanol:toluene;
   c. Two successive 24-hour rinses in absolute toluene.
4. Each squashed tubule was covered with a single drop of permount, and a coverslip was placed over it in such a manner that the permount was distributed uniformly under the coverslip. Mounted preparations were then allowed to dry for a minimum of one week.
Microscopic Examination

Temporary slides were examined with oil immersion using phase-contrast optics at a magnification of 1000X. The meiotic stages examined included diakinesis, metaphase I, and metaphase II. Slides on which these stages occurred were subsequently made permanent as described above.

It was discovered that configurations in many of the cells in diakinesis were obscured by intensity of staining. In order to examine this stage, squash preparations were sealed with a small amount of acetic acid remaining under the coverslips and were examined with phase-contrast optics. They were photographed and subsequently were subjected to air-drying and staining as described above. Stained preparations of diakinesis chromosomes also were photographed.

Photography

All photographs were made from temporary mounts using phase-interference optics. A 35 mm Nikon camera mounted on a Leitz microscope was used in photographic recording. The shutter was controlled by a system which provided for uniformity of photographs with respect to degree of exposure. Negatives and prints were developed using Kodak Dektol and Fixer.

Tabulation

Two sets of data were compiled separately. The number of chromosomes per cell in metaphase I plates was counted to determine the frequency of cells exhibiting reduction in number of bivalents. In
metaphase II cells, arm lengths of the chromatids of chromosomes I and II were compared; numbers of chromosomes in which lengths of chromatid arms were equal and numbers exhibiting unequal chromatid arm length were recorded. Preliminary attempts to distinguish chromosomes I and II in metaphase II preparations were frequently unsuccessful since, in many cells, only one of the two chromosomes was present. Thus, data for chromosomes I and II were grouped in counts of chromosomes with alterations in arm length.
CHAPTER III

RESULTS

A reciprocal translocation between chromosomes I and II was detected and identified during examination of metaphase I and metaphase II plates. The results of examination of metaphase I plates indicated that a reciprocal translocation was present at a frequency approaching 100%; these findings are summarized in Table 1.

The quadrivalent association occurred in the RIV (ring-of-four) configuration (Figs. 1-3). Figures 1-2 represent the cell in diakinesis. Figure 3 is a photograph of the RIV as it appears at late diakinesis.

Of 425 cells examined during metaphase I, 260 (61.17%) exhibited ten bodies, which included nine bivalents and one quadrivalent (Figs. 4-5).

In 115 of the 425 cells, nine bodies were present in the nucleus. Of these 115 cells, all contained the quadrivalent and 88 could be identified as lacking one of the smaller bivalents of the karyotype.

In 45 of the 425 cells, there were eight bodies, including the quadrivalent and seven bivalents. In 33 of these 45 cells with eight bodies, the two missing bivalents were identified as two of the three smaller bivalents of the karyotype.

Thus, a total of 381 of the 425 cells examined both contained the quadrivalent and exhibited a reduction in number of bivalents in
Table 1. Frequency of Reciprocal Translocation between Chromosomes I and II in Metaphase I Spermato­cytes of F1 Crictetus griseus after Paternal X-irradiation at 400 R

<table>
<thead>
<tr>
<th>No. Bodies in Nucleus</th>
<th>No. Cells Counted</th>
<th>No. Cells Containing Quadrivalent in which Missing Bivalents Were Identified</th>
<th>No. Cells Containing Quadrivalent in which Missing Bivalents Were Not Identified</th>
<th>Total No. Cells Containing Quadrivalent</th>
<th>Total % Cells Containing Quadrivalent</th>
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<tr>
<td>8</td>
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<td>425</td>
<td>381</td>
<td>39</td>
<td>425</td>
<td>100.00</td>
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Figure 1. Ring-of-Four (RIV) Quadrivalent Association in a Diakinesis Spermatocyte.

Figure 2. Ring-of-Four (RIV) Quadrivalent Association in a Second Spermatocyte at Diakinesis.
Figure 3. Ring-of-Four (RIV) Quadrivalent as Seen in a Spermatocyte at Late Diakinesis.

Figure 4. Metaphase I Spermatocyte with Quadrivalent RIV and Nine Bivalents.
which the missing bivalents could be identified. Only five cells con-
tained eleven bodies; all had the quadrivalent RIV plus ten bivalents. The remaining 39 cells contained the quadrivalent and exhibited a reduction in number of bivalents, but the missing bivalents could not be identified.

Metaphase II plates were examined for changes in arm length of individual chromatids to determine which chromosomes were involved in the translocation. These examinations revealed alterations in arm length of the two largest chromosomes, I and II (Fig. 6).

In examination of metaphase II plates, attempts to distinguish chromosomes I and II were frequently unsuccessful because, in many cells, only one of the two chromosomes was present and comparisons could not be made. Thus, data for chromosomes I and II were grouped in counting chromosomes with alterations in arm length. Numbers and percentages of chromosomes I-II exhibiting alterations in arm length are recorded in Table 2. A total of 291 chromosomes I-II were examined in 193 metaphase II cells. Of these 291 chromosomes, 146 (50.17%) had arms of equal length and 145 (49.82%) had arms of unequal length.

Of the 193 metaphase II cells examined, 74 cells did contain both chromosomes I and II. These cells were used to determine the type of exchange occurring among the components of the RIV (see Fig. 7 and Tables 3-4).
Figure 5. A Second Metaphase I Spermatocyte with Quadrivalent RIV and Nine Bivalents.

Figure 6. Metaphase II Spermatocyte Exhibiting Altered Chromatid Arm Lengths in Chromosomes I and II.
Figure 7. Possible Crossovers within the Ring-of-Four (RIV) Quadrivalent Association.
Table 2. Percentage of Metaphase II Chromosomes I-II of F₁ Cricetulus griseus Spermatocytes Exhibiting Alterations in Arm Length after Paternal X-irradiation at 400 R

<table>
<thead>
<tr>
<th>No. Metaphase II Cells Examined</th>
<th>No. Chromosomes Examined</th>
<th>% Chromosomes with Arm Lengths Equal</th>
<th>No. Chromosomes with Arm Lengths Unequal</th>
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</thead>
<tbody>
<tr>
<td>193</td>
<td>291</td>
<td>146</td>
<td>145</td>
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<tr>
<td></td>
<td></td>
<td>50.17</td>
<td>49.82</td>
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Table 3. Gametes Formed by Translocation Heterozygote when Crossing Over Does Not Occur at the First Meiotic Division

<table>
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<tr>
<th>Pattern of Segregation</th>
<th>Meiotic Gametes</th>
<th>Gametes</th>
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<tbody>
<tr>
<td>Alternate (I-II/II-TII)</td>
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<td>G'F'E'D'U'T'</td>
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<td>ABCDEFG</td>
<td>G'F'E'D'U'T'</td>
</tr>
<tr>
<td></td>
<td>TUWXYZ</td>
<td>A'B'C'V'W'X'Y'Z'</td>
</tr>
<tr>
<td></td>
<td>TUWXYZ</td>
<td>A'B'C'V'W'X'Y'Z'</td>
</tr>
<tr>
<td>Adjacent II (I-TII/T-II)</td>
<td>ABCDEFG</td>
<td>G'F'E'D'U'T'</td>
</tr>
<tr>
<td></td>
<td>ABCDEFG</td>
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<td></td>
<td>A'B'C'V'W'X'Y'Z'</td>
<td>TUWXYZ</td>
</tr>
<tr>
<td>Adjacent I (I-TI/II-TII)</td>
<td>ABCDEFG</td>
<td>TUWXYZ</td>
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<tr>
<td></td>
<td>ABCDEFG</td>
<td>TUWXYZ</td>
</tr>
<tr>
<td></td>
<td>G'F'E'D'U'T'</td>
<td>TUWXYZ</td>
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<tr>
<td></td>
<td>G'F'E'D'U'T'</td>
<td>A'B'C'V'W'X'Y'Z'</td>
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</table>
Table 4. Gametes Formed by Translocation Heterozygote when Crossing Over Occurs at the First Meiotic Division

<table>
<thead>
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<th>Pattern of Segregation</th>
<th>Meioyte</th>
<th>Gametes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternate (I-II/T_I-T_{II})</td>
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<td>ABCDEFG</td>
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<td>ABC'V'W'X'YZ</td>
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<td></td>
<td>TUVWXYZ</td>
<td>ZYXWVCSA</td>
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<td></td>
<td>TU'D'E'F'G</td>
<td>TUDEFG</td>
</tr>
<tr>
<td>Adjacent II (I-T_{II}/T_I-T_{II})</td>
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<td>ABCDEFG</td>
</tr>
<tr>
<td></td>
<td>ABC'V'W'X'YZ</td>
<td>ABCVWXYZ</td>
</tr>
<tr>
<td></td>
<td>Z'Y'X'W'V'C'B'A</td>
<td>ABCDEFG</td>
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<tr>
<td></td>
<td>G'FEDCB'A'</td>
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<td></td>
<td>TU'D'E'F'G</td>
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<tr>
<td>Pattern of Segregation</td>
<td>Meiocyte</td>
<td>Gametes</td>
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<td>------------------------</td>
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</tr>
<tr>
<td>Adjacent I (I-T&lt;sub&gt;1&lt;/sub&gt;/II-T&lt;sub&gt;II&lt;/sub&gt;)</td>
<td>ABCDEFG</td>
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<td>ABC'V'W'X'YZ</td>
<td>Balanced</td>
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<tr>
<td></td>
<td>G'E'D'U'T'</td>
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<tr>
<td></td>
<td>Z'Y'XWVUT'</td>
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<td>Balanced</td>
<td>G'FEDCB'A'</td>
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<td>ABCVWXYZ</td>
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<td>TUDEFG</td>
<td>TUVWXYZ</td>
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</table>
CHAPTER IV

DISCUSSION

Reciprocal translocations reduce the randomness of selection, which is prohibited when chromosomes are connected in rings or chains during metaphase I. A well-known example is found in *Oenothera*. *O. lamarckiana* \((n = 7)\) has one bivalent and a ring-of-12 at metaphase I. According to Strickberger (1968), with complexes such as the ring-of-12 in *O. lamarckiana* or the ring-of-14 in *O. biennis* and *O. muricata*, segregation is almost exclusively alternate, with duplications and deficiencies being generally absent and entire translocation complexes segregating as a unit in each gamete. This, of course, is an extreme example in which there is total absence of random segregation. Translocations involving fewer chromosomes exhibit more randomness, and those with no translocations exhibit total randomness of segregation.

The Chinese hamster (*Cricetulus griseus*) has a normal complement of 11 chromosome pairs. The presence of the ring-of-four (RIV) quadrivalent in 100.00% of the 425 metaphase I spermatocytes examined and the reduction in number of bivalents in 98.82% of the metaphase I spermatocytes (Figs. 1-5; Table 1) indicate the presence of a reciprocal translocation in the spermatocytes of the F1 individual examined in this study. Of the five cells \((1.18%)\) which had 11 bodies in the nucleus, all had the RIV quadrivalent; the presence of the extra chromosome is attributed to "leak-over" from another cell during preparation.
The number of cells available for examination was limited by difficulties inherent in the preparation technique. Only cells whose metaphase plates were in the proper one-dimensional orientation after mounting could be counted (i.e., those in which chromosomes were properly flattened and facing the observer). Precipitation of stain and bunching and folding of chromosomes obscured configurations in many preparations and limited the number of usable cells.

Examination of metaphase II plates revealed alterations in arm length of the two largest chromosomes, I and II. If a reciprocal translocation between chromosomes I and II had been induced in the irradiated parental male and had been transmitted to the F₁ individual examined, alterations in arm length should be detectable in metaphase II meiotic cells of the F₁ male, with the frequency of chromosomes I and II exhibiting unequal arm lengths corresponding to one-half the frequency of cells carrying the reciprocal translocation. Such differences in arm length are expected to be negligible in the normal homologs contributed by the non-irradiated parental female.

The metaphase II cells were examined to determine whether the lengths of the paired arms of chromosome I and/or chromosome II were equal or unequal. Since, of the 193 cells examined, a large number (119) contained only one of these two large chromosomes, differentiation between the two chromosomes was impossible in these cells and data for both chromosomes were grouped.

The frequency of chromosomes I-II with arms of unequal length in metaphase II spermatocytes of this F₁ male was 49.82%, or 145 of 291 chromosomes examined (Table 2). Thus, the frequency of spermatocytes
carrying the translocation, both as calculated from the frequency of metaphase II chromosomes I-II exhibiting unequal arm lengths (2 x 49.82% = 99.64%) and as calculated from numbers of metaphase I cells in which the quadrivalent (RIV) was detected (100.00%) indicates that a reciprocal translocation between chromosomes I and II, presumably induced by paternal X-irradiation at 400 R, is carried by virtually all of the germ cells of the F₁ male.

Somatic (lung) cells from the same animal have been cultured by Dr. R. H. Fetner; mitotic metaphase cells were photographed and arm lengths were measured and compared by computer analysis. Lung cells from a normal hamster also were cultured and analyzed. Since the reciprocal translocation occurred between chromosomes I and II, the two largest chromosome pairs in this species, the four largest chromosomes of the 22 chromosomes of F₁ lung cells were designated I, T₁, II, and T₁. Computer analysis revealed that the translocation consisted of the reciprocal exchange of a longer segment of genetic material from chromosome I to chromosome II and of a shorter segment from chromosome II to chromosome I (R. H. Fetner, personal communication). Thus, in order of decreasing size, the products of the aberration are T₃, I, II, and T₁.

In cataloging the chromosomes of the 74 meiotic cells in which both chromosomes I and II were present in metaphase II, six different categories initially were established, based on comparisons of arm lengths within a chromosome (Fig. 7; Tables 3-4).

(1) One cell in which both chromosome I and chromosome II
appeared shorter than normal and intrachromosomal arm lengths were equal.

(2) Seven cells in which one chromosome had short and equal arm lengths and the other had long and equal arm lengths. This category results when there is no crossing over and segregation at metaphase I is of the alternate type, resulting in the occurrence of the two translocation chromosomes in the same cell during metaphase II.

(3) Three cells in which one chromosome had short and equal arms and the other had long and unequal arms. This category results from alternate segregation in which there are no crossovers involving $T_I$, while $T_{II}$ is involved in one crossover event.

(4) Nineteen cells in which one chromosome had long and equal arms and the other had unequal arm lengths. This category could result from (a) adjacent II segregation in which there were no crossovers in chromosome I and one crossover in $T_{II}$ or in which there were no crossovers in $T_{II}$ and one crossover in chromosome I, or (b) adjacent I segregation in which there were no crossovers in chromosome II and one crossover in $T_{II}$.

(5) Twenty-three cells in which both chromosomes were long and equal. This situation could result if there were no crossing over (or, less likely, if there were two crossovers) between chromosomes, followed by any of the following segregation patterns: (a) Alternate (I-II); (b) Adjacent II (I-$T_{II}$); or (c) Adjacent I (II-$T_{II}$). The situation described in category (1), in which arm lengths of chromosomes I and II were equal and were both shorter than normal, could also result from
this set of circumstances if one assumed that different regions of the cell were in different focal planes. This explanation seems likely since category (1) was observed only once and since no form of segregation with or without crossing over would be expected to yield a metaphase II cell in which both chromosomes are shorter than normal and chromatid arms are equal in length.

(6) Twenty cells in which arm lengths of both chromosomes were unequal. This category could result from any combination of chromosomes undergoing adjacent I, adjacent II, or alternate segregation, whenever the crossover frequency is equal to one crossover per chromosome.

The expected results of alternate and adjacent segregation are illustrated in Figure 7 and Tables 3-4. If no crossing over occurred in any of the four chromosomes involved or if, more rarely, a double crossover occurred, then only in alternate segregation would one obtain gametes that would result in viable zygotes. Either adjacent I or adjacent II segregation would result in both losses and duplications of genetic material, with the production of nonviable zygotes. It is true that in humans the duplication of a part of a chromosome in translocation trisomy 21 results in viable Down's syndrome individuals (Rosen- crans, 1971; Hecht, 1972), but in this case there is no loss of genetic material. Carr (1971) reported that 27% of spontaneous abortions in humans result from unbalanced gametes and noted that these unbalanced gametes are more likely to involve translocations between larger chromosomes.

Assuming one crossover per chromosome (or four crossovers per
RIV), the results are as follows: (a) Half the gametes resulting from alternate segregation are balanced; (b) none of the gametes resulting from adjacent I segregation are balanced; and (c) half the gametes resulting from adjacent II segregation are balanced. Thus, the reduction in fertility as a result of the reciprocal translocation is demonstrated.

The zygotes expected from a mating between this male F₁ hamster and a normal female are shown in Table 5. Of the 36 possible combinations of male and female gametes, four combinations would resemble the normal mother and would result in viable zygotes; four would resemble the balanced F₁ father and would also result in viable offspring; and 18 would be unbalanced to the extent that viable offspring would be impossible. The remaining 10 combinations could yield viable but physically defective offspring (comparable to human individuals with Down's syndrome resulting from partial duplication or Cri-du-chat syndrome resulting from partial deletion). If these combinations should result in viable offspring, semisterility is observed; if not, then fertility may be greatly reduced, depending upon the extent to which partial loss or duplication of chromosome material is consistent with formation of viable zygotes by the organism.

Examination of progeny of this F₁ male would have been informative in determining the stability of the demonstrated translocation and its effects on the fertility of the F₁ male. Unfortunately, no such data are available since mating records have been maintained only for female hamsters of this colony.
Table 5. Zygotes Expected from a Mating between Male Fj Hamster Examined in this Study and Normal Female after Alternate, Adjacent II, or Adjacent I Segregation

<table>
<thead>
<tr>
<th>Segregation</th>
<th>I-II</th>
<th>I'-II'</th>
<th>Eggs</th>
<th>I-II'</th>
<th>I-I'</th>
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<tbody>
<tr>
<td>Alternate</td>
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<td>I-I'</td>
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</tr>
</tbody>
</table>

Sperm: Chromosome I normal-short (I-TI), Chromosome II normal-long (II-TII)
Egg: Both chromosome pairs normal (I-I', II-II')
(*) = Not necessarily lethal but causing partial duplications or deletions, possibly resulting in defective offspring analogous to Down's or Cri-du-chat syndrome in man
CHAPTER V

RECOMMENDATIONS

1. This work should be repeated with the following additions and modifications:
   
a. Mitotic screening of male offspring of the irradiated male to detect those individuals with chromosomal abnormalities.
   
b. Maintenance of breeding records for any abnormal male offspring identified by mitotic screening, to determine effects of the chromosomal abnormalities on reduction in fertility.
   
c. Unilateral orchiectomy in lieu of sacrifice of affected offspring, to permit continuation of breeding studies in conjunction with cytological studies of meiotic cells.
   
d. Examination of meiotic cells of male offspring of the F1 animal carrying the abnormality to determine the extent to which partial duplication and/or loss of genetic material is consistent with viability, in view of observations of viable, though phenotypically abnormal human individuals who are partially trisomic (e.g., Down's syndrome resulting from translocation) or who carry partial deletions (e.g., Cri-du-chat syndrome).
2. The use of fluorescent banding techniques would expedite the identification of those chromosomes involved in translocations and would facilitate the identification of any chromosome sections which might have been lost in the exchange. This technique also would permit cytological detection of inversions in the F₁ and subsequent generations.
LITERATURE CITED


